

Reproduction in Men with Klinefelter Syndrome: The Past, the Present, and the Future

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ABSTRACT

Klinefelter syndrome (KS) is the most common chromosomal aberration in men. There are approximately 250,000 men with KS in the United States, and the prevalence of KS in male reproductive practices is 3 to 4%; however, most men are never diagnosed. KS has an effect on normal development, growth, social interactions, bone structure, and sexual and reproductive function, thus a multidisciplinary approach to men with KS is important in providing state of the art care to children and men with KS.

Over the last 10 years, with advancements in artificial reproductive techniques and the successful delivery of healthy children from men with KS, the involvement of reproductive endocrinologists and urologists in the care of patients with KS is becoming commonplace. The new areas of intense research investigate optimal methods of hormonal manipulations, preservation of fertility in adolescents, and development of universal early screening programs for KS.

This review provides the latest update in our understanding of the pathophysiology, natural history, and evolving paradigms of therapy in adolescents and men with KS.

KEYWORDS: Klinefelter syndrome, meiosis, spermatogenesis

Klinefelter syndrome (KS) is the most common numerical chromosomal aberration among men, with an estimated frequency of 1:500 to 1:1000 of live deliveries.¹ KS is characterized by X chromosome polysomy with X disomy being the most common variant (47,XXY). Ninety percent of men with KS have non-mosaic X chromosome polysomy.²

Although classic description of men with KS emphasized tall eunuchoid body proportions, low testosterone, sparse facial and pubic hair, small, hard testicles,

micropenis, sterility, and mild to moderate cognitive deficits, it is now well known that this original description is not accurate and men with KS represent a broad spectrum of phenotype, professions, income, and socioeconomic status.¹ Severe intellectual deficits are rare, and often auditory processing delay and language dysfunction seen in men with KS are misdiagnosed as cognitive deficits.² Thus most if not all internists, pediatricians, urologists, and reproductive endocrinologies have seen men with KS who were not diagnosed appropriately. It is

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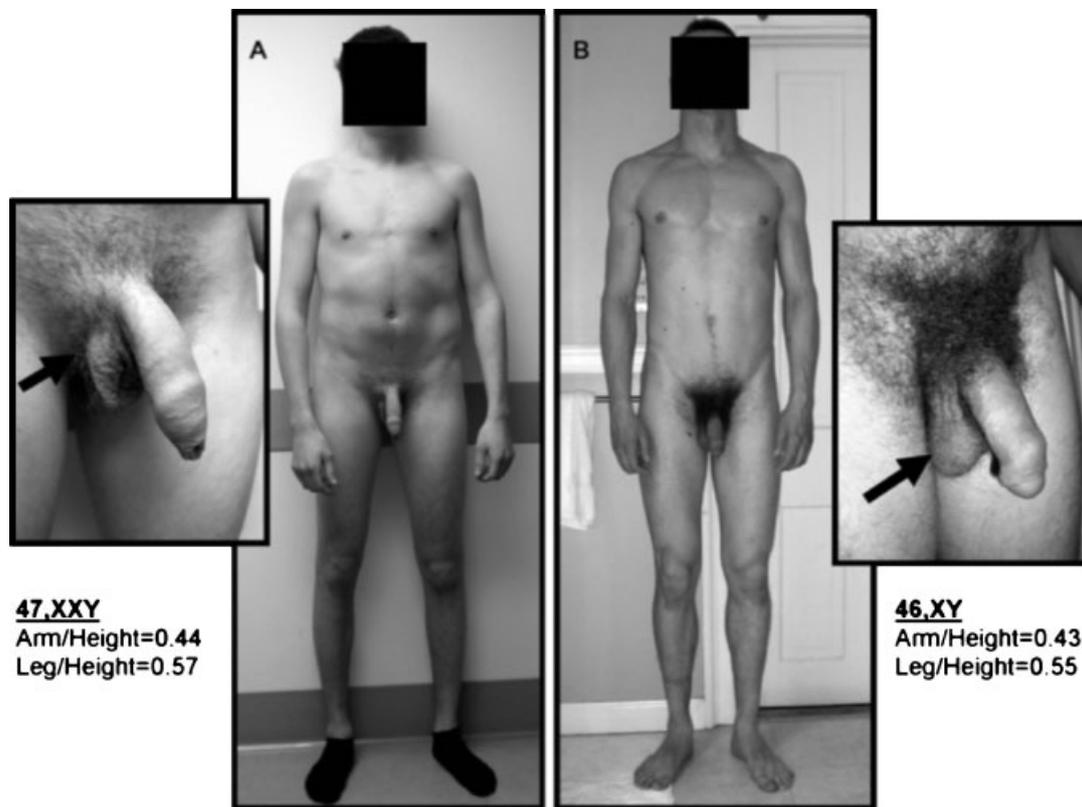


Figure 1 The classic descriptions of men with KS are based on the most severe cases of phenotypic abnormalities. Most teenagers and young adults seen in our practice have typical body proportions, arm span, and penile length as those of their peers. The only obvious difference that is seen in all men with KS is clearly visible difference in testicular size between men with KS (very small testes) and men with 46,XY karyotype (normal size). The photograph shows two 21-year-old men seen in our practice. The patient with 46,XY had a history of constitutionally delayed puberty. Both of them required testosterone treatment early during puberty. The patient with KS continues testosterone replacement therapy. Both of them are top-of-the-class college students.

estimated that only 10% of adolescents with KS are diagnosed before puberty.¹ The exact number of men who are never diagnosed is difficult to assess because not all men who are infertile or have low testosterone undergo cytogenetic evaluation. The main reason for delay or missed diagnosis is varied phenotypic features of KS among men with the same chromosomal arrangements (47,XXY) and subtle complaint by most men or adolescents with KS. Men with more than two X chromosomes (48,XXXY; 49,XXXXY) are more affected than are men with classic 47,XXY karyotype.³ The cardinal problems in men with KS—progressive testicular failure and thus azoospermia or cryptozoospermia, small testes (5 to 7 cm³), and low testosterone—are common denominators in men with KS and should prompt cytogenetic evaluation (Fig. 1).

