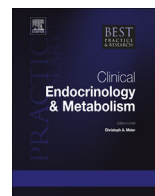




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# Infertility considerations in klinefelter syndrome: From origin to management

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Klinefelter syndrome (KS) is defined as the presence of one or more extra “X” chromosome in a male patient. It affects approximately 1 in 600 newborn males and the most common chromosomal abnormality, leading to male hypogonadism and infertility. There is a lack of data supporting best practices for KS patients’ care. In this paper we review controversial issues in KS research ranging from mechanisms of variation in KS phenotype to abnormalities resulting in reduced sperm production to successful sperm retrieval disparities after testicular sperm extraction (TESE). Translation to live birth and offspring health is also examined. Finally, medical therapies used to optimize the hormonal status and chances of fertility in KS patients are reviewed. We will also discuss the experimental spermatogonial stem cell (SSC) treatments, which are considered the future for TESE negative patients.

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## Introduction

Klinefelter syndrome (KS) is most often characterized by chromosomal aneuploidy 47, XXY. The original phenotype was described by Klinefelter et al. in 1942, with the genotype (47, XXY) later being identified in 1959 [1,2]. Despite the wide phenotypic variance in KS patients, common findings include small, firm testes, hypergonadotropic hypogonadism, and azoospermia. This presents significant challenges to the KS patient desiring biological paternity, which has been previously deemed to be unachievable. However, recent works have shown sperm can be retrieved with a testicular biopsy, which can then, in turn, be used for intracytoplasmic sperm injection (ICSI) with relative success [3]. Here we attempt to provide a comprehensive review of the literature regarding infertility considerations in patients with Klinefelter syndrome. While numerous related studies exist, this work seeks to review a broad spectrum of topics ranging from proposed genetic mechanisms leading to chromosomal abnormalities and sperm reduction to proposed infertility treatment approaches as well as future therapeutic options that are currently considered experimental.

### *Incidence*

It is estimated that between 1:500 to 1:1000 live born boys have KS; therefore, KS, making it the most common chromosomal abnormality linked to male factor infertility [4–7]. The prevalence of KS has been previously stated several ways in the literature: 0.1–0.2% of newborn males, 3–4% of infertile males, and 8–12% of azoospermic patients [4,8,9]. However, it is essential to note that these numbers are considered underestimates, given that phenotypic variability often leads to the KS underdiagnosis. The lifetime diagnosis rate in affected patients has been estimated to only be 25% [6].

### *Variance of phenotype*

It is widely understood that KS phenotypic presentation is variable and that the classically observed phenotype may be the “extreme” version given the proportion of patients that go undiagnosed. The original phenotype was described as “enlarged breasts, sparse facial and body hair, small testes, and the inability to produce sperm” [1]. KS patients suffer from androgen deficiency, elevated LH and FSH, and most reliably, azoospermia (>95%) with infertility (>99% of patients) [10]. Additional phenotypic manifestations of KS include decreased muscle mass, predisposition to type 2 diabetes, metabolic syndrome, increased risk for ischemic heart disease, cancer (breast and mediastinal), and global learning, speech, and psychiatric impairments [10]. Several groups have examined whether the extra X chromosome's paternal origin results in phenotypic variance amongst KS patients. While some of these groups concluded the extra X origin does not impact the phenotype, others have suggested that an extra paternal X chromosome may lead to the late onset of, and increased time to, pubertal progression [11–14].

### *Etiopathogenesis of klinefelter syndrome*

Numerous advances have been made in understanding the impact of genetics on the KS phenotype. Similar to females, it is known that KS males undergo chromosome inactivation facilitated by the product of the X-inactive specific transcript gene (Xist) [15–17]. This is supported by the presence of Xist in KS males but not in non-KS males, as well as a similar methylation pattern to females in KS patients and KS animal models. Therefore, it is logical that genes escaping X chromosome inactivation would negatively contribute to the KS phenotype. Several genes (Eif2s3x: Eukaryotic translation initiation factor 2 subunit 3, X-linked, Ddx3x: DEAD-Box Helicase 3 X-Linked, Kdm5c: Lysine (K)-Specific Demethylase 5C, Kdm6a: Lysine (K)-Specific Demethylase 6A) have been suggested, which may escape X chromosome inactivation and influence the KS phenotype [4,18]. Additionally, conflicting associations have been made between the preferential inactivation of the short versus long allele of the androgen receptor (AR) gene and how the level of cytosine-adenine-guanine.

