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REVIEW *Andrology*

Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome

S. Franik[1,*], Y. Hoeijmakers[1], K. D'Hauwers[2], D.D.M. Braat[1], W.L.M. Nelen[1], D. Smeets[3], H.L. Claahsen – van der Grinten[4], L. Ramos[1], and K. Fleischer[1]

[1]Department of Obstetrics and Gynaecology, Radboudumc Nijmegen, Nijmegen, The Netherlands [2]Department of Urology, Radboudumc Nijmegen, Nijmegen, The Netherlands [3]Department of Genetics, Radboudumc Nijmegen, Nijmegen, The Netherlands [4]Department of Pediatrics, Amalia Children's Hospital, Radboudumc Nijmegen, Nijmegen, The Netherlands

*Correspondence address. E-mail: s.franik@radboudumc.nl

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STUDY QUESTION: Should fertility preservation be offered to children with Klinefelter syndrome (KS)?

SUMMARY ANSWER: Current evidence shows that fertility preservation should not be offered to adolescents with KS younger than 16 years because of lower retrieval rates for germ cells by testicular sperm extraction (TESE) compared with retrieval rates for adolescents and adults between 16 and 30 years.

WHAT IS KNOWN ALREADY: KS, the most common chromosomal disorder in men leading to non-obstructive azoospermia, is caused by the presence of at least one additional X chromosome. The onset of puberty in adolescents with KS leads to progressive degeneration of the testicular environment. The impact of the subsequent tissue degeneration on fertility potential of patients with KS is unknown, but in previous literature it has been suggested that fertility preservation should be started in adolescents as early as possible. However spermatozoa can be found by TESE in about 50% of adults with KS despite severe testicular degeneration. This review discusses the current evidence for fertility preservation in children and adolescents and possible prognostic markers for fertility treatment in KS.

STUDY DESIGN, SIZE, DURATION: An extensive literature search was conducted, searching Pubmed, Embase, Cinahl and Web of Science from origin until April 2016 for 'Klinefelter syndrome' and 'fertility' and various synonyms. Titles and abstracts have been scanned manually by the authors for eligibility.

PARTICIPANTS/MATERIALS, SETTING, METHODS: In total 76 studies were found to be eligible for inclusion in this review. Information from the papers was extracted separately by two authors.

MAIN RESULTS AND THE ROLE OF CHANCE: Various studies have shown that pre-pubertal children with KS already have a reduced number of germ cells despite a normal hormonal profile during childhood. The presence of spermatozoa in the ejaculate of adolescents with KS is extremely rare. Using TESE, the retrieval rates of spermatozoa for adolescents younger than 16 years old are much lower (0–20%) compared with those for adolescents and young adults between 16 and 30 years old (40–70%). Although spermatogonia can be found by TESE in about half of the peri-pubertal adolescents, there are currently no clinically functional techniques for their future use. Children and adolescents need to be informed that early fertility preservation before the age of 16 cannot guarantee fertility later in life and may even reduce the chances for offspring by removing functional immature germ cells which may possibly develop into spermatozoa after puberty. Furthermore, except for the age of patients with KS, there are no identified factors that can reliably be used as a predictive marker for fertility preservation.

LIMITATIONS, REASONS FOR CAUTION: Most of the evidence presented in this review is based on studies including a small number of adolescents with KS. Therefore, the studies may have been underpowered to detect clinically significant differences for their various outcomes, especially for potential predictive factors for fertility preservation, such as hormone levels. Furthermore, the population of patients with KS

diagnosed during childhood might be different from the adult population with KS where the diagnosis is based on infertility. Results based on comparisons between the two groups must be interpreted with caution.

WIDER IMPLICATIONS OF THE FINDINGS: Despite the limitations, this review summarizes the current evidence for managing fertility preservation in patients with KS to provide optimal health care.