Although the management of children, adolescents, and men with KS has been a domain of endocrinologists and geneticists with only marginal interest given to the syndrome in reproductive biology and clinical reproductive medicine, successful sperm recovery from men with KS and successful pregnancies using this

sperm for intracytoplasmic sperm injection (ICSI) stimulated renewed interest in epidemiology, pathophysiology, and management of KS over the past decade.^{4,5} This article focuses on new developments in reproductive biology and medicine in men with KS.

PATHOPHYSIOLOGY, EPIDEMIOLOGY, AND MECHANISMS OF SPERMATOGENIC FAILURE

The 47,XXY karyotype of KS arises spontaneously when paired X chromosomes fail to separate—nondisjunction—in the I or II phase of meiosis during oogenesis or spermatogenesis.⁶ Less than 3% of X chromosome polysomy occurs during early divisions of the fertilized egg. Postfertilization nondisjunction is also responsible for mosaicism, which is seen in ~10% of patients. Advanced maternal age and possibly paternal age have been linked to increased risk of KS.⁷ Children born from assisted reproductive technology (ART) have increased risk of sex chromosome aberrations, but it is unclear if this phenomenon is a direct result of in vitro fertilization

(IVF) or is a reflection of the increased risk of non-disjunction seen in older couples.⁸ Future epidemiologic data should answer this question.

The X chromosome carries genes that are involved in testis function, brain development, and growth among many others. Men with KS are usually infertile because of primary testicular failure. Typical patient with KS will present with low serum testosterone, high luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, and often elevated estradiol.³

Men with KS are at a higher risk of autoimmune diseases, diabetes mellitus, leg ulcers, osteopenia and osteoporosis, tumors (breast and germ cells), and historically increased mortality, although it is unknown if the morbidity associated with KS is a result of hypogonadism and hyperestrogenism or rather abnormal function of X chromosome-linked genes.^{5,6} Although some authors believe that the cognitive impairment and emotional problems are caused by low testosterone, as prepubertal hypogonadism was shown to negatively affect brain development ascribed by decreased thickness of the left temporal lobe gray matter, it is unlikely that low testosterone is a main reason for learning problems, as men with Kallmann syndrome, who often had un-

detectable levels of testosterone during early adolescence, have no cognitive problems.

Most men with KS are diagnosed as adults when they present with infertility or hypogonadism.² Delay in diagnosis may be secondary to phenotypic variation of patients with KS. The underlying mechanism of broad phenotypic variation of the same chromosomal aberration, 47,XXY, is unknown, but it may be explained by differences in hormonal profile, as well as differences in genetic background and skewed inactivation of the additional genetic material on the X chromosome.¹⁵ The X chromosome is the only chromosome in humans where one sex (female) has double the amount of genetic material. The X chromosome undergoes inactivation through noncoding RNA X chromosome inactivating transcript (XIST)⁹ (Fig. 2). X chromosome inactivation (XIC) must occur in men with X chromosome polysomies, because in females one of the X chromosomes undergoes random XIC in embryonic tissues, and the presence of two active X chromosomes in animal and hybridoma models is lethal, thus it is unlikely that both X chromosomes are active in men with KS. The XIC in females had to compensate for evolutionary loss of most of the X chromosome genes from the Y chromosome,