(CAG) repeats (as repeat length increases, receptor activity decreases) in the highly polymorphic N-terminal region impact phenotype [4,19]. Some studies have demonstrated the preferential inactivation of the short allele (therefore higher CAG repeats) and a relationship between increasing CAG

repeat length as well as phenotypic severity. However, other studies have failed to demonstrate a preferential inactivation pattern or the relationship between CAG repeat length and phenotype [14,20–23]. Despite the myriad of evidence, a definitive connection between AR CAG repeat length and phenotype cannot be made. Lastly, the SHOX (short-stature Homeobox containing gene on chromosome X) gene, which is located in the pseudoautosomal region (PAR1) and escapes X inactivation, has been implicated in the phenotype of KS patients as it regulates brain natriuretic peptide and fibroblast growth factor receptor 3 [24–26]. This effect could be amplified given the data suggesting that KS patients experience higher copy number variants than control patients in regions expressing genes that escape inactivation, such as PAR1 [4].

Approximately 80–90% of KS males have classic genotype, 47, XXY [6,7]. The remaining patients have either high grade aneuploidies (e.g. 48, XXXY), X chromosome abnormalities (e.g. 47, iXq,Y), or are mosaic (e.g. 47, XXY/46,XY) [4]. The presence of extra X chromosomes results from non-disjunction: the failure of chromosomes to separate during the anaphase portion of meiosis I, II, or mitosis [4]. Bojesen and Gravholt highlighted that meiotic nondisjunction is either a paternal or maternal process, with roughly 50% attributed to each sex [27]. It is important to remember that nondisjunction from the paternal source can only lead to the 47, XXY genotype if nondisjunction occurs during meiosis I (fertilization products following paternal meiosis II nondisjunction include XXX and XYY) [28]. Alternatively, mosaicism is thought to be a result of “loss of an X chromosome of a 47, XXY secondary to anaphase lagging” or from early mitotic nondisjunction in a 46, XY zygote [4].

## Mechanisms of reduced sperm production and defining the KS spermatozoa karyotype

### *Examining the karyotype of klinefelter syndrome spermatozoa*

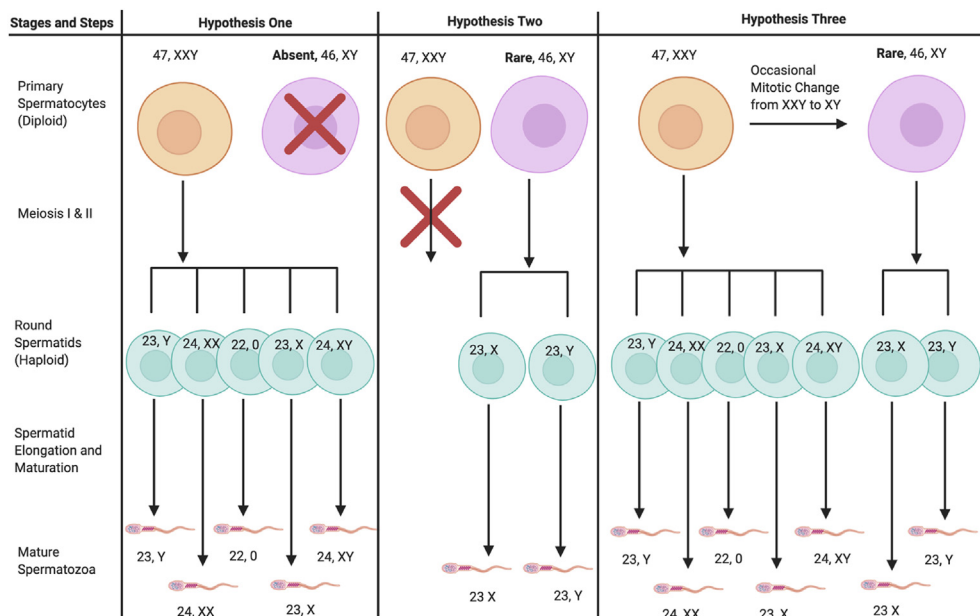
Despite the diagnosis of non-mosaic Klinefelter syndrome, numerous groups have identified discrepancies between a given patient's peripheral blood and spermatozoa karyotype. It is now known that the rate of aneuploidy in KS sperm is approximately 5–7% compared to the established 1% in fertile men and the 4% in men with genetically normal, non-obstructive azoospermia [29–31].

Examining the sperm aneuploidy rate of 5–7% raises the question of the mechanism of haploid sperm production. In one of the earliest studies, Foresta et al. performed fine-needle aspiration (FNA) in 10 KS patients. They subsequently performed fluorescence *in situ* hybridization (FISH). Only 2 out of 10 patients were positive for spermatogenesis, while the other eight patients demonstrated Sertoli cells only. When looking at the two patients, all spermatogonia and primary spermatocytes were 47, XXY, while the secondary spermatocytes, spermatids, and spermatozoa were distributed between 23, X, 23, Y, 22,0, 24, XY, and 24, XX. This work supports the idea that XXY spermatogonial stem cells can fully undergo meiosis [32].

