

New concepts in Klinefelter syndrome

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Purpose of review

Klinefelter syndrome, 47,XXY and its variants, is the most common chromosomal aberration among men, with estimated frequency of 1 : 500 among newborns. Men with Klinefelter syndrome present with sequels of hormonal and spermatogenic testicular failure like infertility, low testosterone, erectile dysfunction, and low bone mineral density. This review is aimed to provide the practicing urologist with an important source of clinically relevant information about Klinefelter syndrome.

Recent findings

Sperm can be found in over 50% of men with Klinefelter syndrome, thus men with Klinefelter syndrome are not sterile. Recent evidence suggests that children with Klinefelter syndrome are born with spermatogonia and lose large numbers of germ cells during puberty. Early diagnosis and treatment can improve the quality of life and the overall health of men with Klinefelter syndrome.

Summary

Growing interest in Klinefelter syndrome among translational scientists and clinicians will result in better understanding of the pathophysiology of testicular failure. In some states, screening programs for Klinefelter syndrome are already in place, which will increase the number of patients with Klinefelter syndrome seen by practicing urologists in the near future. Diagnosis and management of patients with Klinefelter syndrome is within the scope and training of urologists. Development of randomized clinical trials comparing different forms of interventions in men and children with Klinefelter syndrome will allow us to standardize the care of these patients.

Keywords

hypogonadism, Klinefelter syndrome, meiosis, spermatogenesis

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Introduction

Klinefelter syndrome, despite its high prevalence and plethora of urological symptoms, has drawn marginal attention among urologists. However, over the last 10 years, with advancements in artificial reproductive techniques and the successful delivery of healthy children from men with Klinefelter syndrome, the involvement of urologists in the care of patients with Klinefelter syndrome is increasingly important. In the past, Klinefelter syndrome was managed mostly by endocrinologists; however, successful sperm recovery from men with Klinefelter syndrome indicating that adolescents with Klinefelter syndrome have spermatogonia has stimulated growing interest in Klinefelter syndrome [1,2].

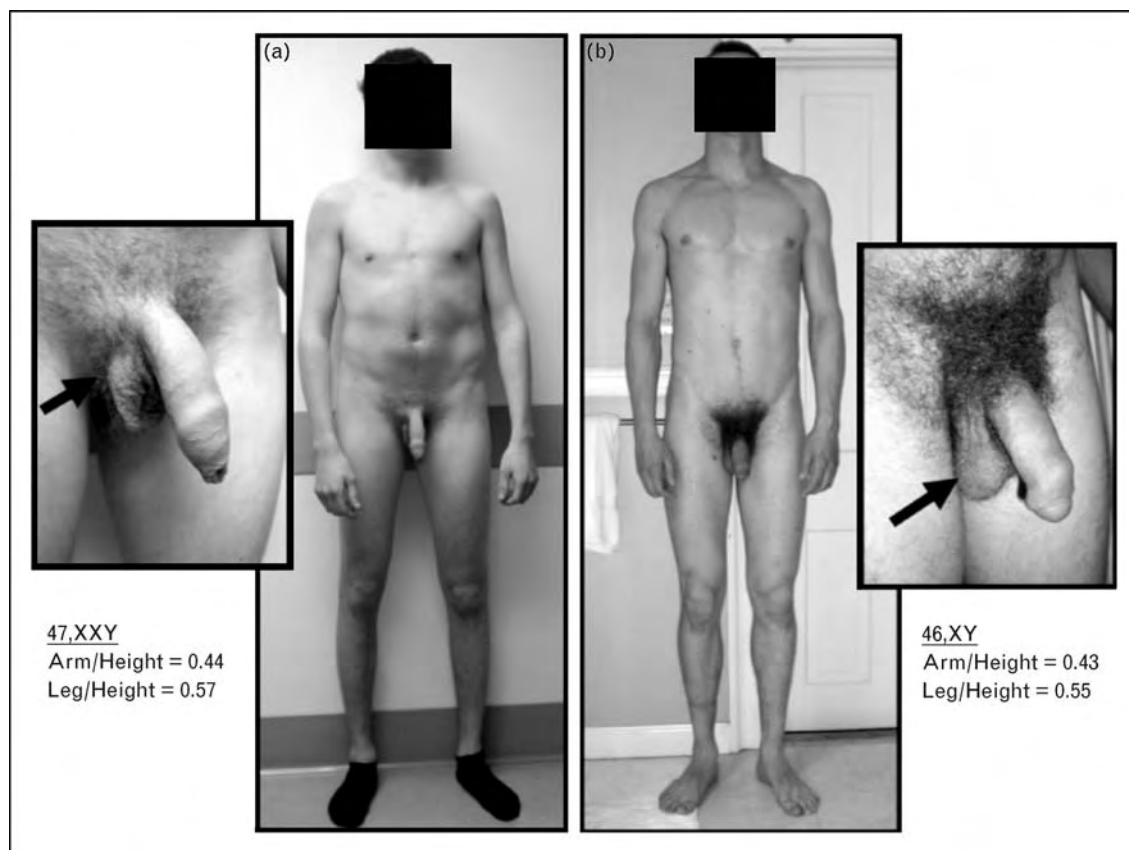
Urologists are in a unique position to address long-term care of patients with Klinefelter syndrome in respect to their reproductive and sexual function. Thus, we feel it is important to provide practicing practitioners as well as

translational scientists with an update on pathophysiology and management of Klinefelter syndrome. This study focuses on new developments in reproductive biology and medicine in men with Klinefelter syndrome.