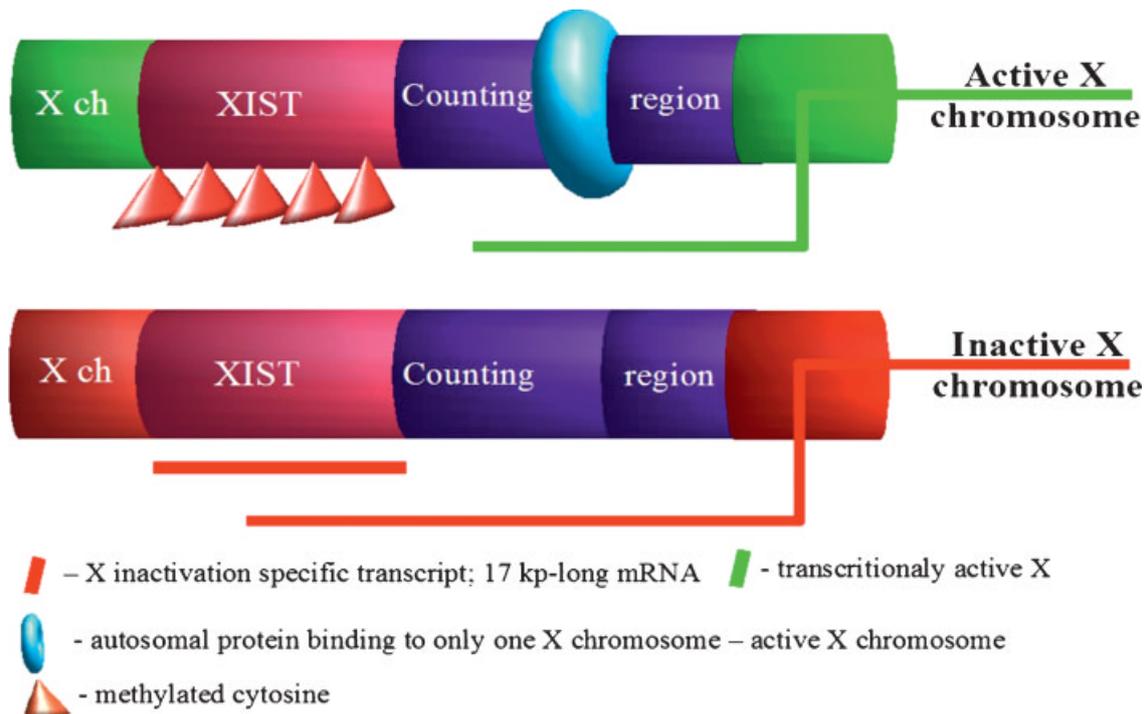


Figure 2 Proposed organization of the X inactivation center on the X chromosome. An unknown molecule/protein complex binds to the counting region on only one X chromosome, marking it as an active chromosome; the rest of the X chromosomes will become inactive. The interactions between counting region and XIST result in methylation of cytosines within the XIST region and inactivation of this region. Because XIST is undermethylated on remaining chromosomes, the XIST transcript is transcribed and initiates cascade of multifocal "painting" of the X chromosome(s), which results in chromatin changes and inactivation of the chromosomes. The fact that the counting region is active on only one chromosome allows for inactivation of any number of additional X chromosomes. The fidelity of this inactivation is not perfect and allows for significant level of promiscuity, thus men with more than one additional X chromosome are more affected because most likely their chromosomes are not completely inactivated.

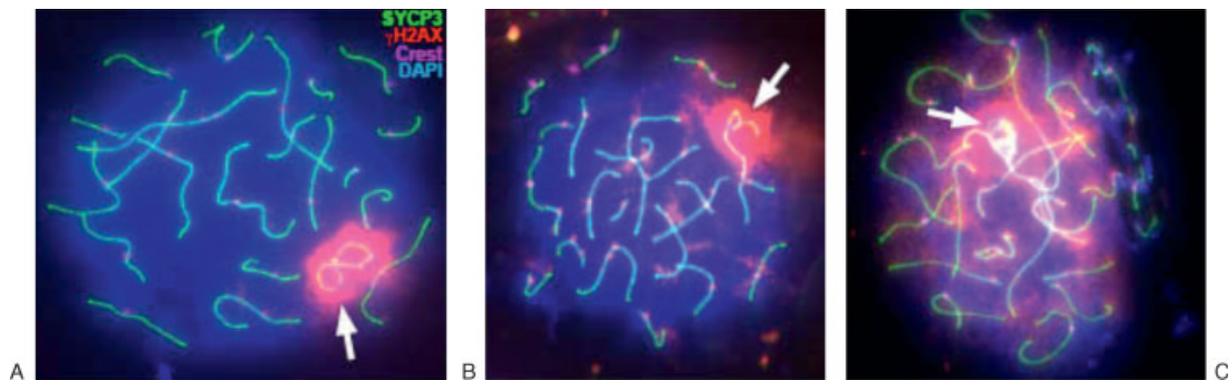


Figure 3 (A–C) Meiotic spread preparation from three different patients who underwent testicular sperm extraction. H2AX (red color) is a highly specific marker for sex bodies, an area of synapses between X and Y pseudoautosomal regions. This preliminary data, obtained in collaboration with Dr. Peter N. Schlegel and Dr. Paula Cohen, who provided the images, indicate that sex bodies are formed in spermatoocytes of normal patients and patients with spermatogenic failure.

thus compensatory mechanisms that ensure normal expression of X-linked genes in males and females had to develop. In normal females and males, transcriptional output from one active X chromosome is doubled and balanced to achieve 1:1 expression ratio between autosomal and sex chromosomes. Thus, adequate inactivation of one of the X chromosomes is critical to achieve normal development.^{10,11} Because men with multiple X chromosomes (48,XXXY; 49,XXXXY) are more affected than are men with classic 47,XXY, it is prudent to assume that control of X chromosome inactivation is suboptimal in men with X chromosome polysomy, and aberrant expression of X chromosome-linked genes plays a role in spermatogenic failure seen in men with 47,XXY.¹² It is well accepted that the X chromosome bears more than 1100 genes that are critical for normal function of the testis and brain.¹³ Inactivation of additional X chromosome is initiated within the XIC–X chromosome inactivation center by activation of XIST promoter (Fig. 2). Transcription of XIST RNA allows for multifocal painting of X chromosome and subsequent recruitment of inactivation proteins with H3 and H4 deacetylation and methylation linking the expression of XIST to chromatin remodeling and gene silencing.¹⁴ Multifocal sites of inactivation along X chromosome allow for physiologic escape of certain genes from inactivation, but at the same time this natural promiscuity in inactivation control may be responsible for reproductive and learning problems seen in KS. Although errors in inactivation of the entire X chromosome may be lethal, ~15% of X chromosome genes escape inactivation in normal individuals and more often in cancer. Not all genes in normal females are inactivated, as some of the genes on the Y chromosome have their homologs on the X chromosome in men; for example, *ZFK* gene codes for protein involved in development of sperm and oocyte. Because normal females lack a Y chromosome, thus *ZFK* is normally active on both active and inactive X chromosomes. Many genes on

the X chromosome are highly expressed in the testis, ovaries, and brain, thus it is not surprising that those organs are affected by X chromosome polysomy.^{11,14–18} Thus, abnormal inactivation of the X chromosome could explain some reproductive and cognitive sequela of KS. Understanding molecular mechanisms of X chromosome inactivation should allow us to better predict the extent of reproductive failure and hopefully offer some treatment in the future.

Over the past decade, developments in microsurgical techniques and advances in ART allowed more than 50% of patients with KS to have their own children through the combination of microsurgical testicular sperm extraction (TESE) and use of freshly retrieved sperm for IVF.^{19,20} However, this technique requires an expensive surgical procedure and hormonal stimulation of a female partner despite the uncertainty if sperm is present in testis.

