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REVIEW

Fertility management of Klinefelter syndrome

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ABSTRACT

Introduction: Klinefelter syndrome (KS) represents the most common chromosomal abnormality in the general population, and one of the most common genetic etiologies of nonobstructive azoospermia (NOA) and in severe oligospermia. Once considered untreatable, men with KS and NOA now have a variety of treatment options to obtain paternity.

Areas covered: The cornerstone of treatment for both KS and NOA patients remains the surgical retrieval of viable sperm, which can be used for intracytoplasmic sperm injection to obtain pregnancy. Although the field has advanced significantly since the early 1990s, approximately half of men with KS will ultimately fail fertility treatments. Presented is a critical review of the available evidence that has attempted to identify predictive factors for successful sperm recovery. To optimize surgical success, a variety of treatment modalities have also been suggested and evaluated, including hormonal manipulation and timing of retrieval.

Expert opinion: Individuals with KS have a relatively good prognosis for sperm recovery compared to other men with idiopathic NOA. Surgical success is heavily dependent upon surgical technique and the experience of the andrology/embryology team tasked with the identification and use of testicular sperm.

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Fertility preservation; hormonal manipulation; Klinefelter syndrome; microTESE; sperm retrieval rate; surgical sperm retrieval

1. Introduction

Klinefelter syndrome (KS) is the most common chromosomal abnormality associated with infertility in men, occurring between 1/500-1/1000 newborn males, and represents ~8% of men with nonobstructive azoospermia [1–3]. The diagnosis also remains the most common karyotypic finding for severely oligospermic men. It is defined as a chromosomal disorder in males with more than one X chromosome, most commonly 47 XXY, though mosaicism and other aneuploidies exist in 10–20% of cases [1,3]. Classically, these men develop primary testicular failure in adolescence causing hypergonadotropic hypogonadism, small firm testes, azoospermia, gynecomastia and incomplete development of secondary sex characteristics. However, the phenotypic range of KS is quite broad, causing only 25% of affected males to be diagnosed during their lifetime, and less than 10% of these patients diagnosed prepuberty [4]. Due to the prevalence of chromosomal abnormalities in men with azoospermia, it is standard of care to obtain a karyotype for any patient with a total sperm count below 5 million [5]. In this setting, it is common for reproductive urologists to render a patient's initial diagnosis of KS [6].

Until recently, the diagnosis of nonobstructive azoospermia, whether idiopathic or secondary to KS, was deemed untreatable in terms of fertility potential. Although sperm could occasionally be found in testis biopsies in these men, and similarly in the epididymis of men with obstructed reproductive tracts, any sperm surgically procured performed poorly with *in vitro* fertilization (IVF) [7]. However, with the development of intracytoplasmic sperm injection (ICSI) in the 1990s, the initial barriers for the use of testicular or epididymal sperm were lifted. By 1998, the first live births using IVF-ICSI for KS patients were reported [8].

Consequently, KS is no longer considered an untreatable form of male factor infertility. In this review, we will address the clinical considerations regarding the medical and surgical management of KS patients desiring paternity. Although much progress has occurred since the first successful live births in the late 1990s, many controversies remain in terms of optimization and preservation of fertility in this unique cohort. Fortunately, the evidence base regarding these remaining clinical questions has begun to mature during the last 5 years. With an understanding of these primary data, KS patients can be appropriately counseled and managed well before surgical intervention becomes necessary. We will primarily focus, however, the discussion on how KS patients can obtain genetically related children through surgery, since all other elements of care are centered around the success of sperm retrieval.

We searched Pubmed, Embase, and Cochrane for literature related to KS in December 2018. Relevant studies and data will be presented in the subsequent review and organized into discrete sections: surgical sperm retrieval, optimization of surgical outcomes, including hormonal management and lastly sequelae of sperm retrieval.

2. Surgical sperm retrieval

2.1. Is surgery always necessary?

In the vast majority of cases, patients with non-mosaic KS will require surgical retrieval of sperm. To reliably produce sperm

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Article highlights

- Men with Klinefelter Syndrome will have viable sperm found at the time of surgical sperm retrieval in at least 40% to 50% of cases
- No clear or robust presurgical indicators have been found to predict which patients may have sperm identified at the time of surgery
- Appropriate and careful hormonal manipulation may optimize the chances of surgical success
- Peripubertal sperm retrieval does not appear to improve sperm recovery rates and should not be routinely offered
- Fertility preservation for the peripubertal Klinefelter male remains an area of ongoing research, and should only be considered under a monitored protocol

in the ejaculate, and therefore avoid surgery, enough sperm production needs to occur in order to ensure some cells survive the journey through the male reproductive tract. Men with mosaicism will have variable amounts of sperm production, with presentations ranging from complete azoospermia to severe oligospermia. In these cases, ejaculated spermatozoa can be used for assisted reproduction. Non-mosaic KS will at times present with sperm in the ejaculate, but typically in very small amounts often requiring extensive searching by an experienced andrologist. Kitamura et al. published a series of 52 men with non-mosaic KS, reporting that 7% of their cohort had sperm in the ejaculate [9]. Similarly, in a larger series documented by Lanfranco et al., 8.4% of 131 non-mosaic KS patients had ejaculated sperm [1].

Interestingly, a handful of case reports have documented spontaneous pregnancy from non-mosaic KS individuals, the first from Kaplan et al. in 1963 [10]. However, only two case reports exist in the literature that confirmed paternity [11,12]. Clearly, this is an exceedingly rare event, and although mosaicism was ruled out in lymphocytes, the possibility of occult mosaicism in the testis remains [1]. In patients who have rare sperm discovered in the ejaculate, successful pregnancies using these sperm for ICSI have occurred [9,13,14].