Others have hypothesized that mature spermatozoa exclusively come from XY spermatogonia and that mature sperm can only result from this subpopulation in the testicle. This would mean that seemingly nonmosaic KS patients possess testicular mosaicism supported by studies that utilize FISH technology and demonstrate XXY and XY subpopulations of spermatogonial stem cells (SSC). These studies have surveyed the testes for spermatozoa and show that the only patients positive for spermatozoa are with both XXY and XY spermatogonia present [33–35]. Hirota and colleagues demonstrated trisomy-biased chromosome loss (TCL) *in vitro* [36], which we believe may also happen in the KS testis during *in vivo* spermatogonial mitosis. At this time, all theories (Fig. 1) provide compelling evidence, and further work is needed to definitively demonstrate whether XXY spermatogonia can undergo spermatogenesis to completion.

### *Proposed mechanisms for reduced sperm production in the klinefelter syndrome patient*

There are numerous theories to explain azoospermia in the KS patient. While diverse in thought, they all point to impaired spermatogenesis and its impact on the SSC. In a comprehensive review, Willems et al. describe three hypotheses to explain germ cell loss: intrinsic germ cell issues, the testicular environment's effect, and the X gene dosage effect [37]. As described above, testicular



**Fig. 1.** Competing Spermatogenesis Hypotheses in Klinefelter Syndrome. Illustration of three hypotheses explaining the potential pathways for spermatogenesis in patients with non-mosaic Klinefelter Syndrome. (1.) All spermatogenesis derives from 47, XXY Spermatocytes, which results in multiple chromosomal options in produced sperm. No 46, XY spermatocytes are present. (2.) All successful spermatogenesis derives from rare 46, XY spermatocytes, which may be present even in non-mosaic Klinefelter patients resulting in sperm with only normal 23, X, and 23, Y genotypes. (3.) A novel hypothesis was presented for the first time in this article. Both 47, XXY spermatocytes and rare 46, XY spermatocytes are responsible for undergoing spermatogenesis with an occasional mitotic change between 47, XXY, and 46, XY at the diploid spermatocyte developmental level.

mosaicism with focal patches of XY spermatogonia and the XXY spermatogonia's inability to undergo meiosis may explain the decreased SSC count. To examine the function of KS germ cells, Laurentino et al. analyzed the DNA methylation pattern and the transcriptome of the germ cells. They were able to show that while KS germ cells have a normal transcriptome profile before spermatogenesis, they express abnormal and variable DNA methylation of imprinted regions such as *H19*, *MEG3*, and *MEST* [38]. This is consistent with prior work linking abnormal DNA methylation of imprinted regions (*H19*, *MEST*, and *SNRPN*) to infertility [39].

It is well known that during progression to puberty, the KS testicular architecture deteriorates and becomes characterized by widespread fibrosis, seminiferous tubule degeneration, and Leydig cell hyperplasia. While a lack of data exists for a specific mechanism, there are emerging theories that the poor testicular environment may lead to decreased spermatogonia and therefore reduced sperm production [37]. Dysfunctional Leydig cells are thought to contribute to this problem. Despite the hyperplasia seen on KS patient biopsies, it has been shown that these cells are frequently dysfunctional and perhaps does not always differentiate into mature Leydig cells. This leads to insufficient testosterone levels to support spermatogonial survival and differentiation [37,40]. Alternatively, several groups have implied that while testosterone production is intact, the ability to transport it systemically is impaired by the compromised vascular network within the KS testicle. This was suggested by Foresta et al. when they compared the ultrasound studies of 92 nonmosaic KS patients to control patients and showed that on average, KS patients had reduced arterial diameter in the brachial, common carotid, common femoral artery, the abdominal aorta compared to control patients. Further analysis failed to establish any correlative factors to these reduced arterial diameters [23].

Additionally, Tuttleman et al. showed that KS patients have higher intratesticular testosterone (ITT) to serum testosterone ratio when compared to Sertoli cell-only and control patients. Given the fact that KS patients demonstrate higher ITT to serum testosterone ratios, it would appear that KS patients have

difficulty converting ITT to serum testosterone. The group then investigated vascular architecture via the 41, XXY\* KS mouse model and showed significantly depressed blood vessels to testicular tissue surface area. This supports the notion that KS Leydig cells may still produce testosterone. However, there is an impaired ability to release testosterone into the systemic circulation [41]. More work is necessary to clarify the functionality of KS Leydig cells and their impact on spermatogenesis completion.