The fact that the sperm is found in the testes of men with KS has challenged the previous assumption that men with KS are always sterile and raised the question whether children with KS are born with severely depleted number of spermatogonia or if there is a period in life when the spermatogonia undergo massive apoptosis.²¹ This question is an important issue from a clinical standpoint because better understanding of mechanisms of testicular failure in men with KS will allow us to offer scientifically based treatment options to these patients.

Based on current data, it is reasonable to assume that most men with KS are born with spermatogonia.^{22–24} However, during early puberty, most likely after the initiation of the first wave of spermatogenesis, the spermatogonia undergo massive apoptosis²⁴ a process which corresponds to rapid increase in FSH levels during early puberty in boys with KS. This hypothesis is based on three facts: identification and recovery of sperm in adult men with KS indicates that spermatogonia are present in at least half of men with KS; rare

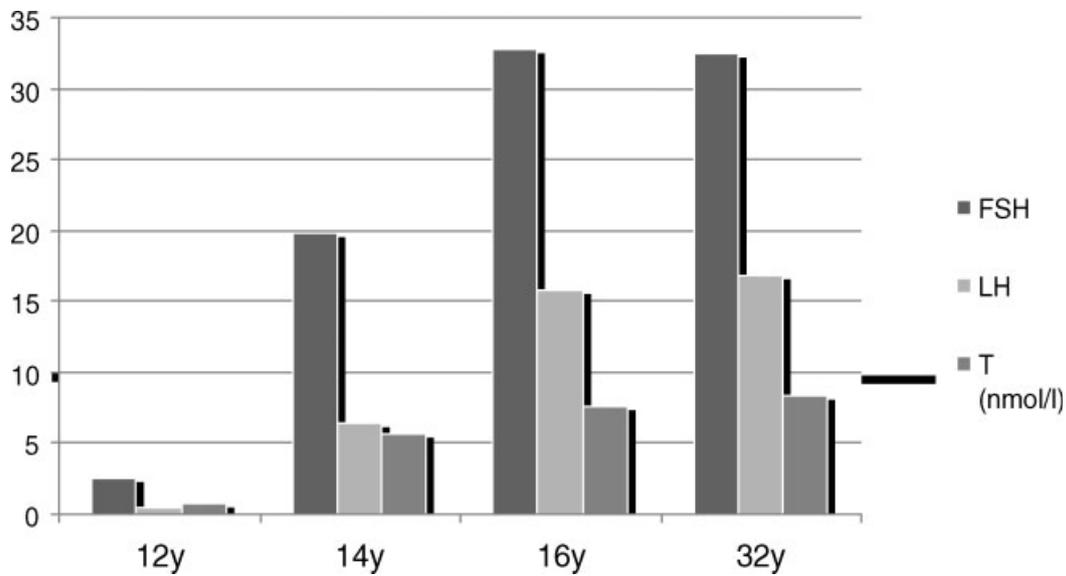


Figure 4 Changes in hormonal level in 85 adolescents and young men seen over 3 years. FSH and LH measured in serum (mIU/mL); total testosterone level converted to nmol/L to facilitate combining all hormone levels to be presented on one graph. The thick black line represents the upper normal levels of LH and FSH, which in fertile men should be below 10 mIU/mL, and low normal level of testosterone, which should remain above 10 nmol/L in adolescents and adult men. The damage to testis occurs within a 2-year window at early puberty, and by 16 years of age, most boys have similar levels of FSH as that of adult men who presented with infertility.

identification of sperm in ejaculate of men with KS; and biopsy data from KS boys at different ages and development stages indicating that boys with KS have spermatogonia at birth and that the damage to germinal epithelium occurs early during puberty.^{21,24} Recently, we have identified in two boys who had an adequate number of sperm in the ejaculate during early puberty that cryopreservation of ejaculated sperm was possible, thus providing evidence that the spermatogenic failure in KS occurs early in puberty and at least some boys complete a full wave of spermatogenesis. Significant effort has been undertaken by our and other groups to investigate the molecular mechanisms of loss of spermatogenesis. Three potential mechanisms are suggested: intratesticular hormonal imbalance with hypersensitivity to increasing intratesticular testosterone and estradiol concentration; Sertoli cell dysfunction; and defects in spermatogonial stem cell renewal. A less likely although possible explanation of spermatogenic failure would be the loss of spermatocytes during meiosis as a result of abnormal pairing of X and Y chromosomes (Fig. 3).

Low testosterone and elevated estradiol levels are cardinal symptoms of KS. In most men, LH and FSH elevation starts early during puberty^{21,24-26} (Fig. 4). Based on our experience, most boys have abnormally elevated FSH and LH at Tanner stage III. It is unknown at this point why men with KS have lower testosterone despite elevated LH, especially as 80% of Leydig cells in men with KS have normal morphology.²⁷ One possible explanation is the lack of adequate feedback from germ

cells. It is known that Leydig cells require adequate paracrine stimulation from Sertoli cells and germ cells.²⁸ A lack of germ cells initiates a cascade of events resulting in abnormal steroidogenesis production as is often seen in men after pelvic irradiation and aggressive chemotherapy.