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Key words: Klinefelter syndrome / fertility preservation / sperm retrieval / TESE / adolescent

Introduction

Klinefelter syndrome (KS) was first described in 1942 by Harry F. Klinefelter and has a prevalence of about 2 in 1000 males (Klinefelter *et al.*, 1942; Bojesen *et al.*, 2003). About 90% of the cases are due to the 47,XXY karyotype, while the remaining 10% show a 46,XY/47,XXY mosaicism or higher-grade X aneuploidies (Jacobs and Strong, 1959; Linden *et al.*, 1995; Lanfranco *et al.*, 2004; Visootsak and Graham, 2006). The phenotype of KS varies greatly: KS mosaicism often causes fewer clinical symptoms in comparison with 47,XXY karyotype or higher-grade X aneuploidies. Typical symptoms include: (i) gynecomastia and a low sexual drive due to testosterone deficiency; (ii) small testes and infertility due to Sertoli cell dysfunction; and (iii) psychosocial problems such as concentration problems and learning difficulties (Paulsen *et al.*, 1968; Smyth and Bremner, 1998). Because of the variable phenotype, KS is often not diagnosed. Only 25% of the adult men with KS have been diagnosed as such, with less than 10% of them being diagnosed before puberty (Bojesen *et al.*, 2003). Most often, the diagnosis of KS is suspected in adulthood after a diagnostic workup of infertility. Approximately 90% of the adult men diagnosed with KS suffer from non-obstructive azoospermia (NOA) and about 10% from subfertility due to oligospermia (Forti *et al.*, 2010). With the availability of assisted reproductive technology (ART) such as testicular sperm extraction (TESE) in combination with intracytoplasmic sperm injection (ICSI), nowadays about half of the men diagnosed with KS can father their own biological children (Aksglæde *et al.*, 2011). To date, no markers or clinical parameters have been found to predict spermatogenesis in males with KS.

In this review, we discuss the current literature on fertility preservation and possible prognostic markers for fertility treatment in KS.

Spermatogenesis and testicular function in Klinefelter syndrome

Many studies have investigated the hormonal profile and histological aspects of testis tissue of KS at all ages (Table I; also compare Davis *et al.* (2015)). The degeneration of germ cells in KS might be caused by a negative effect of the extra X chromosome during chromosome segregation by aberrant sexual chromosome silencing or an adverse influence of the supporting somatic cells on germ cells (Turner, 2015). It remains unclear whether or not germ cell development is normal in early embryonic development. Murken *et al.* (1974) found that degeneration of germ cells starts in fetal life; another study by Coerdt *et al.* (1985) showed that there is a significantly reduced number of germ cells in testicular biopsies from 47,XXY midterm fetuses, although the number and density of testicular tubules and mesenchymal structures appear to be normal (Murken *et al.*, 1974; Coerdt *et al.*, 1985). Two further studies describe normal histology in early fetal testes biopsies (Gustavson *et al.*, 1978; Flannery *et al.*, 1984). In healthy neonates, the pituitary-gonadal axis is strongly activated, producing pubertal or even adult levels of serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and consequently testosterone and inhibin B until the age of 6 months. This is also called 'the mini-puberty' (Andersson *et al.*, 1998). After the mini-puberty, the hormone levels decrease to pre-pubertal levels until the pituitary gonadotrophin axis is reactivated during puberty. In 2004, Lahlou *et al.* showed that in infants with KS testosterone levels were elevated during the first months of life, indicating the presence of a mini-puberty (Lahlou *et al.*, 2004). However, their testosterone levels were significantly lower when compared with infants with a normal karyotype, indicating a disturbed function of the Leydig cells (Lahlou *et al.*, 2004). In contrast, several papers describe a normal development of Sertoli and Leydig cells but with a reduced number of germ cells during early fetal life (Mikamo *et al.*, 1968; Edlow *et al.*, 1969; Ratcliffe, 1982). During childhood, there are normal prepubertal hormonal levels of testosterone, sex hormone binding globulin (SHBG), FSH, LH, inhibin B, anti-Müllerian hormone (AMH), estradiol (E2) but a reduced number of spermatogonia in KS (Ferguson-Smith, 1959; Topper *et al.*, 1982; Stewart *et al.*, 1986; Christiansen *et al.*, 2003; Wikström *et al.*, 2004, 2006a; Bastida *et al.*, 2007; Zeger *et al.*, 2008; Gies *et al.*, 2012a,b; Van Saen *et al.*, 2012a,b). To conclude, the reproductive potential of pre-pubertal children with KS seems to be already compromised, but the precise mechanism remains as yet unknown (Oates, 2012; Davis *et al.*, 2015; Gies *et al.*, 2016).