It is unclear if ejaculated spermatozoa perform as well as testicular-derived cells in terms of reproductive outcomes. Recent controversy has emerged for infertile men with an otherwise normal karyotype who present with excessive sperm DNA damage. An argument has been raised that testicular sperm should be procured in these cases since DNA damage typically accrues subsequent to spermatogenesis. Presumably, during transit through the reproductive tract, excessive oxidative stress results in impaired DNA integrity [15]. Conflicting evidence exists for the role of surgical management in these men with otherwise ample amounts of ejaculated sperm that can be used for assisted reproduction [16,17]. One meta-analysis examined this concept in men with rare ejaculated sperm, which is more relevant to the ~8% of KS patients who have viable sperm in their ejaculate. Abhyankar and colleagues summarized the results for 272 ICSI cycles that used either ejaculated or testicular sperm for fertilization [18]. They found no difference in fertilization or pregnancy rate between the differing sperm sources for men with rare ejaculated sperm. It is important to realize that these cohorts were predominately men with idiopathic impairment of sperm production. No specific data for the KS population exist regarding this question. In the absence of evidence, it is difficult to justify the use of a surgical procedure when usable sperm are available in the semen sample. Currently at our center, we repeat the semen analysis the morning of surgery to confirm the absence of ejaculated sperm. Surgical intervention is canceled if motile sperm are discovered and are sufficient in numbers for the anticipated number of retrieved oocytes of the female partner. For the vast majority of KS patients who do not fall into this category, however, surgical sperm retrieval remains the only option.

2.2. Rationale for surgical sperm procurement

2.2.1. How are sperm produced in the 47 XXY male?

It has long been recognized that men with nonobstructive azoospermia harbor pockets of seminiferous tubules that have intact and complete sperm production [19,20]. The biological basis for this observation remains unknown, and is particularly perplexing given that these seminiferous tubules contain cells with the same genetic makeup and developmental history as tubules devoid of sperm. The process of spermatogenesis involves the maintenance of a unique cellular niche for spermatogonial stem cells (SSCs) and their differentiating progeny. Additionally, this lineage must successfully complete meiosis for the production of haploid gametes, which require an immune-privileged space. The regulation and creation of such an environment includes tight endocrine, paracrine, and autocrine signaling along with appropriate temperature control. Breakdown of any part of this system will result in impaired production. Thus, the umbrella diagnosis of nonobstructive azoospermia, which includes KS, contains an array of etiologies with differing pathophysiologies.

Tournaye and colleagues first reported the presence of testicular sperm in biopsies of non-mosaic KS patients[21]. Similar to idiopathic nonobstructive azoospermia, KS testes were also found to have foci of productive seminiferous tubules [19,22]. Countering theories have been proposed to explain this observation: 1) XXY SSCs are able to complete meiosis and produce functional spermatozoa, or 2) a small population of XY SSCs exist which ultimately lead to the sperm found during surgery. Conflicting evidence has been presented for both hypotheses.

In an early study by Foresta et al., fluorescence *in situ* hybridization (FISH) was conducted on testis fine needle aspirates of 10 KS patients[23]. In two patients germ cells were identified, which were found to be XXY when probes for X, Y, and chromosome 8 were used. Secondary spermatocytes, spermatids, and sperm were found to have variable numbers of X and Y chromosomes. The investigators concluded that these post-meiotic cells stemmed from XXY spermatogonia, thereby supporting the concept that XXY SSCs could progress through meiosis. In a more recent study, computerized cell scanning of testis biopsies was utilized and allowed for FISH and morphology analysis of several thousand cells[24]. Similar to prior work, their cohort of KS patients was relatively small (six with spermatogenesis and five without). Their data demonstrated that most SSCs and primary spermatocytes were XXY, 89% and 77%, respectively. Approximately half of post-meiotic cells analyzed in KS patients demonstrated

normal haploid genomes, although the number of cells recovered for analysis was quite low (114 cells in total). Interestingly, they observed several pachytene figures with a 47 XXY content. This would suggest that XXY germ cells can enter meiosis, though it is unclear if they can finish the process to finish spermatogenesis.

The alternative explanation, that a small population of XY SSCs exist that ultimately lead to sperm production, is supported by both human and animal data. These diploid cells may arise from mitotic errors during expansion of the stem cell population, in a rare but advantageous nondisjunction event. Alternatively, XY SSCs may already be present in a mosaic state not otherwise detected with a standard karyotype. Any subsequent production of aneuploid gametes is thought to occur due to inappropriate support from Sertoli and Leydig cells, termed the 'testicular environment hypothesis.' Sciurano et al. examined non-mosaic KS testis biopsies with a FISH probe against the X-chromosome along with immunofluorescent labeling of several meiotic proteins[25]. Of 11 subjects, spermatogenesis was identified in 6. All observed meiotic spermatocytes were diploid, whereas Sertoli cells exhibited the expected XXY pattern. In a larger study that also utilized FISH, evidence for both XXY and XY spermatogonia was found in a cohort of 24 men[26]. Sperm were found in subjects who had both XXY and XY SSCs within their seminiferous tubules, whereas those without recovered sperm only had XXY cells. In a similar study that also observed pachytene figures, only men with an XY testis cell line had sperm recovered during biopsy, with all observed meiotic events also stemming from XY SSCs[27]. The notion that only diploid precursor cells can support meiosis and finish spermatogenesis is bolstered by studies in murine models. Mroz et al. demonstrated the rare appearance of SSCs in adult XXY mice, all of which carried an XY genome[28]. Additionally, in germ cell transplantation experiments, it has been shown that the testes of XXY mice can support and carry an XY line through spermatogenesis if such cells are present[29].