Recently, in our laboratory we have shown that expression of the LH receptor is normal in men with KS as well as in men with nonobstructive azoospermia who were matched based on similar testosterone levels. The receptor mutation or resistance to LH is unlikely, as not all men with KS have low testosterone. Most likely, the aberrant expression of steroidogenic enzymes and/or negative effects of testosterone production secondary to elevated intratesticular estradiol levels are responsible for the hypogonadism seen in men with KS. Hyperestrogenism is commonly seen in KS, with increased estrogen to testosterone ratios and delayed increase in testosterone levels during puberty being responsible for characteristic body proportions, and gynecomastia.²⁹ In isolated Leydig cells, estradiol suppresses testosterone production by 30 to 40%, and inhibition of estradiol by selective estrogen receptor (ER) antagonist reverses this process (Fig. 5). Most recently, we have shown that expression of aromatase CYP19, an enzyme converting testosterone to estradiol, is 4 times higher in testis of men with KS. Our data bring evidence supporting previously published studies by Schlegel's group indicating that lowering intratesticular estradiol levels using aromatase inhibitors has a beneficial effect on testosterone production in men with KS and can potentially improve spermatogenesis.³⁰

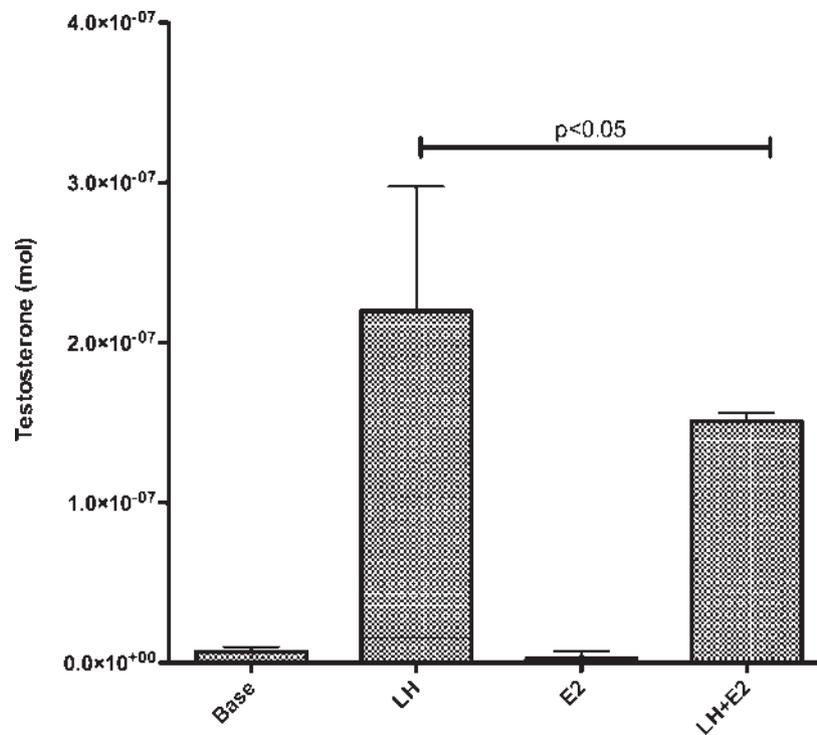


Figure 5 Testosterone production by isolated Leydig cells is negatively affected by increasing estradiol levels. The Leydig cells were incubated with medium only ("Base"), LH, estradiol only, and LH and estradiol in a concentration of 100 nmol/L estradiol. The estradiol decreases testosterone production by 31%. Results of this experiment provide a scientific basis to aromatase treatment in men with elevated estradiol and low testosterone.

Sertoli cells are critical for normal sperm production, and Sertoli function is impaired in men with KS. The trafficking of androgen receptors is impaired with some studies indicating that the function of androgen receptor is impaired in boys with KS, and most of androgen receptor (AR) staining is localized to the cytoplasm. Although normal testosterone levels are necessary for AR dimerization and trafficking, it is unknown at this point if cytoplasmic presence of AR is a result of defects in AR trafficking or low intratesticular testosterone levels.^{21,25,31}

Sperm found in testis of men with KS have only a slightly increased frequency of sex chromosome polysomies, and most boys born from fathers with KS have a normal karyotype.^{19,32,33} This indicates that during meiotic division, the checkpoint mechanisms are able to overcome X chromosome polysomy resulting in sperm with a single X chromosome.²³ During normal meiotic divisions, the X and Y chromosomes undergo pairing at pseudoautosomal regions in the area called the sex body (Fig. 3). Chromosome pairing (which in autosomes occurs along the entire chromosome length and is a prerequisite for normal chromosomal recombination) and chromosome segregation is more complex in males because the length and sequence of X and Y chromosomes differ and require creating a loop of X chromosome that is not paired. X chromosome polysomy in spermatogonia may interfere with Y and X chromo-

some pairing and result in loss of germ cells with 47,XXY. It seems that men with sperm found during TESE have 46,XY spermatogonia indicating occurrence of the repair process during spermatogonial renewal.³⁴ Alternatively, a low degree of intratesticular mosaicism 46,XY/47,XXY, which is not detected by peripheral blood cytogenetics could be responsible for presence of 46,XY spermatogonia. Our preliminary data on chromosomal spreads together with published data by Bergere et al and Yamamoto indicate presence of repair mechanism that during spermatogonial renewal allows for loss of the additional X chromosome and is the most likely explanation for the existence of normal sex chromosome haploid sperms in men with KS.²³ Having better knowledge about repair mechanisms during spermatogenesis in men with KS should allow us to develop new therapeutic interventions in the future.

Diagnosis

KS has rather subtle symptoms, and a high level of awareness about the prevalence of KS in the population of men with delayed puberty, sexual dysfunction, low testosterone, and infertility should allow for increased detection of KS.

Often, just a physical examination and noticing very small testes compared with those of the patient's age

group should be enough to initiate cytogenetic evaluation (Fig. 1).