During puberty, major histological changes in the testes occur. Initially, the testes of KS adolescents grow up to a volume of 6 ml due to the proliferation of Sertoli cells and interstitial cells (Wikström *et al.*, 2004). However, rising serum testosterone levels are subsequently followed by an accelerating decline of germ cells, hyalinization of the tubules, degeneration of Sertoli cells and hyperplasia of Leydig cells (Wikström *et al.*, 2004). This degeneration of the interstitial stroma is accompanied by a decrease in the testis volume to a prepubertal size of 2–4 ml (Ratcliffe *et al.*, 1986; Robinson *et al.*, 1986). It is still unclear whether or not the rise in serum or intratesticular testosterone concentrations during puberty is associated with accelerated destruction of the seminiferous tubules during this period (Davis *et al.*, 2015).

Table I Sexual development in patients with KS.

Study ID	Number of participants	Age of participants	Results
Coerdt *et al.* (1985)	35	AD 20–22 weeks	Low number of germ cells Elevated number of tubules without germ cells
Ratcliffe (1982)	1	4 weeks	Normal sertoli and leydig cells Low number of spermatogonia
Lahlou *et al.* (2004)	18	0–8 months	Low serum testosterone Normal LH, FSH, inhibin B, AMH
Ross *et al.* (2005)	22	1–23 months	Low serum testosterone Normal LH, FSH
Aksglaede *et al.* (2007)	10	2–4 months	Elevated testosterone, LH, FSH Normal SHBG, inhibin B
Salbenblatt *et al.* (1985)	17 10 9	8–10 years 11–12 years 13–14 years	Normal FSH, LH, T, E2 Normal FSH, LH Elevated T, E2, FSH, LH Normal T, E2
Christiansen *et al.* (2003)	11	10–16 years	Low serum inhibin B
Wikström et al. (2004, 2006a, 2006b)	14	10–14 years	Low type A dark spermatogonia Degeneration of sertoli and leydig cells, progressive pattern of hyalinization and fibrosis with onset of puberty
Gies *et al.* (2012a,b)/Van Saen *et al.* (2012a,b)	7	10–16 years	Normal and elevated FSH Normal, low or absent inhibin B Low or normal testosterone Massive fibrosis and hyalinization in all but one testicular biopsies
Lanfranco *et al.* (2004)	189 (mosaic and non-mosaic)	18–75 years	Elevated E2, SHBG Normal LH, FSH Low testosterone, inhibin B Hyperplasia of leydig cells, hyalinization and fibrosis of seminiferous tubules, residual foci of spermatogenesis
Van Saen *et al.* (2012a,b)	22 (non-mosaic, TESE negative)	24–43 years	Massive fibrosis and hyalinization in all testicular biopsies, residual foci of spermatogenesis in 4 patients
Foresta *et al.* (1999)	10	28–37 years	Elevated LH and FSH Low testosterone