Insufficient evidence exists to rule out either the testicular environment hypothesis or the concept that XXY spermatogonia can contribute to the production of viable sperm. Data supporting either conclusion have used similar and sound methodologies. It is also conceivable that these two explanations may not be mutually exclusive. Given the possibility that XXY SSCs may progress and achieve sperm production, the question arose if sperm obtained from KS men will result in an XXY child, or worse should other chromosomal abnormalities result during an abnormal meiotic process.

2.2.2. Are sperm from KS men safe to use?

Once procurement of sperm from KS individuals was found to be possible, it was unclear if offspring derived from these cases would carry a higher incidence of genetic abnormality [30,31]. The initial experience in the late 1990s, however, demonstrated a surprisingly reassuring result. Palermo et al. were one of the first groups to successfully treat couples affected by KS, documenting the delivery of three infants (two boys and one girl) with normal karyotypes[8]. The following year, two other centers reported the successful delivery of three male infants with a normal karyotype [32,33]. In 2001, Cruger et al. reported the successful use of ejaculated spermatozoa from a KS man, which resulted in the birth of a healthy girl[14]. Fullerton et al. reviewed the existing data in 2010 to assess the risk toward offspring following use of KS sperm[34]. They found a total of 101 live births of otherwise healthy infants. Only one report documented an abnormal XXY fetus in a triplet pregnancy, which was selectively reduced and allowed for the successful delivery of twins[35]. An even more contemporary series supported these initial outcomes in a cohort of 65 KS couples[36]. Despite a chromosomal abnormality in all somatic cells and a theoretic 50% risk of aneuploidy in germ cells, the risk of a Klinefelter male fathering a child with sex chromosome abnormality is remote, estimated at <1% without embryo testing.

During the late 1990s, the presence of XY SSCs in the Klinefelter testis was not known, and so it was unclear how healthy pregnancies proved to be the norm than the exception. In an attempt to quantify the risk to potential offspring, several groups examined the chromosomal makeup of available sperm from KS patients. Levron et al. used FISH to examine a total of 112 sperm from 5 KS individuals for chromosomes X, Y, and 18[37]. They observed an increased rate of aneuploidy, accounting for 6.3% of cells tested. In fertile men, the rate of aneuploidy in sperm is quite low, typically well below 1% (when a limited number of chromosomes are reviewed)[38]. In a follow-up study by Vialard and colleagues, the aneuploidy rate of KS sperm was also found to be approximately 5%[39]. Interestingly, this study also analyzed the aneuploidy rate of individuals with nonobstructive azoospermia with a negative genetic evaluation. These individuals, who are routinely offered surgical sperm retrieval for IVF-ICSI, demonstrated a similar rate of aneuploidy as the KS cohort at 4%. These results have been substantiated by several other groups utilizing similar methods, providing for a reasonably concordant rate of sperm aneuploidy within the primary literature [26,27].

It appears that the risk of aneuploidy in resulting offspring is similar between KS individuals and other men with nonobstructive azoospermia. As discussed previously, this phenomenon could be due to diploid XY SSCs contributing to sperm derived within the KS testis. Given the rate of hyperhaploidy seen in testicular sperm from men with Klinefelter's, it is more likely that there is progressive selection of sperm that are euploid, and subsequently embryos that are euploid, during normal reproduction. It is also possible that normal haploid sperm tend to fertilize at a higher rate, and resulting euploid embryos are better equipped to progress when comparing outcomes with aneuploid sperm. This latter natural selection during reproduction is likely why we do not observe a 6 – 7% rate of aneuploidy in children born from KS couples. Nevertheless, enough data now exist to reassure patients that the risk to potential offspring is similar to nonobstructive azoospermia in general, and not elevated due to the KS diagnosis. Therefore, preimplantation genetic testing (PGT) at blastocyst stage (day 5) might be considered optional versus classic day 3 transfer without PGT. A comprehensive treatment plan, however, should still include referral for genetic

counseling of KS men and any male partner found to have a chromosomal abnormality.

2.2.3. Success rate of surgical sperm retrieval

Similar to individuals with idiopathic nonobstructive azoospermia, a significant proportion of KS men undergoing sperm retrieval will not have sperm found during surgery. In both these conditions, the majority of seminiferous tubules will be devoid of mature sperm, and success is dependent upon the sampling of the rare foci that contain intact spermatogenesis. Prior to the late 1990s, traditional testicular sperm extraction (TESE) utilized a small incision of the surrounding tunica albuginea of the testicle. The testicular parenchyma would be biopsied through this incision, essentially providing a random specimen in which to search for usable sperm. Early attempts to procure sperm from KS individuals resulted in a relatively disappointing sperm retrieval rate (SRR), typically ranging from 25% to 40% [21,35,37,40,41].

Given that small, isolated patches of spermatogenesis exist in KS and nonobstructive azoospermia patients, the microdissection TESE (microTESE) procedure was developed to improve visualization of the seminiferous tubules and to allow for selective biopsy of the most promising tissue[19]. Early reports of microTESE for the nonobstructive azoospermia population reported an increase of SRR from 45% to 63% while minimizing the amount of tissue excised (mean weight of 9.4 mg as opposed to 720 mg)[19]. In 2009 Ramasamy et al. published one of the largest series of 68 men with KS who underwent micro-TESE[42]. Successful retrieval occurred in 66% of the cohort, with a pregnancy and live birth rate of 57% and 45%, respectively. In a similar series, one patient who desired a large family had successful sperm retrieval on five consecutive microTESE attempts[22]. One study also purported that in the KS population, proceeding to bilateral microTESE following an unsuccessful attempt on one side tends to be more successful than for chromosomally normal NOA men[43].