KS can be diagnosed in the postnatal period by karyotyping, the presence of Barr body in the mucosal scraping, fluorescence in situ hybridization (FISH), and molecular techniques. Karyotyping has been a gold standard in KS diagnosis but the test is expensive, labor and time consuming, and has relatively low sensitivity for 47,XXY/46,XY mosaicism¹⁶; however, currently used tests are too expensive to be offered as a screening tool. Recently, Barr body cytology has been proposed as a cheap and sensitive test for KS screening, however this test had 95% specificity and 82% sensitivity for the diagnosis of KS.⁹

FISH in the diagnosis of chromosomal polyploidy has similar specificity and sensitivity to the karyotype, but FISH requires expensive probes, experienced technologists, and imaging software. Molecular techniques, especially quantitative polymerase chain reaction (PCR), have been used in preimplantation genetic diagnosis (PGD) of multiple diseases like familial mental retardation, cystic fibrosis, muscular dystrophy, and chromosomal numerical aberrations among others.¹⁷ Recent literature on prenatal screening for common aneuploidies (13, 18, 21, and sex chromosomes) confirms that molecular techniques are as sensitive as karyotype and FISH; however, molecular techniques are less expensive and faster, hence more amendable to prenatal screening.^{18–20} We believe that the availability of a fast and a cost-effective postnatal screening and diagnostic test for KS can significantly improve the quality of care in those patients.

The advantage of using PCR-based technology is the long-standing experience in PCR and the availability of equipment in virtually every research and clinical laboratory, low volume of blood needed for the DNA extraction (important for screening in children and neonates), and low cost. The optimal test for diagnosis of KS would allow all children born from older parents or through ART to be screened at birth for KS, as early diagnosis has a positive impact on child development.

Growing evidence suggests that early diagnosis and therapeutic interventions in boys and men with KS may have a beneficial effect on their physical, academic, and social development and health.⁷ Unfortunately, only 10% of men affected by KS are diagnosed in preadolescence and adolescence, the time when treatment may be the most effective.⁸ It is possible that early detection and screening for KS in target populations (children with learning disabilities, developmental problems, or men with hypogonadism, sterility, or diabetes mellitus [DM]) could offer early treatment to affected men.⁷

In our laboratory, we have developed an alternative method of testing for X chromosome polysomy that uses differences in the methylation pattern of two genes located on the X chromosome: familial mental retardation gene 1 (*FMR1*) and X chromosome inacti-

vating transcript (*XIST*). Pena proposed the use of *FMR1* gene in the diagnosis of KS in a letter to the *Journal of Andrology*.¹⁰ However, to our knowledge, there has been no publication evaluating this technique in KS. Our test costs \$5 per assay and has 100% specificity for detection of X chromosome polysomy.³⁵

LABORATORY AND AUXILIARY EVALUATION

All men with KS should have a full hormonal evaluation including follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol, prolactin, and insulin-like growth factor-1 (IGF-1). Cortisol levels should be routinely measured as there is growing evidence that adrenal steroidogenic deficiency may be seen in 47% of men with KS.³⁶

Peripheral blood cytogenetics are adequate for diagnosis; however, mosaicism may not be detected if lower than 10%. We and others have noticed an increased risk for Y chromosome microdeletions in men with KS, and screening for Y chromosome is routinely done in our practice.^{37,38}

Because of the decreased level of testosterone and significantly increased risk of osteopenia and osteoporosis in men with low testosterone, bone density testing is routinely performed to assess risk for osteopenia and osteoporosis. If osteopenia or osteoporosis is diagnosed, then additional laboratory tests including calcium, phosphorus, parathyroid hormone (PTH) calcium, and vitamin D3 should be measured. Most of the men with KS are taller than predicted height; however, in our population a significant number of adolescents have short stature and low body mass index (BMI). Of 100 adolescents seen over the past 2 years, we identified three cases of growth hormone deficiency. Because growth hormone has a synergistic effect on genital growth, IGF-1 should be routinely measured. Men with KS have an increased risk of deep vein thrombosis, and in 85 adult men we have seen over the past 3 years, three men had pulmonary embolism and deep vein thrombosis. At this point, it is unclear if screening for mutations leading to hypercoagulability is indicated in all men with KS, however all patients with KS should be informed about the increased risk of deep vein thrombosis and have their hematocrit checked to avoid increased viscosity.³⁹

Patients with KS have an increased risk of extratesticular germ cell tumors, and a chest x-ray should be obtained if one suspects intrathoracic process; however, overall the incidence is extremely rare.

Risk of breast cancer in men with KS is controversial. We have previously shown that KS is a risk factor for breast cancer, but other authors have reached different conclusions.^{40,41} Out of precaution and because no cost concerns are involved, we recommend that all patients are taught early how to perform breast

self-examination and alert their physician if an abnormal nodule or nipple discharge is found.

THE PHYSIOLOGIC APPROACH TO THE MANAGEMENT OF MEN WITH KS

Management of men with KS is challenging because one's reproductive goals have to be included in optimal medical treatment. Most men present with infertility; however, one has to remember that KS has a significant impact on one's overall health and life long management has to be considered an integral part of the reproductive medicine practice of men with KS.

Treatment options in adolescents and adults differ, and especially in younger adolescents fertility preservation should be discussed with parents. At present, fertility preservation in adolescents should be reserved to large academic centers, and each team needs to solve complex ethical, legal, and logistics issues that arise when a child with a genetic defect is subjected to the surgical procedure, for which benefits, although very likely, are not certain at this point. The treatment of adult men with KS is much more standardized and accepted. Since recovery of sperm through TESE and successful live births of children conceived through the combination of TESE and ICSI, it is critical that reproductive endocrinologists and urologists are familiar with the current literature and success rates of combined TESE and ICSI in men with KS, as all too often men with KS are directed toward adoption. The best success rate of IVF in KS seems to be obtained using of fresh sperm through testicular biopsy performed the same day as egg retrieval. In the largest study reported to date by Schlegel et al, the retrieval rate was much higher, 69% (29 of 42), than the retrieval rate of 42% (5 of 12) published by Friedler et al.^{19,42} Fertilization rate was higher: 85% using fresh sperm in Schlegel's study and 58% using cryopreserved sperm. Although the article by Friedler et al shows no statistical difference between fresh and cryopreserved sperm, there is a trend toward a lower rate of fertilization and implantation, but with only five patients in the study, valid statistical conclusions are difficult to make.