Sperm recovery in ejaculates and TESE in KS

Adolescents

To our knowledge, four studies have been published reporting sperm recovery attempts by ejaculation in adolescents and young adults with KS (Table II). In three studies, no spermatozoa were found in the ejaculates of 27 participants (in total) aged 10–25 years (Aksglaede *et al.*, 2008; Gies *et al.*, 2012a,b; Nahata *et al.*, 2016). In the fourth study, a severe oligoasthenoteratozoospermia was found in one 17-year-old adolescent, whereas in the remaining 27 participants aged 13–19 years, no spermatozoa were found (Rohayem *et al.*, 2015). Information about the effectiveness of TESE in adolescents with KS is more abundant (Table II). The first case report of a successful TESE in a 15-year-old boy with non-mosaic KS was published in 2001 (Damani *et al.*, 2001). A subsequent study investigated the effectiveness of TESE in 14 boys with non-mosaic KS aged 10–14 years. They found no spermatozoa in the study group, but spermatogonia were found in 50% of the children (Wikström *et al.*, 2004). Another study confirmed these findings by reporting the absence of spermatozoa in all seven boys with non-mosaic KS aged 13–16 years. Again, spermatogonia were found in 4 out of 7 boys (Gies *et al.*, 2012a,b). Rives *et al.* reported the presence of spermatozoa by TESE in 1 of 5 boys with non-mosaic KS aged 15–16 years, and the presence of elongated spermatids in another one (Rives *et al.*, 2013). Van Saen *et al.* reported retrieval rates of spermatogonia of 72% (5/7) in adolescents with KS aged 13–16 years. However, the overall number of spermatogonia in the boys with KS was significantly reduced compared with the number of spermatogonia found in normal adolescent boys. No spermatozoa were found in the adolescents boys with KS (Van Saen *et al.*, 2012a,b). Another study by Rohayem *et al.* investigated the retrieval of spermatozoa by microscopically assisted TESE (mTESE) in a study population of 50 adolescents aged 13–19 years. They reported a spermatozoa retrieval rate of 38% (19/50). However, in the subgroup of adolescents aged 13–14 years, the spermatozoa retrieval rate was 10% (1/10), whereas in the subgroup of adolescents aged 15–19 years the spermatozoa retrieval rate was much higher with 45% (18/40) (Rohayem *et al.*, 2015). Another recent study investigated the spermatozoa retrieval rate by mTESE in 10 adolescents and young men with KS aged 12–25 years and found spermatozoa retrieval rates of 50% (5/10) (Nahata *et al.*, 2016). A recent study by Plotton *et al.* investigated the effectiveness of TESE in 21 males with non-mosaic KS aged 15–20 years; spermatozoa

Table II Presence of spermatozoa in ejaculates or TESE biopsies of adolescents and young men with Klinefelter syndrome.

Study ID	Number of participants	Age of participants	Results
Damani *et al.* (2001)	1	15 years	1/1 (100%) spermatozoa by TESE
Wikström *et al.* (2004)	14	10–14 years	0/14 (0%) spermatozoa by TESE 7/14 (50%) spermatogonia by TESE
Aksglaede *et al.* (2008)	12	15–20 years	0/12 spermatozoa in ejaculate
Gies *et al.* (2012a,b) Van Saen *et al.* (2012a,b)	7	10–16 years	0/7 (0%) spermatozoa in ejaculate 0/7 (0%) spermatozoa by TESE 5/7 (72%) spermatogonia by TESE
Rives *et al.* (2013)	5	15–17 years	2/5 (40%) spermatozoa or spermatids by TESE
Mehta *et al.* (2013)	10	14–22 years	7/10 (70%) spermatozoa by TESE after treatment with letrozole
Plotton *et al.* (2015)	21	15–20 years	12/21 (57%) spermatozoa by TESE
Rohayem *et al.* (2015)	50	13–19 years	1/28 (4%) spermatozoa in ejaculate 19/50 (38%) spermatozoa by mTESE
Nahata *et al.* (2016)	15	12–25 years	0/15 (0%) spermatozoa in ejaculate 5/10 (50%) spermatozoa by mTESE
Totals	135	10–25 years	1/62 (2%) spermatozoa in ejaculate 12/21 (57%) spermatogonia by (m)TESE 46/118 (39%) spermatozoa by (m)TESE

retrieval rates of 57% (12/21) were achieved (Plotton *et al.*, 2015). Even higher sperm retrieval rates of 70% were reported in a small study investigating the effectiveness of pretreatment with testosterone and the aromatase inhibitor letrozole in 10 adolescents with non-mosaic KS aged 14–22 years (Mehta *et al.*, 2013).

To summarize, spermatozoa are extremely rare in ejaculates of adolescents and young adults with KS. The spermatozoa retrieval rate by TESE in adolescents with KS varies between 0% and 70% in different small studies, mostly depending on the age of the participants (Table II). The presence of spermatogonia in testicular biopsies in adolescents with KS has been reported to be about 50%.

Adults

Four case reports have shown the presence of spermatozoa in the ejaculate of adults with non-mosaic KS and their ability to father spontaneously their genetically own child by reporting live birth (Bourne *et al.*, 1997; Crüger *et al.*, 2001; Tachdjian *et al.*, 2003; Komori *et al.*, 2004). Furthermore, five larger studies reported uniformly successful ejaculation of spermatozoa in 7.4–8.4% of their study population of 351 men (in total) with KS (Kamischke *et al.*, 2003; Kitamura *et al.*, 2000; Lanfranco *et al.*, 2004; Aksglaede *et al.*, 2008; Selice *et al.*, 2010). Two of those men were diagnosed as having a mosaic pattern; the other 349 had non-mosaic KS confirmed by lymphocyte karyotyping.