Several meta-analyses have now been conducted regarding the expected SRR for men with nonobstructive azoospermia. Bernie et al. found that conventional TESE was twofold more effective in retrieving sperm than fine-needle aspiration, whereas microTESE was 1.5-fold more effective at retrieval than conventional multi-biopsy (random) TESE[44]. In the subset of nonobstructive azoospermia men with KS, the effect of different sperm retrieval methods has also been explored. Fullerton et al. analyzed 13 studies accounting for 373 men with KS[34]. When comparing microTESE to standard TESE, SRR improved to 55% as opposed to 44% for the entire cohort. Similarly, in a broader review, Mehta et al. found an overall SRR of 61% using microTESE versus 47% for standard biopsy [45]. In a more contemporary analysis, representing the largest set of data from 37 studies, Corona and colleagues document an overall SRR of 44% (CI 39–48), with a resulting live birth rate of 43% (CI 34–53) for those who had sperm recovered[46]. Interestingly, they did not observe a significant difference in outcomes between conventional TESE and microTESE.

The discrepant results reported by Corona et al. may reflect differences in practice, as microTESE success is based upon the experience of the surgeon in addition to the experience of the supporting staff, such as the andrology team that conduct extended searches for sperm [46,47]. The importance of these factors can be gleaned from the data of Schiff et al., who reported the highest SRR of 69% for a cohort of 42 men [22]. In this study, all procedures were performed by a single high-volume surgeon. Additionally, a dedicated andrology and embryology team were present in the OR to provide immediate feedback and help guide the extent of dissection. These contributing factors are difficult to account for in meta-analysis and speaks to the importance of an experienced and dedicated team to ensure optimal outcomes.

3. Optimization of surgical outcomes

3.1. Identifying appropriate surgical candidates

Given that not all men will have sperm found, the identification of preoperative predictive factors became a prominent goal of reproductive urologists. Despite significant effort, no reliable physical exam finding or laboratory test has proven useful. Although isolated series have found associations, these reports were not reproduced when applied to larger cohorts. The most heavily studied parameters include data typically obtained during an azoospermia workup, mainly: preoperative FSH, total testosterone, testis volume, and age.

It has been shown that FSH, and feedback from inhibin B, correlate inversely with the size of the germ cell population within the testis [48,49]. It was intuitive, therefore, to assume that a markedly elevated FSH level would predict fewer, or the absence thereof, of germ cells and their progeny. In one of the larger single-surgeon experiences, Ramasamy et al. examined their cohort of 792 men who presented with nonobstructive azoospermia[50]. They found that subjects with an FSH of 15–30 IU/mL had the same SRR as men with an FSH > 45 IU/mL. Similarly, FSH level did not predict pregnancy or live birth outcomes. These results countered the findings of earlier, smaller studies that evaluated the role of FSH[51]. When Ramasamy and colleagues focused only on men with KS, FSH remained an insignificant factor in terms of predicting retrieval success[42]. The failure of FSH to predict sperm retrieval reflects the effectiveness of microTESE in finding rare sites of sperm production, and the recognition that small foci of sperm production are inadequate to alter serum FSH levels.

Along similar lines, it is thought that decreasing levels of inhibin B are associated with the elevated FSH levels characteristic of men with KS. Normally, germ cells, including elongating spermatids during spermiogenesis, feedback upon surrounding Sertoli cells, which ultimately secrete inhibin B based upon the signaling of these surround sperm precursors[52]. However, just as in the case of FSH, inhibin B proved to be a poor predictor of spermatogenesis in men with hypergonadotropic hypogonadism. In a group of 185 individuals with nonobstructive azoospermia, Vernaeve et al. found no significant relationship for levels of inhibin B, with and without FSH as an adjunct, in predicting SRR[53]. In their study, inhibin B produced a receiver operating characteristic curve (ROC) with an underlying area of 0.51. Additionally, other investigators have found discordant values of inhibin B and FSH for men with nonobstructive azoospermia, further limiting its

clinical value [51,54]. In series where a more limited sperm retrieval procedure is done, overall characteristics of the testis, such as FSH or inhibin B may better predict the presence of sperm in the limited sample of testicular tissue that is searched/retrieved.

One popular reasoning for the poor predictive value of FSH and inhibin B is that they represent a global view of sperm production. The isolated pockets of intact spermatogenesis observed during microTESE are likely not enough to engage the feedback network characteristic of normal individuals. In line with this hypothesis, a recent meta-analysis by Li and colleagues confirmed the insignificance of preoperative FSH for predicting SRR[55]. Utilizing the data from 21 studies, the area under the curve (AUC) for the FSH ROC was only 0.61. More sophisticated investigations have begun regarding gene promotion and polymorphisms in receptor sequences, especially regarding the FSH-beta subunit and the FSH receptor, although conclusive evidence has yet to emerge that will impact clinical management of the KS patient [56–58].