Even by the most conservative estimates, in at least 50% of adult men with KS, viable sperm can be found and successfully used for IVF. This obviously is one of the most tremendous successes stories in reproductive medicine. To date, the follow-up of boys born from fathers with KS has not shown any phenotypic abnormalities or increased risk of KS.^{19,43} Optimal timing of sperm retrieval as well as optimal hormonal treatment prior to sperm retrieval has not been established to date. Injectable testosterone is detrimental to the sperm recovery rate; however, this may simply reflect that often men with KS who had to be treated with testosterone injections early may have more severe testicular failure with delayed puberty and poor develop-

ment during puberty. More data are needed about optimal hormonal treatment of men with KS. In our practice, we stop injectable testosterone in men with KS prior to any treatment for infertility. Some of the men who are used to very high levels of circulating testosterone are placed on topical testosterone, usually AndroGel (Solvay, Marietta, GA), which achieves physiologic levels of testosterone and does not suppress FSH and LH as much as does injectable testosterone. FSH, LH, testosterone, and estradiol are checked 4 weeks after AndroGel is started to achieve LH and FSH within upper normal limits. As elevated levels of FSH increase expression of aromatase CYP19, moderate suppression of circulating FSH levels by AndroGel may decrease estradiol production and have a positive impact on testicular function in men with KS. An aromatase inhibitor like Arimidex (anastrozole, AstraZeneca, Wilmington, DE) is used in all patients for a minimum of 6 months to decrease intratesticular estradiol levels and increase testosterone production. Aromatase inhibitors have been shown to increase testosterone and improve sperm recovery rates.³⁰ Aromatase inhibitors are generally well tolerated and have been used in teenagers with short stature without any significant side effects.⁴⁴ Elevated estradiol has a negative effect on spermatogonial divisions, but it is unknown if hyperestrogenism is a primary reason for depletion of spermatogonia in men with KS.⁴⁵ We have also shown in the laboratory that suppression of estradiol increases intratesticular testosterone production, thus aromatase inhibitors offer physiological treatment in KS. Prospective studies evaluating the use of aromatase inhibitors in adolescents are under way in our center and preliminary analysis shows preservation of testicular volume, however more data are needed.

Some practitioners use human chorionic gonadotropin (hCG) to stimulate intratesticular testosterone and sperm production. It is possible that increasing intratesticular testosterone may increase the chances of sperm recovery, but because of concern about concomitant increase in estradiol levels, the hCG should be used with aromatase inhibitors.⁴⁶

In patients who are not interested in fertility treatment, the treatment focus is on testosterone replacement therapy, health maintenance, adequate bone health, and decreasing the risk of deep vein thrombosis. Because of space limitations, principles of long-term management of men with KS will be covered in a separate publication. Because sterility is often a main concern of parents and the adolescent patient, our center and other centers have developed a program for the preservation of fertility in boys with chromosomal aberrations using the same principles of practice as we use in children and adolescents who will undergo chemotherapy or radiation treatment.^{47,48}

Sperm cryopreservation in postpubertal adolescents and adults faced with need for chemotherapy is

common in many centers and is becoming a standard of care in medical and radiation oncology despite the fact that not all forms of chemotherapy result in sterility. KS results in sterility in more than 97% of men, and thus every effort should be considered for preservation of fertility in children diagnosed with KS.⁴⁹

Our own experience together with published reports indicate that the loss of spermatogonial cells in men with KS occurs progressively and that most boys with KS are born with spermatogonia that undergo massive apoptosis most likely occurring during early puberty.^{21,24} Thus, it is highly likely that during early puberty, there is a period when spermatogenesis starts to occur and sperm is present in ejaculate. This time frame is an opportunity to obtain ejaculated sperm or sperm from testicular biopsy for cryopreservation. Ejaculated sperm cryopreservation has been an established standard of care in the preservation of fertility in adult men. There is an excellent track record for the fertilization capacity of cryopreserved sperm, and sperm stored for more than 20 years has been successfully used for fertilization. This procedure offers clear benefits to our patients with respect to their biological reproductive options and may have positive impact on the psychological development of an adolescent faced with a diagnosis that for years has been synonymous with sterility. Although it is most likely that the amount of sperm preserved will be insufficient for intrauterine insemination, the number of sperm is more than sufficient for IVF. This prospect of having cryopreserved sperm facilitates the discussion of the impact of KS on fertility in younger men, who in our practice may have an easier time accepting the diagnosis knowing that they are not sterile. Having banked sperm may also affect the interpersonal relationships of adolescents, as they are not labeled sterile anymore. In our practice, most adolescent patients are interested in a preservation program, and all parents were interested in preservation of fertility, although this may represent a bias of our practice. Having sperm available simplifies the IVF procedure itself, avoids general anesthesia, and reduces the cost required to procure sperm in adult men.