Epidemiology

Klinefelter syndrome is the most common numerical chromosomal aberration among men, with an estimated frequency of 1 : 500–1 : 1000 of live deliveries [3]. Klinefelter syndrome is characterized by X chromosome polysomy with X disomy being the most common variant (47,XXY). Ninety percent of men with Klinefelter syndrome have nonmosaic X chromosome polysomy [3,4].

Although the classic description of men with Klinefelter syndrome 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

Figure 1 The classic descriptions of men with Klinefelter syndrome 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 their peers. The only obvious difference that is seen in all men with Klinefelter syndrome is clearly visible difference in testicular size between men with Klinefelter syndrome (very small testes) and men with 46,XY karyotype (normal size). The photograph shows two 21-year-old men seen in our practice. The 46,XY had history of constitutionally delayed puberty. Both of them required testosterone treatment early during puberty. The patient with Klinefelter syndrome continues testosterone replacement therapy. Both of them are top of the class college students.

description is not accurate and men with Klinefelter syndrome represent a broad spectrum of phenotypes, professions, incomes and socioeconomic status [3]. Severe intellectual deficits are rare. Often, the auditory processing delay and language dysfunction seen in men with Klinefelter syndrome are misdiagnosed as cognitive deficits [5]. Most commonly, men with Klinefelter syndrome will present to their urologist with infertility: azoospermia or severe oligospermia, low testosterone and complications of low testosterone such as erectile dysfunction and poor libido. Boys will present with concerns about genital and pubertal development [3]. Spermatogenic and steroidogenic dysfunction are cardinal and the most prevalent signs of Klinefelter syndrome. Harder testes, with volume less than 10 ml, in older adolescent and younger men should always be evaluated further regardless of penile size, body proportions, or level of androgenization (Fig. 1). Men with more than two X chromosomes (48,XXXY; 49,XXXXY) are more affected than men with the classic 47,XXY karyotype [5].

Pathophysiology, epidemiology and mechanisms of spermatogenic failure

The 47,XXY karyotype of Klinefelter syndrome arises spontaneously when paired X chromosomes fail to separate – nondisjunction in stage I or II of meiosis, during oogenesis or spermatogenesis [6]. Less than 3% of X chromosome polysomy occurs during early divisions of the fertilized egg. However, postfertilization nondisjunction is responsible for mosaicism, which is seen in approximately 10% of Klinefelter syndrome patients. Men with mosaicism are less affected and often are not diagnosed. Advanced maternal age and possibly paternal age have been linked to increased risk of Klinefelter syndrome [7].

The X chromosome carries genes that play roles in many body systems including testis function, brain development and growth [8]. Men with Klinefelter syndrome are usually infertile because of primary testicular failure. A

typical patient with Klinefelter syndrome will present with low serum testosterone, high LH and FSH level, and often elevated estradiol; however, the decline in testosterone production is progressive over the life span and not all men suffer from hypogonadism [9**].

Men with Klinefelter syndrome are at a higher risk of autoimmune diseases; diabetes mellitus, leg ulcers, osteopenia and osteoporosis, tumors (breast and germ cells), and historically have increased mortality [10*,11]. It is unknown if the morbidity associated with Klinefelter syndrome is a result of hypogonadism and hyperestrogenism or rather due to abnormal function of X chromosome-linked genes.

It is well accepted that the X chromosome bears over 1100 genes that are critical for the normal function of the testis and brain [12]. Inactivation of additional X chromosome is initiated within the X chromosome inactivation center (XIC) by activation of the XIST promoter. Many genes on the X chromosome are highly expressed in the testis, ovaries and brain; thus, it is not surprising that these organs are affected by X chromosome polysomy [13–18]. Understanding the molecular mechanisms of X chromosome inactivation has important clinical applications, because at this point, we do not have a clinical or molecular test that can predict an extent of reproductive or cognitive failure in an individual patient [19].

In the last decade, developments in microsurgical techniques and advances in artificial reproductive technologies (ART) allowed over 50% of patients with Klinefelter syndrome to have their own children through the combination of microsurgical testicular sperm extraction (TESE) and use of freshly retrieved sperm for in-vitro fertilization (IVF) [20,21]. The fact that sperm can be found in the testes of men with Klinefelter syndrome has challenged the previous assumption that men with Klinefelter syndrome are always sterile. This has raised the mechanistic question whether children with Klinefelter syndrome are born with a severely depleted number of spermatogonia or whether there is a period in life when the spermatogonia undergo massive apoptosis that results in depletion of the spermatogonial population and subsequent azoospermia [22].