The alterations in the blood-testis barrier (BTB) and seminiferous tubule structure cannot be excluded as contributing factors toward azoospermia in the KS population. Giudice and colleagues studied a population of 35 nonmosaic KS patients and were able to show a decrease in expression of the BTB proteins CLD11 and CNX43 and hypothesized that this could be the result of decreased markers of maturity in the somatic compartment (AR on Sertoli cells and INSL3 on Leydig cells) [42]. Van Saen et al. examined 27 adult KS patients to explore the derangements in KS seminiferous tubules. They showed that there was a decreased expression of ACTA2, which is a contractile marker for peritubular myoid cells (PMCs). The authors note that with the loss of contractile function, these PMCs can “evolve” to a cell type similar in form and function to a fibroblast. Finally, they note that PTMCs normally secrete glial cell line-derived neurotrophic factor (GDNF), which is a well-established growth factor for SSC proliferation [43].

Overall, this is very intriguing evidence that supports previously proposed hypotheses. The authors' opinion is that the phenomenon of germ cell depletion and azoospermia seen in KS patients is a multifactorial issue which likely combines elements of each hypothesis. These mechanisms and their ability to impact KS germ cell health all warrant future studies to better delineate spermatogenesis involvement.

### Fertility implications of klinefelter syndrome

Before the utilization of testicular sperm extraction (TESE), either conventional (cTESE) or microscopic (mTESE), a diagnosis of Klinefelter syndrome was thought to be synonymous with a near 0% chance of achieving biological paternity. In a review by Gravholt et al., the authors summarize that the frequencies of infertility and azoospermia amongst the KS population are 99% and 95%, respectively [10]. However, in rare scenarios, some KS patients can produce spermatozoa in their ejaculate leading to spontaneous pregnancy. While it is not surprising that mosaic KS patients may have spermatozoa in their ejaculate, in two different studies, Kitamura et al. and Lanfranco et al. were able to demonstrate that 7.7% and 8.4% ( $n = 4$  out of 52, and  $n = 11$  out of 131) respectively of nonmosaic KS patients were also positive for spermatozoa on semen analysis [44]. Subsequently, the use of ejaculated sperm for intracytoplasmic sperm injection (ICSI) has anecdotally been shown to result in pregnancy [5,44–46]. The results of these case reports have appropriately raised questions as to whether underlying testicular mosaicism exists despite non-mosaicism on peripheral blood lymphocyte karyotyping [5].

The use of testicular biopsy has dramatically increased the odds of finding spermatozoa in KS patients. Despite the changes in the testicular architecture of KS patients, several groups have shown that focal areas of spermatogenesis often exist [33,47,48]. Sciarano and colleagues examined 11 non-mosaic, adult KS patients that received testicular biopsies. They showed that 55% of patients had focal spermatogenesis areas when analyzed with H&E staining with light microscopy analysis [33].

#### *Variation in the success of sperm retrieval from TESE procedures*

While there has been controversy in the literature, many studies suggest microscopically obtaining testicular biopsies (mTESE) leads to higher success rates of sperm retrieval. Given the findings that KS patients only have focal areas of spermatogenesis, it makes sense that a targeted, microscopic biopsy would lead to a higher sperm retrieval rate (SRR) than the conventional TESE, which is a random biopsy. In its early days, Schlegel et al. demonstrated the technique for mTESE and the ability to focus on seminiferous tubules likely to have normal sperm production instead of sclerotic tubules [47]. They showed an increased SRR (63% from 45%) while simultaneously excising lower tissue amounts when comparing mTESE to cTESE (9.4 mg versus 720 mg). This is especially important in the atrophic testes of KS patients, as less biopsied tissue leads to less disruption, and in theory, a smaller chance of testicular devitalization. Following the inception of mTESE, numerous groups have published series on the success of retrieving sperm in KS patients. Of note, some of the most extensive series from Schiff

et al., Bakircioglu et al., Ramasamy et al. have demonstrated SRR of 69%, 47–57%, and 66%, respectively [48–51]. However, other series of similar sizes have demonstrated lower SRR ranging from 17% to 28% [52,53]. The etiology for this wide range of SRRs is likely multifactorial but is thought to be influenced by preoperative hormonal treatments (either positive or negative effects), surgeon experience, degree of supporting staff, and andrology laboratory capabilities as opposed to patient-specific factors given the lack of data to keep the influence of patient factors on mTESE success [5].