Li et al. also reviewed the importance of testis volume upon SRR[55]. Since the seminiferous tubules constitute the majority of testis volume, early investigators attempted to correlate testis volume with SRR [59,60]. In a large single-site series, Bryson et al. reviewed their experience of 1,127 men with nonobstructive azoospermia, a significant proportion of whom had KS[61]. The SRR was 55%, 56%, and 55% for testis volumes of <2, 2 to 10, and >10 cc, respectively. In the subsequent analysis by Li and colleagues, the poor predictive performance of testis volume was substantiated, with an ROC AUC of 0.64. When restricting to only men with KS, Corona et al. also found no significant correlation between SRR and testis volume for a combined cohort of 1,248 patients[46].

Leydig cell function also does not appear to correlate well with the presence of sperm during surgery. Several small retrospective series have purported a role for baseline testosterone level in men with KS. Ramasamy et al. found that among their cohort of 68 men, individuals with normal preoperative testosterone tended to have higher retrieval rates (86 versus 63%, $p = 0.06$)[42]. In a more contemporary series, Chehrazi et al. found that those with successful surgeries had a mean testosterone level of 340 ng/dL as opposed to 233 ng/dL in those who failed ($p < 0.001$)[62]. It is worth mentioning that the SRR reported by Chehrazi and colleagues was significantly less than historical rates, raising the concern for generalizability of their results. Rohayem et al. also documented a higher SRR with compensated Leydig cell function (specifically, LH < 17.5 and T > 7.5 nmol/L) in a cohort of 135 men who had retrieval rates comparable to previous reports[63]. Ultimately, as was the case for testis volume and FSH levels, preoperative testosterone concentrations did not hold significance during subsequent meta-analyses[46].

The need for robust predictors of SRR remains an ongoing research goal for reproductive specialists. Several groups have begun to examine differences in the androgen receptor to explain the variation in KS phenotype, including CAG repeat length and novel mutant variants [64–67]. Unfortunately, none of these have provided reproducible insight. It should be noted that some correlation with testicular histopathology has been linked to retrieval success rates, although these data cannot be practically applied as it requires a preemptive testis biopsy procedure [55,68]. If the testis is going to be opened for diagnostic biopsy, why not obtain tissue for sperm recovery as well? Additionally, even if testis histopathology is known pre-op, approximately one in five men with the worst-case scenario (tubular hyalinosis) will have sperm found[68]. The predicted SRR remains high enough that couples normally proceed with sperm retrieval anyway, and so a preliminary diagnostic biopsy rarely changes clinical management.

3.2. Age

It has been theorized that SRR may improve if KS patients are advised to undergo sperm retrieval at a younger age [45,69]. This concept gained early popularity due to the results of several retrospective series, in addition to the perceived demise of testicular function during puberty. In their large retrospective series, Bryson et al. found that men with KS had a higher rate of retrieval if they were younger than 30, documenting an SRR of 81% versus 33% for those older than 30 ($p < 0.01$)[61]. Similarly, two other groups reported significant improvement of surgical success when stratifying outcomes based on age, collaborating the inverse relationship with SRR [42,63,70]. It was extrapolated that SRR may be further optimized if retrieval occurred during puberty, since clinical evidence for testicular failure begins to occur during this time. Conversely, published data did not identify any age, above which, sperm retrieval was not possible.

Although the process of testicular failure in KS is beyond the scope of this review, the key points that drove the practice of peripubertal sperm retrieval warrants discussion. Proponents of early surgery argue that stem cells are present in prepubertal boys, but seem to disappear in a large proportion of adults following the period of adolescence. To begin with, conflicting reports of germ cell numbers have been reported for fetal and postnatal KS testes [71–74]. In the XXY mouse model, germ cell loss coincides with the mitotic expansion of the SSC population in the early post-natal period, with virtually complete demise of these cells prior to the age that meiosis commences [75–77]. A recent study by Winge et al. provided new data regarding the timing of SSC loss[78]. Postnatally, primordial gonocytes can be identified due to expression of the OCT3/4 marker. In normal development, expression of this marker is continually lost as these cells differentiate into pre-spermatogonia, the progenitors of the SSC population. Notably, this differentiation step is marked by the expression of MAGE-A4. Interestingly, Winge et al. found similar dropout rates of OCT3/4 in KS and normal postnatal testes, but those with KS did not demonstrate the concomitant increase of MAGE-A4 concentration characteristic of gonocyte differentiation[78]. These data may explain the conflicting studies published several decades ago, which purported the presence of germ cells in fetal and postnatal KS boys, as they may have been describing the presence of OCT3/4 positive gonocytes. More importantly, the lack of MAGE-A4 in KS suggests that these observed precursor cells may never become functional due to their lack of differentiation, likely to

succumb to apoptosis as time progresses. Along these lines, Muller and colleagues provided evidence that nearly all preliminary germ cells disappear by the age of 2 years in those with KS[72].

Several other groups, however, have presented evidence that counters the findings of Muller et al., positing that most germ cell loss occurs upon entrance into puberty. Following the postnatal period, pre-pubertal KS boys typically demonstrate hormonal profiles that are similar to their unaffected counterparts, including serum concentrations of testosterone, LH, FSH, inhibin B, insulin-like 3, and AMH [79–81]. With the onset of puberty, KS boys experience an initial rise of androgen levels and growth of testicular volume. However, these changes level off earlier in the course of development, with progressively worsening hypergonadotropic hypogonadism. Inhibin B levels drop to undetectable levels while FSH increases[82]. Coincidentally, the initial growth of testicular volume begins to regress[83]. During this period, seemingly normal seminiferous tubule architecture is replaced by varying degrees of hyalinization and Leydig cell hyperplasia, which often manifests on testicular ultrasound as hypoechoic nodules[79].