The underlying mechanism leading to the ejaculation of sperm in about 8% of apparently non-mosaic KS has not yet been revealed. One possibility could be that a blood mosaicism has simply been overlooked, because most frequently only 20–30 lymphocytes are tested. Another possible explanation could be a higher rate of hidden mosaicism in testicular tissue compared with lymphocytes, as has been shown in a study investigating 5 men with KS where mucosal cells and testicular tissue had a much higher rate of mosaicism compared with lymphocytes (Garcia-Quevedo *et al.*, 2011).

The effectiveness of TESE in adults with KS has been carefully reviewed in 2013 (Aksglaede and Juul, 2013). A total of 22 studies investigating TESE or mTESE in 741 adults with KS, reported 374 successful spermatozoa retrievals by (m)TESE procedures, which is an overall sperm retrieval rate of 50% (Aksglaede and Juul, 2013). However, for adults where no spermatozoa have been found by TESE, there is still an 18% chance of finding spermatogonia (Van Saen *et al.*, 2012a,b). The live birth rate per embryo transfer for an ICSI procedure for KS after successful sperm retrieval by TESE was shown to be similar to the success rate reported for other causes of non-obstructive azoospermia: 28% of 33 patients compared with 26% of 113 controls (Yarali *et al.*, 2009).

In conclusion, about 8% of adult men with apparently non-mosaic KS are able to ejaculate spermatozoa (Table III), and sperm retrieval rates by (m)TESE are reported to be about 50%.

Experimental approaches for sperm recovery by using immature germ cells in children and adolescents

During the past decade, important achievements have been made regarding fertility preservation. Different approaches for preserving fertility with immature germ cells have been studied (Wyns, 2010; Goossens *et al.*, 2013). Cryopreservation or vitrification of either testicular tissue or spermatogonial stem cell (SSC) suspension is currently the method of choice for malignancy, although it is still experimental (Brook *et al.*, 2001; Larsen *et al.*, 2002; Kvist *et al.*, 2006; Picton *et al.*, 2015). Pre-clinical studies harvesting immature germ cells in primates have shown the capability of generating sperm by transplantation of the previously extracted tissue (Schlatt *et al.*, 2009). However, for KS boys there are some concerns regarding the feasibility of this technique (Gies *et al.*, 2012a,b). It is unknown whether the XXY karyotype originally affects somatic cells or germ cells. If the karyotype affects the somatic cells, a re-transplantation of harvested germ cells might be unsuitable because of the subsequent hyalinization of testicular tissue. This needs

Table III Presence of spermatozoa in ejaculates of adult men with Klinefelter syndrome.

Study ID	Number of participants	Age of participants	Results (spermatozoa in ejaculate)
Bourne et al. (1997)	1	29 years	1/1 (100%)
Kitamura et al. (2000)	25	28–37 years (TESE positive, others unknown)	4/52 (7.7%)
Crüger et al. (2001)	1	28 years	1/1 (100%)
Tachdjian et al. (2003)	1	27 years	1/1 (100%)
Kamischke et al. (2003)	57 (one mosaic)	Mean age 29 years	4/57 (7.4%)
Lanfranco et al. (2004)	131 (one mosaic)	18–74 years	11/131 (8.4%)
Aksglaede et al. (2008)	27	15–52 years	2/27 (7.4%)
Selice et al. (2010)	84	15–58 years	7/84 (8.3%)
Totals	328	15–74 years	31/327 (9%)

Table IV Results of studies investigating age as a predictive factor for presence of spermatozoa in TESE samples.