In light of the variation observed among the KS patient series, numerous meta-analyses have been performed. Fullerton et al. examined 13 studies with a total of 373 non-mosaic KS patients and showed a mean SRR of 44% (range: 16–60%) [54]. One of the secondary endpoints of the study was the difference in SRR between cTESE and mTESE. Further analysis of the data showed that mTESE yielded a significantly higher SRR (42% versus 55%). Similarly, Mehta et al. reviewed 16 studies, including a total of 497 patients, and found a mean SRR of 51% with mTESE and cTESE yielding mean SRRs of 61% and 47%, respectively [55]. Finally, the most extensive systematic review and meta-analysis to date was performed by Corona et al. They analyzed 37 studies, which showed an overall mean SRR of 44%. However, their secondary analysis did not demonstrate a statistically significant difference between SRR from cTESE and mTESE (43% vs. 45% respectively per TESE cycle) [56]. When comparing these studies, it is prudent to appreciate several factors. All three systematic reviews capture very similar SRRs for cTESE (42%, 47%, and 43%). However, there is a fair degree of variance between SRRs for mTESE studies (55%, 61%, and 45%). Notably, the Corona et al. study includes an additional eight mTESE studies. Of those eight studies, four of them had SRR <50%, and two of them had cohort sizes of >100 patients. Therefore, the SRR from mTESE in the Corona paper is likely influenced mainly by the results of two studies [56]. We recommend the practitioner exercise caution when interpreting average mTESE SRR values as they appear to be variable and impacted by surgeon experience and facility capabilities. For instance, Boeri et al. demonstrated a 21.4% SRR rate in 103 nonmosaic, KS patients [57]. Despite using various surgical techniques, the study describes a thorough laboratory (intraoperative with subsequent laboratory examination) examination of all specimens. Therefore, when counseling KS patients on their odds for successful SRR, it is paramount to be realistic about their center's success to create reasonable expectations for the patient.

### *The success of IVF/ICSI and offspring health*

The meta-analysis by Corona et al. additionally examined the live birth rate following IVF/ICSI in KS patients. There was a 43% (CI: 36–50%) (218/410 ICSI cycles) pregnancy rate followed by a 43% (CI 34–53%) (211/410 ICSI cycles) live birth rate (LBR). There were no factors deemed to positively or negatively predict the LBR, including the use of fresh versus cryopreserved sperm [56]. Included in this analysis is work by Greco et al. that examines the karyotype of 16 babies born from sperm retrieved from 15/38 nonmosaic KS patients. While the preimplantation genetic diagnosis was not offered to these patients, amniocentesis and fetal karyotyping showed that all had a normal karyotype [58]. This is echoed by the work of numerous other groups, including a series of 65 patients and a review of 101 live births [3,5,54,59–62]. These results align with the fact that there does not appear to be an increased risk of aneuploidy in KS patients than in genetically normal patients with non-obstructive azoospermia [5].

### **Current therapeutics for the infertile klinefelter syndrome patient**

While future work is still needed to ascertain the etiopathogenesis of KS, there are current medical management options available to the practicing clinician to optimize sperm retrieval efforts. The implementation of medical and surgical therapies is not without controversy and therefore, the appropriate age for medical and surgical interventions for KS patients will also be discussed.

### *Overview of testosterone replacement therapy and combination therapies*

The typical adult KS patient is characterized by hypergonadotropic hypogonadism. Testosterone replacement therapy (TRT) has negative implications on spermatogenesis when administered in

proximity to planned fertility. However, as reviewed by Fainberg et al. and Herati et al., it is also known that elevated intratesticular testosterone levels are needed for spermatogenesis to occur [5,63]. Therefore, increasing testosterone levels while also preserving or even enhancing spermatogenesis is paramount for the reproductive urologist to keep in mind. Aside from traditional TRT, pharmacologic options include human chorionic gonadotropin (hCG), selective estrogen receptor modulators (SERMs), aromatase inhibitors (AI), and intranasal testosterone. Of note, it is thought that topical testosterone therapy has less of a depressive effect on spermatogenesis compared to injectable TRT [5]. This is supported by works showing that topical testosterone therapy does not appear to drastically decrease the FSH or LH level in KS patients, which, in theory, would allow for ongoing spermatogenesis [64]. However, more work is required to compare the hormonal profile and SRR of KS patients receiving injectable versus topical TRT. Overall, there is a lack of data examining the use of TRT plus hCG in the Klinefelter syndrome patient.

In our current practice (any hypogonadotropic patient including KS), when long term TRT is expected, after sperm cryopreservation if possible, we recommend stopping TRT for one month at least once a year (ideally every 6 months) and continuing with standard-dose hCG (1000–2000 IU subcutaneously every other day for an entire month). If attempted pregnancy is planned, TRT should be stopped 6 months in advance and replaced with continuous hCG therapy.