An early investigation by Wikstrom et al. attempted to delineate the exact timeframe for germ cell loss in a cohort of 14 KS boys aged 10 to 14[83]. All members of their cohort underwent one testis biopsy, half of whom underwent surgical intervention while Tanner stage 1. SSCs were observed only in the younger group, prior to testicular growth (<2 cc in volume) and the rise in testosterone levels. No meiotic cells were detected in any of the study subjects, which led the authors to conclude in a subsequent analysis that germ cell loss occurs at the onset of meiosis[84]. This concept gained popularity, with multiple centers publishing results of sperm retrieval in peripubertal KS boys (Table 1). As can be appreciated from Table 1, the SRR has not proven superior to what is seen in adults even though the mean ages at retrieval typically surpassed the expected period of spermarche[85].

The question remains if the peripubertal boys in which sperm are found correspond to adult patients with successful retrieval. Are we finding sperm that would have been found anyway later in life? These data have led many leaders in the field to question the practice of peripubertal sperm retrieval [80,88,89]. The concern for this concept is further bolstered by the recent meta-analysis by Corona and colleagues, which found no predictive power of age in terms of SRR[46]. Additionally, adolescent KS patients do not appear to be overly concerned about fertility preservation, and it is unclear what the effect will be of a negative retrieval attempt in these young men[85]. Further, many have questioned the value of sperm cryopreservation, where sperm may not survive freeze-thaw, additionally arguing that sperm retrieval at an early age may reduce the chance of having functional sperm usable at a later point for reproduction.

If a small window of opportunity exists to optimize SRR, there must be clinical data to trigger surgical intervention. Also, success will only be realized if sperm retrieval is attempted after spermarche and prior to the onset of germ cell loss. In an enlightening study conducted by Gies et al., the ability to detect such a window of opportunity was directly tested[90]. Seven boys, aged 10–14, were prospectively followed every 4 months and assessed for Tanner stage, testis volume, inhibin B/FSH concentrations, and for spermaturia. Testicular biopsy was conducted once testis growth arrested, inhibin B concentration began to fall, or when FSH began to rise. Despite using these traditional indicators of failing spermatogenesis, no patients were found to have mature sperm within their testis tissue. All patients were observed to already have significant fibrosis and hyalinization on histopathology. Clearly, the histologic changes within the testis preceded the arrest of testis growth and the decline in inhibin B. The authors concluded that these changes likely occurred at the beginning of puberty and could not be predicted by exam or hormonal profile[90]. It is possible that these changes even occurred prior to the onset of sperm production. At this time, it is reasonable to conclude that current clinical data are insufficient to predict the ideal theoretical period in which SRR may be maximized, and that such a window of time may be too short to practically act upon.

More recently, Van Saen et al. provided strong evidence that germ cell loss occurs continuously throughout childhood, which directly refutes the notion of a precipitous decline of spermatogenic cells at the onset of puberty[91]. They used modern immunohistochemistry against MAGE-A4 to mark SSCs that may eventually produce sperm, assessed from biopsies during four periods of life: fetal, prepubertal (age 4–7), peripubertal (age 12–16), and adult (>18). They found that though MAGE-A4 positive cell populations were comparable between KS and controls for fetal samples, very small numbers were present in prepubertal KS boys. Additionally, only 30% of peripubertal boys had identifiable SSCs. Hyalinization and fibrosis were present beginning at the peripubertal stage,

Table 1. Data for viable sperm recovery among peripubertal boys with KS, sorted by mean age at time of surgery. SRR = sperm recovery rate.

Study	Design	Age Range	Mean Age	Cohort Size	SRR
Wikstrom 2004[83]	Prospective	10 – 14	11.8	14	0%
Van Saen 2018[91]	Retrospective	12 – 16	14.3	20	5%
Damani 2001[69]	Case Report	15	15	1	100%
Gies 2012[90]	Prospective	13 – 16	15.1	7	0%
Mehta 2013[102]	Retrospective	14 – 22	15.5	10	70%
Rives 2013[85]	Retrospective	15 – 16	15.8	5	20%
Rohayem 2015[63]	Retrospective	13 – 19	16.5*	50	38%
		15 – 19	17.1*	40	45%
Nahata 2016[86]	Prospective	15 – 23	17.6	10	50%
Plotton 2015[87]	Prospective	15 – 22	18.9	25	52%

*Approximate values derived from primary literature tables & figures when 2-year age ranges were presented rather than exact ages.

well after the decline of the germ cell population in childhood. These data further challenge the idea that SSR can be optimized by intervening early in puberty, and explain why clinical data (such as testis volume and Tanner stage) do not correlate with sperm recovery.

In the future, it may be possible to offer prepubertal children testis tissue cryopreservation, which may produce sperm assuming the technology is developed to mature, transplant, and or culture the spermatogenic cell line[92]. This research has garnered significant interest in the oncofertility realm, where boys with presumably normal fertility potential are offered intervention prior to gonadotoxic therapy. It should be noted that although we have had significant progress in other mammalian species, in cell culture we have yet to fully maintain and mature the human germ line all the way to sperm production[92]. If the goals of these research endeavors are obtained, it is debatable if they would be applicable to the KS population, in which the germ cell population is already significantly compromised by early childhood [90,91]. Some have indicated that a critical number of SSCs may be required to initiate a successful SSC culture [93,94]. However, Van Saen et al. have already demonstrated incredibly small numbers of SSCs in prepubertal KS boys, ranging from 0.03 to 0.06 germ cells per seminiferous tubule[91]. Only time will tell if these technologies will ultimately deliver for the oncofertility population, and if they will be applicable to the KS male.