Study	Spermatozoa found by TESE/ total number of participants	Mean age of TESE positive participants in years (range or SD)	Mean age of TESE negative participants in years (range or SD)
Vernaeve et al. (2004)	24/50	29.5 (27–32)	32.8 (30–36)
Okada et al. (2005)	26/51	31.0 (25–40)	38.0 (28–43)
Bakircioglu et al. (2006)	42/74	31.6 ± 3.4	35.0 ± 5.1
Kyono et al. (2006)	6/17	30.2 ± 3.9	37.6 ± 4.4
Ferhi et al. (2009)	8/27	28.6 (25–32)	33.9 (29–42)
Selice et al. (2010)	9/24	30.0 ± 7.2	29.5 ± 7.8
Plotton et al. (2015)	23/41	24.3 ± 7.4	23.7 ± 7.4

to be investigated before approaches such as transplanting testicular tissue or SSCs for maturation could be considered for fertility preservation of Klinefelter boys. Another approach called *in vitro* maturation is still experimental and not yet proven to be safe in humans. Despite great achievements in animal studies, no complete *in vitro* spermatogenesis has yet been accomplished in humans (Wyns, 2010; Aponte et al., 2013). A study investigating *in vitro* maturation of human germ cells from non-obstructive azoospermic patients showed proliferation of spermatogonia and the differentiation of elongated spermatids. But the spermatids had a low fertilization potential and almost all derived blastocysts showed severe chromosomal aberrations (Sousa et al., 2002).

To summarize, fertility preserving approaches for KS children using spermatogonial stem cells remain highly experimental. Unfortunately, it is unlikely that techniques such as tissue grafting, SSC transplantation, xenografts or *in vitro* maturation of SSCs will produce viable spermatozoa in KS men within the next few years.

Predicting factors for sperm retrieval

So far, no consensus has been reached about the optimal age for successful sperm retrieval in KS (Seo et al., 2004; Vernaeve et al., 2004; Okada et al., 2005; Bakircioglu et al., 2006; Kyono et al., 2006; Ferhi et al., 2009; Selice et al., 2010; Plotton et al., 2015; Gies et al., 2016). Four studies including a total of 169 participants observed that an age above 34 years may have a negative impact on sperm retrieval (Okada et al., 2005; Bakircioglu et al., 2006; Kyono et al., 2006; Ferhi et al., 2009). Another four studies including 151 KS men reported no differences regarding successful sperm retrieval by TESE in participants aged between 15 and 33 years old (Seo et al., 2004; Vernaeve et al., 2004; Selice et al., 2010; Plotton et al., 2015). A study by Plotton et al. compared the effectiveness of TESE in 25 participants aged 16–24 with 16 participants aged 25–39 years and found similar sperm retrieval rates of 13/25 (52%) and 10/16 (63%), respectively (Plotton et al., 2015). For adolescents with KS younger than 15 years, current evidence suggests that the retrieval rate of germ cells by (m)TESE is very low compared with adolescents of 15 years and older (Wikström et al., 2004; Gies et al., 2012a,b, 2016, ; Rives et al., 2013; Rohayem et al., 2015). It is clear that the degeneration of the testicular environment seems to accelerate with the onset of puberty, but there is no evidence yet for a negative influence on spermatogenesis. As Oates (2012) stated, there have been no studies that have investigated sperm retrieval from ejaculation or TESE for the same patient at multiple times in subsequent years during sexual development (Oates, 2012). Although the study with the lowest average age in Table IV (Plotton et al., 2015) has the highest retrieval rate of spermatozoa (56%), an age-dependent decline of retrieval rates cannot be estimated at present (Table IV).

It has to be noted that the population of KS patients diagnosed during childhood may be different from the population with KS diagnosed at

adulthood. Children with KS are usually diagnosed due to behavioral and intellectual problems, while adults are usually diagnosed due to infertility without other symptoms (Groth *et al.*, 2013; Close *et al.*, 2015). Therefore caution is required when comparing results obtained in boys with those obtained in adults.