#### *Selective estrogen receptor modulators and aromatase inhibitors*

Another way to manipulate the hormonal balance of KS patients is with the use of SERM and aromatase inhibitors (AI). The goal of these medications is to support endogenous testosterone production while conserving the appropriate testosterone to estrogen ratio (T:E). Two studies demonstrate the use of these agents as a fertility aid in KS patients. A study by Mehta et al. examined 10 KS patients aged 14–22 who received topical testosterone (goal of >400 ng/dl) for a range of 1–5 years as well as 1 mg of daily anastrozole with a goal T:E ratio of >10 as endorsed by earlier work [51,64]. Of the ten patients receiving mTESE, seven of them had retrievable sperm. At the time of hormonal optimization, the mean testosterone and FSH levels for these patients were 600 ng/dl and 33.5 mIU/ml, respectively [64]. A larger-scale study by Ramasamy et al. looked at a graduated regimen of an AI (anastrozole vs. testolactone) with or without the addition of hCG or clomiphene in KS men with T level <250 ng/dl. The patients receiving treatment were classified as “responders” (posttreatment testosterone >250 ng/dl) versus “non-responders” (posttreatment testosterone <250 ng/dl), and the SRRs for each group were shown to be significantly different (77% vs. 55%,  $p = 0.05$ ) [51].

#### *Intranasal testosterone*

Although it has yet to be studied in the KS population, nasal testosterone is an emerging therapy that appears to restore androgen levels without having a profound impact on the hypothalamic-pituitary–gonadal (HPG) axis or spermatogenesis. Unlike other TRT formulations, nasal testosterone (Natesto™) has a brief half-life of 10–100 minutes and therefore is thought to mimic the true pulsatile nature of testosterone release. In an open-label, single-arm trial by Ramasamy et al., 60 non-KS hypogonadal patients were enrolled to receive Natesto™ (125 ml per nostril TID) for six months (3 and 6 months follow up) [65]. The study showed that sperm concentration and total motile sperm count were not significantly affected after 3 and 6 months of Natesto™ treatment [65]. However, there was an insignificant downward trend in these two parameters. While more data and long-term follow-up are needed, nasal testosterone appears to be an option for hypogonadal individuals including KS patients who desire fertility. Future work could examine this therapy's use in KS patients as they could be considered index patients for this therapy.

#### *Proposed treatment approach*

When considering the full armamentarium of hormone therapy agents, some have suggested adopting an HPG axis reset [63]. The goal of this is to suppress the constant expression of gonadotropins seen in hypergonadotropic hypogonadal patients and to reset the axis in a pulsatile manner

that allows for improved intratesticular testosterone without a hypergonadotropic state that desensitizes the Leydig and Sertoli cells. Herati et al. examined two non-mosaic KS patients being considered for mTESE. Before their procedure, each patient was treated with testosterone enanthate 200 mg/mL per week and 3000 IU of hCG three times a week for at least six weeks. Following treatment, both patients had T levels above 300 ng/dl (from 120 to 150 ng/dl) and their FSH levels declined from 21.8 to 56.6 mIU/mL to 1.1–2.6 mIU/mL respectively [63]. Successful sperm retrieval was then performed in each patient. While it is difficult to say whether HPG axis reset increased the chances of sperm retrieval, the combined therapy used to increase T levels and suppress gonadotropin levels to a physiologically normal level is intriguing and warrants future study. Ideally, this would be performed in patients with a previously negative mTESE in the pre-treatment setting.

Various treatment approaches have been recommended to optimize a patient's likelihood for successful sperm retrieval. For instance, Masterson et al. demonstrated their recommended algorithm for fertility management in adolescent KS patients [66]. Their algorithm evaluates whether a patient has been previously treated with TRT. For patients previously on TRT, they recommend discontinuing TRT, allowing for a washout period, rechecking the laboratory workup, and treating hormone derangements as necessary [66]. From there, a repeat semen analysis can be performed, followed by TESE in the event of a negative semen analysis. As highlighted by the fertility guideline from the Association for X and Y Variations (AXYS) Clinical and the Research Consortium on clinical practices (<https://genetic.org>), maintaining normal testosterone levels is paramount for sperm development. However, TRT is known to inhibit spermatogenesis through inhibition of LH, FSH, and testicular testosterone synthesis. Therefore, workup should begin with obtaining a laboratory workup for each patient, including a semen analysis, testosterone, estradiol, FSH, and LH level. Then, careful consideration should be given to using selective estrogen receptor modulators with or without aromatase inhibitors in patients with low testosterone and elevated estrogen, respectively, to maintain a T:E ratio of at least 10 in patients with an initially negative semen analysis.

### **Controversies surrounding the retrieval of spermatozoa in the peripubertal, KS patient**

Given the well-known decline of spermatozoa in Klinefelter syndrome patients, many investigators have debated the appropriate age to attempt sperm retrieval. While there is a body of literature demonstrating the positive impact of androgen therapy on KS physical development and neurodevelopment [67,68], this topic surpasses this paper's scope. Previously, it was thought that early intervention during the peripubertal period might yield higher sperm retrieval rates given the perceived timeline for SSC degeneration [55]. However, many risks accompany early intervention with TESE despite the positive attitude regarding early fertility preservation from KS patient parents [69]. These include the risk of further testicular damage and the demonstration of statistically significant decreases in testosterone levels [70].