3.3. Hormonal manipulation

3.3.1. Can hormonal manipulation optimize SRR?

Reproductive urologists have utilized a wide array of agents to manipulate the hormonal milieu of their nonobstructive azoospermic patients in hopes of optimizing surgical success. These drugs include gonadotropins (HCG and FSH), aromatase inhibitors, exogenous testosterone, and antiestrogens. The details of exogenous androgens, which are typically suppressive of gonadotropin production and hence spermatogenesis, will be reserved for the next section. The remaining agents attempt to modulate three factors: intratesticular testosterone, testosterone to estrogen ratio (T:E ratio), and the direct stimulation of spermatogenesis. As was the case with predictive factors of SRR, the primary literature supporting these practices is mainly observational in nature with conflicting data. It should also be noted that in the KS male, in which hypergonadotropic hypogonadism is present, the effective levels of LH and FSH are already quite high, and so the addition of exogenous hormones would not be thought to dramatically impact reproductive physiology. Nevertheless, the data exploring the use of HCG and recombinant FSH is worth reviewing.

It is well known that spermatogenesis requires an intratesticular testosterone concentration approximately an order of magnitude higher than the range typically measured in serum [95,96]. As stated previously, some authors have argued that baseline testosterone, and or the level of LH compensation, may be predictive of SRR [42,63,97]. Extrapolating from this, many practitioners attempt to drive endogenous testosterone production to higher levels using HCG, aromatase inhibitors or selective estrogen receptor modulators in hopes of improving surgical success. Two trials have evaluated the use of HCG in nonobstructive azoospermia. Hussein et al. performed a staged intervention, utilizing clomiphene, HCG, and FSH depending upon hormonal response of subjects[98]. Their results demonstrated higher SRR, but have been criticized due to their strict exclusion criteria (subjects with an FSH 1.5x higher than normal were not included). It is unclear if such therapy will work in the case of KS, as these patients often have markedly elevated FSH levels. In addition, the strict selection of subjects may have resulted in the higher SRR, rather than any hormonal intervention. Similarly, Shiraishi et al. utilized a combination of HCG and FSH (if suppression occurred) in nonobstructive azoospermics prior to salvage microTESE if a previous attempt had failed[99]. In this highly selected cohort, they observed a higher SRR for those who received hormonal therapy prior to a repeat microTESE attempt (21 versus 0%, $p < 0.05$). These investigators specifically excluded men with KS.

Relatively little evidence has been generated specific to the KS patient. In the study by Ramasamy et al., which utilized a strategy of titrated HCG, clomiphene, and aromatase inhibitors for KS men with an initial total testosterone below 300 ng/dL, they found that responders (defined as post-treatment testosterone >250 ng/dL) demonstrated a higher SRR than for men who did not respond to medical therapy with an increase in testosterone (77 vs. 55%, $p = 0.05$)[42]. They also found that a T:E ratio above 10 appeared to impart better recovery results. With the lack of clear guidelines and level 1 evidence, reproductive urologists rely upon the above data for management of their KS patients. Since most KS patients have hypergonadotropic hypogonadism, FSH is already typically high and it is thought that the supplementation of FSH will provide little benefit[100]. Addition of drivers of endogenous testosterone are prescribed to achieve a goal preoperative androgen level. Should the T:E ratio begin to shift unfavorably, or if FSH is overly suppressed, aromatase inhibitors may be used as a combined therapy. The duration for such treatment is typically 2 to 6 months, as a full round of spermatogenesis requires 2–3 months. However, the androgen-dependent component of sperm production may be affected by as little as 1–2 months of hormonal therapy[101].

3.3.2. The role of exogenous androgens

Spermatogenesis requires significantly higher intratesticular testosterone levels compared to circulating concentrations assayed from a peripheral blood draw[95]. To support this process, LH must drive androgen synthesis within the Leydig cells of the testis. Exogenous testosterone suppresses LH, and although it can provide an androgen concentration required systemically, it may provide a contraceptive effect in terms of sperm production[96]. For this reason, reproductive urologists routinely face the consequences of impaired spermatogenesis due to exogenous androgens, as is the case for testosterone replacement therapy (TRT) or the abuse of anabolic steroids. Men with KS, however, may require androgen support from an early age, which poses a unique challenge for the management of reproductive potential[45].

Small retrospective series initially presented concerning data regarding SRR in KS men who had previously been treated with TRT. In the study by Schiff and colleagues, KS

men who were previously treated with TRT, predominantly with injectable testosterone, dropped the SRR to 20% (although these results were only based on five patients)[22]. Of note, exogenous injectable testosterone preparations appear to have a more dramatic suppressive effect on gonadotropin production when compared to topical agents. Mehta et al. successfully managed 10 KS adolescents utilizing TRT with gel-based application plus an aromatase inhibitor, documenting an SRR of 70% despite concomitant androgen replacement[102]. In a follow-up study with a cohort of 110 patients, topical testosterone did not appear to significantly decrease LH or FSH, suggesting that gonadotropins remained high enough despite topical TRT to promote any spermatogenesis that may be present[103]. Finally, in a recent study conducted by Garolla et al., SRR was not affected for TRT-treated and untreated KS men[104]. As suspected, on subanalysis, SRR began to decrease in only those patients who had suppression of endogenous LH secretion. These latter data provide evidence that carefully titrated TRT may be appropriate in KS men who plan to initiate fertility treatment in the far future. However, it should be noted that the package insert for gel testosterone products recognizes the decrease in gonadotropins with gel testosterone administration. Although this may be abrogated in some men with an aromatase inhibitor, the risk of decreasing sperm production with even gel testosterone treatment must be considered. Until further evidence is offered, the traditional approach we employ in our practice remains to stop TRT and to transition to HCG ~6 months prior to planned surgical sperm retrieval.