There are potential significant regulatory, logistic, and developmental physiology issues faced by the male reproductive specialist offering an adolescent cryopreservation program. First, it is not yet established when the loss of spermatogonia occurs and if all boys undergo adequate spermatogenesis to have sperm in ejaculated semen or in testicular biopsy material. There is no recognized and well-accepted set of markers, which would allow us to decide on the best timing for the cryopreservation. At present in our practice, we check FSH, LH, T, and inhibin-B levels every 6 months starting 2 years prior to predicted start of puberty. Furthermore, morning urine samples from 2 consecutive days are obtained, spun, and evaluated for the presence of

sperm. Physical exam with measurement of testicular volume is performed every 6 months. When FSH and LH start to increase, the discussion is held with the parents, who decide on the best method of obtaining sperm sample. If the patient self-stimulates, the semen sample is obtained in the office and examined after 30 minutes of centrifugation. If sperm is found, the patient is placed on Arimidex for 6 months, and additional semen samples are obtained at a cryopreservation center and preserved, with the goal of storing at least four to six vials of ejaculated sperm. If no sperm is found but the FSH continues to increase, then microsurgical testicular sperm retrieval is offered. The microsurgical biopsy is preferred because it offers the advantage of minimal testicular damage and only a small volume of testis has to be obtained. The sample is examined in the operating room, and the testicular tissue is cryopreserved if spermatogonia are found. This approach offers the best chance for preservation of fertility.

ETHICAL CONCERNS AND CHALLENGES WHEN OBTAINING SPERM VIA MASTURBATION IN 12- to 14-YEAR-OLD PATIENTS

Ethical concerns and challenges arise when obtaining sperm via masturbation from 12- to 14-year-old patients. The management of patients with anejaculation or idiosyncratic masturbation patterns can prove quite challenging. The physician must be able to ascertain if an adequate level of consent has been obtained and disclose the results in an age-appropriate way. The physician must also have the ability to discuss with empathy the plan for cases where no sperm is found. All of the above issues create additional challenges. These concerns are best approached by open-ended and transparent questions about self-stimulation. In our program, which has more than 1100 young (adolescents and individuals in their twenties) patients with male reproductive and sexual problems, we have a trained RN who discusses issues related to adolescent sexual activity with parents or adolescent boys. In addition, we closely collaborate with an experienced clinical social worker.

From a review of literature, the average age of onset of masturbation is 12 in the American Caucasian population,⁵⁰ thus most adolescents will be able to produce a semen sample. We have previously reported on our experience in obtaining semen through masturbation in adolescent patients in Poland—no parent or adolescents had ethical issues producing a sample for semen analysis.⁵¹ Because some adolescents exhibit idiosyncratic masturbatory patterns, and a significant proportion of adolescents have ejaculatory dysfunction—which usually preclude collection of semen through self-stimulation—two approaches can be attempted. It is possible to discuss different techniques of stimulation

(which in our experience is very difficult) or to use vibratory stimulation. Vibratory stimulation using the FDA-approved medical vibrator allowed for delivery of semen in most men. Alternatively, electroejaculation or vibratory stimulation under anesthesia can be attempted. In a study of patient and parent attitudes toward sperm preservation in boys undergoing chemotherapy, 70% were in favor of using masturbation or electrostimulation as a means of obtaining sperm for cryopreservation.⁵²

The advantage of working with KS patients and their families is that one encounters a highly educated and motivated group of patients and parents who realize that testicular dysfunction is one of the cardinal characteristics of the syndrome, and thus most adolescents play an active role in the discussion of fertility and sexual issues. One cannot overestimate the positive impact on adolescents and parents when they learn that their son actually does have sperm in the ejaculate. This approach also gives parents an option of early understanding of potential emotional and financial needs their son will have to face in the future to achieve paternity. One also needs to provide alternative options of management such as deferring the testicular sperm extraction to adulthood knowing that this approach will require fresh sperm because the number of sperm obtained when a biopsy is done in adult men with KS is so low that freezing is not a viable option. Alternatively, the reproductive specialist needs to be prepared to provide continued support and advice in case no sperm is found in ejaculate or testicular biopsy.

The options for patients who have no sperm in the ejaculate depend on the level of FSH and the age of the patient. Cryopreservation of testicular tissue in the 12- or 13-year-old patient who has at least a decade prior to desiring fertility is reasonable. With the current scientific advancement of *in vitro* maturation of spermatogonia, it is realistic to assume that technology allowing maturing sperm will be available. We currently have an ongoing research program devoted to maturing the spermatogonia from boys with KS. Five testicular biopsies performed in a 13-year-old, 14-year-old, and two 15-year-old patients failed to identify germ cells, but all those boys had FSH above 20 and were advanced in pubertal development (Tanner stage III/IV and V). There were no complications of surgical biopsy, which was performed under operative microscope by an experienced microsurgeon who performs more than 250 microsurgical procedures a year. Lack of sperm recovery in those five boys is consistent with data by Wikstrom et al that the loss of germ cell occurs early during puberty.²⁴ Optimal time of testicular biopsy would be a time when spermatogenesis progresses through completion and motile sperm can be retrieved; thus in our initial group, we purposefully restricted biopsy to adolescents who either were not able to ejaculate or had no sperm in the ejaculate. Currently, we use scrotal ultrasound and

magnetic resonance spectroscopy to follow the adolescents and investigate optimal timing of testicular biopsy, and we focus on the younger group of patients.

FUTURE PROSPECTS FOR KS PATIENTS

Many questions remain to be answered before recommendations about optimal treatment and long-term management of KS can be made. Better understanding of molecular mechanisms governing X chromosome inactivation, regulation of meiosis, and timing as well as the pathophysiology of spermatogonial loss should allow for the development of new treatment options in the future. One can envision Leydig cell transplantation as a viable although futuristic method of correcting low testosterone. The preservation of fertility in adolescent boys with KS combined with the development of a successful method of xenografting of testicular tissue will give us powerful tools to better understand how the loss of germ cells occurs and to provide the option of fertility restoration in the future as is done already in children who undergo chemotherapy. New diagnostic and imaging tests may aid us in timing the surgical and hormonal interventions to one day have the ability to prevent spermatogonial loss. Although those are daunting tasks, we cannot forget that just a decade earlier, most of us considered our patients with KS sterile with no hope for paternity.

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