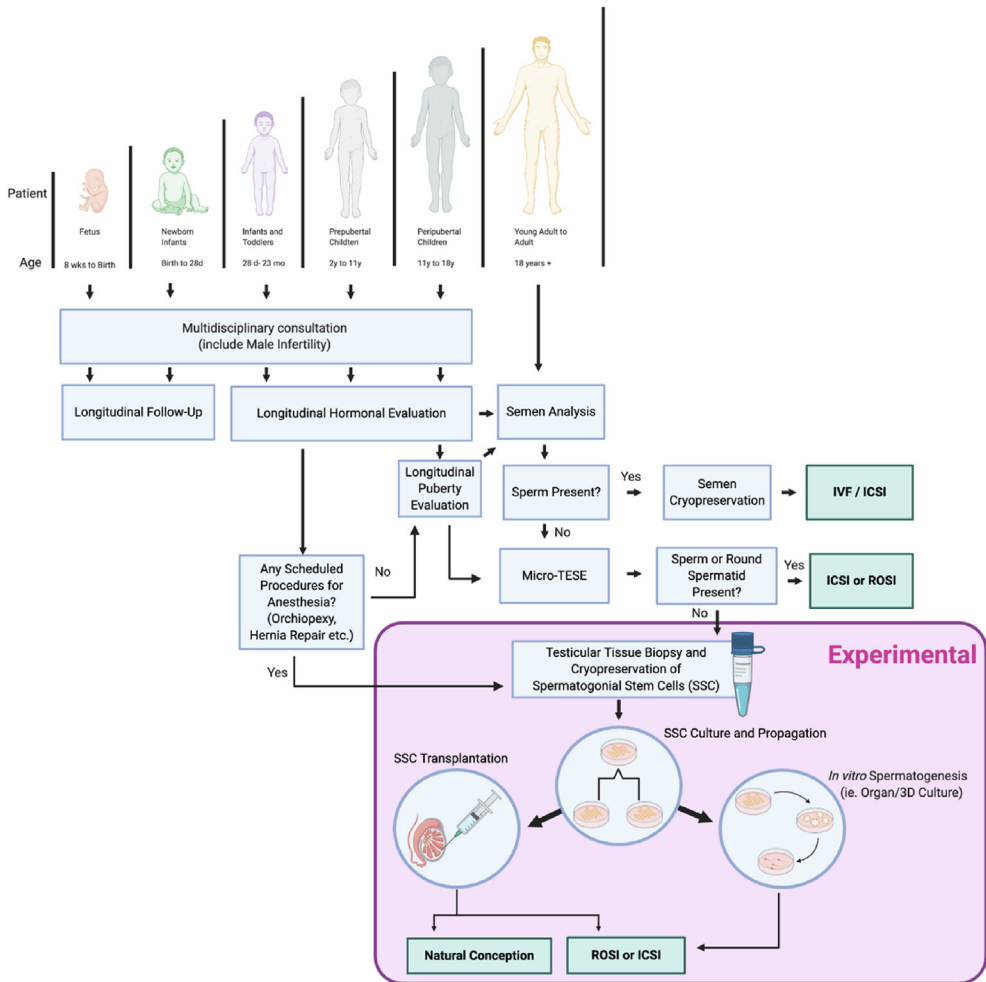
Most importantly, numerous studies show that the SRR in peripubertal patients does not seemingly offer a significant benefit compared to that of the adult population [5,70]. For instance, Plotton et al. demonstrated an SRR of 52% in KS patients aged 15–23 and 62% in patients older than 23, which did not carry statistical significance [71]. Similarly, Nahata et al. examined ten adolescent boys and found a 50% SRR with no distinct predictive factors favoring successful retrieval [72]. Given the widely cited meta-analysis by Corona et al., which demonstrates an SRR of 44% per TESE cycle irrespective of age, there does not appear to be a significant benefit in offering the adolescent KS patient a TESE procedure. Given the risks of the procedure and lack of apparent benefit, the authors recommend that pursuing sperm retrieval in the pre or peri-pubertal KS patients should be postponed to later in life if possible.