The impaired spermatogenesis in half of KS patients could also be caused by an intrinsic problem of the germ cells, possibly linked to (epi)-genetics of the surplus X chromosome instead of being a result of the hyalinization and fibrosis of the testicular environment. Support for this theory would be the stable sperm retrieval rate of around 50% among KS men and the failure of progressive tissue degeneration to be of prognostic value for spermatogenesis (Aksglaede and Juul, 2013). However, in various small studies, the inactivation pattern of the surplus X chromosome was also not shown to be of predictive significance for phenotypic characteristics (Wikström *et al.*, 2006a). Other factors such as hormones (serum FSH, LH, free and total testosterone, E2, inhibin B, SHBG, prolactin), testicular volume and testicular histology have also not shown any predictive value for higher sperm retrieval rates (Vernaeve *et al.*, 2004; Bakircioglu *et al.*, 2006; Koga *et al.*, 2007; Selice *et al.*, 2010; Gies *et al.*, 2012a,b; Van Saen *et al.*, 2012a,b; Bryson *et al.*, 2014; Haliloglu *et al.*, 2014). Only Rohayem *et al.* (2015) described a combination of total serum testosterone above 7.5 nmol/l and LH levels below 17.5 U/l to result in higher retrieval rates of spermatozoa by mTESE in both children and adults with KS (Rohayem *et al.*, 2015). Another large study has identified higher serum testosterone levels and lower levels of LH and FSH as positive predictive markers in men with azoospermia in general, but the predictive value of serum testosterone levels in KS remains unclear (Cissen *et al.*, 2016).

Treatment with testosterone has previously been reported to be a negative influence on future fertility treatment of KS, based on a sperm retrieval rate of 20% within a small study population of five adults with KS (Schiff *et al.*, 2005). In contrast, Plotton *et al.* (2015) found no negative influence of testosterone treatment on spermatogenesis in 41 adolescents and adults. Overall, they reported spermatozoa retrieval by TESE in 9/17 (52.9%) men with KS who had previously been treated with testosterone, and a positive TESE in 14/24 (59.1%) men with KS who never had been treated with testosterone (Plotton *et al.*, 2015). Another study including 10 adolescents and young men with KS aged 14–22 years who received topical testosterone replacement therapy and an aromatase inhibitor, found spermatozoa by TESE in 7 of 10 participants (70%) (Mehta *et al.*, 2013). Continuing topical testosterone replacement therapy during fertility preservation, as chosen by Mehta *et al.* (2013), or stopping the testosterone treatment nine months before performing TESE, as arbitrarily chosen by Plotton *et al.* (2015), has yielded good results (Mehta *et al.*, 2013; Plotton *et al.*, 2015). Therefore, testosterone treatment is not likely to have a permanent negative impact on fertility treatment, but larger studies are necessary to confirm these findings. Current evidence does not justify postponing clinically-indicated androgen treatment in adolescent boys with KS for fear of reducing their future fertility and TESE prospects.

Clinical approach to achieve fertility in KS

Despite the common advice for adolescents with KS to undergo TESE as early as possible as being reported in some literature, we suggest a more expectant approach as previously recommended (Oates, 2012; Gies *et al.*, 2016). Based on the studies described in this work, (pre)pubertal TESE cannot be recommended to date. Parents of children with KS need to be clearly informed that (pre)pubertal TESE is highly experimental and that this approach cannot guarantee fertility later in life. Indeed it even bears the risk of harming the fertility of their offspring, by possibly removing SSCs that may have had developed into spermatozoa later in life. Furthermore, the psychological impact of a TESE on a young adolescent, who may already have psychological problems due to abnormal sexual development, should be taken into consideration. Two recent studies show that fertility is not an issue of awareness in adolescent boys with KS, but that their parents and pediatricians appreciate fertility preservation at an early age, despite the experimental character of the intervention (Rives *et al.*, 2013; Gies *et al.*, 2015). The impact of a TESE procedure on the psychological well-being of adolescents with KS has not been investigated and studies are needed.

Conclusion

Current literature shows that spermatozoa can be found by TESE in half of the patients with KS aged 16–30 years old. Therefore, we suggest that a TESE procedure for fertility preservation should not be performed in KS patients younger than 16 years, except for patients in an academic research setting, but it is best performed before the age of thirty, even if these men prefer to postpone fatherhood.

Authors' roles

S.F. and Y.H. searched the databases, did the data extraction and wrote the first draft of this review. S.F. also wrote the final draft. K.D., D.D.M.B., W.L.M.N., D.S., H.L.C.–v.d.G., L.R. and K.F. contributed to the review by helping with the interpretation of the data, revising the article critically and writing the final draft.

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Conflict of interests

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