4. Sequelae of surgical sperm retrieval

Surgical sperm retrieval is an outpatient procedure with relatively low rates of complication. As with any procedure, hematoma or infection may occur, although the rate for sperm retrieval is relatively low[2]. Specific to KS patients, however, is the concern for subsequent decline in Leydig cell function, possibly transitioning patients to require TRT despite adequate androgen levels preoperatively. The microTESE procedure, which was developed to maximize SRR, has proven to improve the hematoma rate and to decrease the loss of testicular parenchyma[19]. In a comparative study by Amer et al., patients were subjected to conventional TESE on one side and microTESE for the contralateral testis[105]. They found that significantly less tissue was removed for the microTESE procedure (4.6 mg versus 53.6 mg), and a 43% decrease of absolute risk for intratesticular hematoma. Subsequently, Okada et al. confirmed that men who underwent microTESE had significantly less testicular volume loss following surgery compared to those who had conventional TESE[106].

Multiple groups have examined endogenous testosterone production following sperm retrieval. Androgen synthesis does acutely drop following surgery but tends to recover at a variable rate[2]. In a large cohort of 435 men with nonobstructive azoospermia, testosterone levels returned to baseline in 85% of subjects by 1 year post-surgery, and in 95% of the cohort by 18 months[107]. In a recent meta-analysis by Eliveld et al., the risk of hypogonadism following sperm extraction was assessed based upon the data of 15 studies, 8 of which specifically commented on the effects for the KS population [108]. The KS cohort had a mean decrease in testosterone at 6-months post surgery of 118 ng/dL (CI: −169 to −69), which improved to a mean decrease from baseline of 66 ng/dL (CI: −116 to −15) for follow-up beyond 12 months. KS realized the greatest impact on Leydig cell function from surgery as compared to men with nonobstructive azoospermia secondary to other etiologies. As opposed to men with normal karyotypes, KS individuals typically returned to baseline testosterone levels by 26 months post-surgery. These results may be due in part to practice patterns, since TRT is typically transitioned to HCG injections prior to sperm retrieval.

5. Conclusions

KS is the most common chromosomal disorder in men, defined as any additional X chromosome, and is by far the most common karyotypic abnormality identified during workup of male infertility. The hallmark of KS is progressive testicular decline leading to hypergonadotropic hypogonadism. Puberty appears to be the trigger for much of the histologic changes that occur in the testis, ranging from hyalinization, fibrosis, and varying levels of spermatogenic arrest. Despite this process, the prognosis for fertility treatments remain relatively high compared to men with idiopathic nonobstructive azoospermia. For at least half of men, isolated foci of intact spermatogenesis may be discovered during surgical exploration, which can be used with IVF-ICSI to produce viable pregnancies. Reassuringly, the vast majority of children born to KS fathers are karyotypically normal. Many questions remain regarding the process by which KS men produce sperm, the optimal timing of sperm retrieval, and if future technologies may provide a mode of fertility preservation for those diagnosed early. Currently, no robust predictors of surgical success have been identified. Additionally, the notion that SRR may improve if surgery is pursued during the peripubertal period appears discredited now that larger more contemporary cohorts have documented disappointing results. Beyond age, the hormonal optimization of KS individuals remains a work in progress, with low-level evidence pointing to manipulation of endogenous testosterone production while preserving FSH levels and the T:E ratio. Management of these parameters is nontrivial and requires early referral to a reproductive urologist once a man with KS begins to consider family building.

6. Expert opinion

The above review summarizes the natural history of progressive testicular failure in KS and the data supporting fertility treatments that are currently available. Although KS is often labeled as a solitary diagnosis in the reproductive literature associated with severe testicular atrophy, the phenotypic expression of intratesticular function may be quite variable. This is reflected in the wide range of different patterns of testicular histology, from full spermatogenesis to limited development of sclerotic tubules in Leydig-cell hyperplasia. Given the variability within patients, the highly focal nature of sperm production and the variability of technical surgical approaches to patients, a wide range of published SRRs is expected. Furthermore, preoperative

predictors of surgical success are limited for KS patients. Advances in the field have been hindered by the preponderance of observational study designs that have produced an array of conflicting data. These data should be interpreted with great caution, as the poor level of evidence may lead to misinterpretation of their results. This issue is best exemplified by the early drive to harvest sperm in the peripubertal period, which was at best ethically opaque and has not endured the emergence of better data. What we do know is that KS men have a reasonable prognosis as compared to men with idiopathic nonobstructive azoospermia, with viable sperm recovered in at least 40% to 50% of individuals. There may be an opportunity to increase SRRs through hormonal manipulation, but again, the lack of level 1 evidence to support this intervention is concerning. When treating an azoospermic man, an intervention may harm as well as enhance spermatogenesis. Even the most benign-appearing intervention should be undertaken with caution, especially when the intervention (e.g., hCG or exogenous testosterone) decreases gonadotropins and may have a direct adverse effect on sperm production. In our practice, we prefer a conservative approach in which any TRT is halted, and endogenous testosterone production is promoted through a balance of selective estrogen receptor modulators, HCG, and aromatase inhibitors. These parameters, however, do not influence outcomes as much as intraoperative experience of the surgeon in combination with the quality of his or her andrology/embryology team. Finally, the prospects of testicular tissue cryopreservation for future fertility remain highly experimental despite their burgeoning popularity. It is our opinion that such therapies only be offered in the research setting under a monitored experimental protocol.

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