### **Promising experimental avenues for fertility in the klinefelter syndrome patient**

While a significant proportion of KS patients will yield mature sperm for future fertility therapy, the reality remains that many patients will have a negative TESE result. To offer these patients a chance at biological paternity, great attention has been given to SSC technology. A recent systematic review and meta-analysis by Deebel et al. examined 36 manuscripts with a total of 386 patients. They found that spermatogonial cells were present in all of the fetal/infantile patients and 83% of the prepubertal



patients' testes. In 42.7% and 48.5% of peripubertal/adolescent and adult KS patients, respectively. Additionally, 46.4% peripubertal/adolescent and 24.3% adult patients negative for spermatozoa were still positive for spermatogonia [73]. Sadri-Ardekani and colleagues have shown the ability to successfully culture and propagate human SSC for up to 28 weeks while demonstrating an 18,450-fold increase in SSC over 64 days [74]. The successful culture of SSC has been further corroborated by others [75,76]. SSC presence in biopsy specimens could allow for future therapy, such as *in vitro*



**Fig. 2.** Management of Klinefelter Syndrome for Fertility Preservation. Following Klinefelter syndrome diagnosis, all ages from fetal through peripubertal children are recommended to undergo multidisciplinary consultation, including male infertility specialists. Newborns will be followed longitudinally, as will infants and children with attention paid to regular hormonal evaluations up to, and especially during, puberty. In children, if at any point a surgical procedure requiring anesthesia is scheduled, the authors recommend undergoing experimental testicular tissue biopsy and cryopreservation of SSC at that time. In adults desiring fertility, if sperm is present on semen analysis, this may be preserved and used for IVF or ICSI. If no sperm are found, mTESE is the preferred next step. If sperm or round spermatid is found on mTESE, then it may be preserved for ICSI or ROSI. If no sperm are found, then experimental testicular tissue biopsy and cryopreservation of SSC is suggested. In adults and children who underwent experimental SSC cryopreservation, SSCs can be cultured and propagated, with the goal of SSC auto-transplantation leading to potentially increasing the number of 46XY SSCs to support *in vivo* spermatogenesis and subsequent natural conception or ROSI/ICSI. An additional experimental possibility following SSC propagation is *in vitro* spermatogenesis, which is possible in 3D Organoid culture and which may allow for ROSI or ICSI conception. Abbreviations: SSC, Spermatogonia Stem Cells; TESE, Testicular Sperm Extraction; ROSI, Round Spermatid Injection; ICSI, Intracytoplasmic Sperm Injection.

spermatogenesis with subsequent IVF/ICSI. While full *in vitro* spermatogenesis of human SSC has not yet been achieved, differentiation to the level of post-meiotic germ cells (PRM1 and Acrosin positive) has been demonstrated [77]. Despite the shortcomings of *in vitro* spermatogenesis to date, Tanaka et al. have shown that round spermatid injection (ROSI) can be used to achieve a successful pregnancy and live Birth. In their first study, they were able to show the successful birth of 14 children born to 12 women using round spermatid injection [78]. They subsequently published their follow-up study examining 90 babies that were born following ROSI for two years and comparison to naturally born babies [79]. This work demonstrated the absence of significant physical or cognitive-developmental differences between the ROSI and naturally conceived babies. Given the advances in this field, the future use of SSC harvest, *in vitro* spermatogenesis, and ROSI or IVF/ICSI may help TESE negative KS patients achieve biological paternity. Although early testicular biopsy, which should still be considered experimental and needs IRB approval, may help ensure successful retrieval of SSCs for SSC banking, it is not recommended unless the patient needs to undergo anesthesia for another clinical indication (Fig. 2).

## Summary

KS is the most common genetic cause for hypogonadism and infertility in men. KS patients have almost the same life expectancy as the general population, therefore providing optimal quality of life for them is critical. A multidisciplinary clinical care team is necessary to support the hormonal therapy and fertility treatment of KS patients. Despite significant advances in IVF/ICSI therapy for KS patients, there is still a large proportion of patients in which spermatozoa cannot be obtained. Therefore, future work is needed to address hormone therapy optimization and advance reproductive technology for the TESE negative patient.

### Practice Points

- Although utilizing genetic tests such as non-invasive prenatal diagnosis (NIPD) and karyotyping has increased, most KS patients (up to 75%) remain undiagnosed.
- Hypogonadism is a classic characteristic of KS. However, many questions concerning the timing, dose, and route of testosterone, hCG, and AI administration remain to be answered.
- Up to 95% of KS patients are azoospermic, with natural conception being a rare event.
- Up to 50% of KS patients have retrievable spermatozoa which can be isolated and used for ICSI.
- Sperm aneuploidy in the standard KS patient (5–7%) is not much higher than genetically normal patients with non-obstructive azoospermia (4%).

### Research Agenda

- Existing KS mouse models mimic several features of human KS and can be used as valuable research tools.
- The science surrounding *in vitro* propagation and differentiation of KS SSCs should be furthered.
- Pathophysiology of spermatogenesis defects and testicular fibrosis in KS patients should be investigated in more detail.
- Experimental SSC cryopreservation can be offered to KS patients undergoing TESE or younger patients undergoing simultaneous clinical procedures under general anesthesia.

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