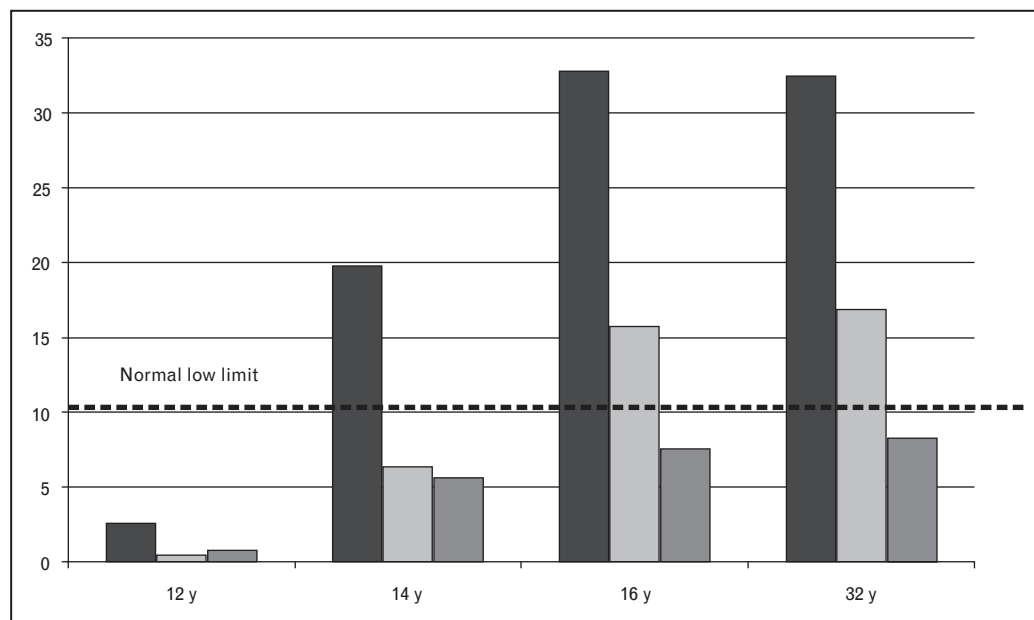
Based on the current data, it is reasonable to assume that most men with Klinefelter syndrome are born with spermatogonia [23–25]. However, during early puberty – most likely after initiation of the first wave of spermatogenesis – the spermatogonia appear to undergo massive apoptosis ([25], Fig. 4). This hypothesis is based primarily on three observations: testicular sperm can be identified and recovered from at least half of adult men with Klinefelter syndrome; in rare cases, sperm can be found in ejaculates of men with Klinefelter syndrome; and data

from boys with Klinefelter syndrome who were biopsied at different ages and development stages indicated that boys with Klinefelter syndrome have spermatogonia at birth but that damage to the germinal epithelium occurs early during puberty [22,25]. Three potential mechanisms have been suggested to explain spermatogenic failure in Klinefelter syndrome: intratesticular hormonal imbalance with hypersensitivity to increasing intratesticular testosterone and estradiol concentrations, Sertoli cell dysfunction, and defects in spermatogonial stem cell renewal. A less likely although possible explanation of spermatogenic failure would be a loss of spermatocytes during meiosis as a result of abnormal pairing of X and Y chromosomes.

Low testosterone and elevated estradiol levels are cardinal symptoms of Klinefelter syndrome. In most men, LH and FSH elevation starts early during puberty [22,25,26*,27] (Fig. 2). Based on our experience, most boys with Klinefelter syndrome have abnormally elevated FSH and LH at Tanner stage III. The most likely causes of hypogonadism seen in men with Klinefelter syndrome are aberrant expression of steroidogenic enzymes and/or negative effects of elevated intratesticular estradiol levels. Hyperestrogenism is commonly seen in Klinefelter syndrome, with increased estrogen-to-testosterone ratios and delayed increase in testosterone levels during puberty being responsible for the characteristic body proportions and gynecomastia [28]. Treatment with aromatase inhibitors can lower intratesticular estradiol levels and have beneficial effect on testosterone production and spermatogenesis in men with Klinefelter syndrome [29].

Sperm found in testes of men with Klinefelter syndrome have only a slightly increased frequency of sex chromosome polysomies, and most boys born from fathers with Klinefelter syndrome have a normal karyotype [20,30,31]. These findings indicate that during early stem-cell proliferation or meiotic division, the checkpoint mechanisms are able to overcome X chromosome polysomy resulting in sperm with a single X (or Y) chromosome [24]. Data by Bergere *et al.* [32] and Yamamoto *et al.* [24] suggest that the most likely explanation for normal haploid sperm in men with Klinefelter syndrome is the presence of a repair mechanism that, during spermatogonial renewal, allows for the loss of the additional X chromosome. Alternatively, rare errors in mitotic proliferation of spermatogonia in which the ‘error’ actually results in a normal karyotype could explain the development of normal sperm.

Klinefelter syndrome can be diagnosed prenatally by amniocentesis and in the postnatal period by karyotyping, fluorescence in-situ hybridization (FISH) and molecular techniques. Karyotyping is a gold standard in Klinefelter syndrome diagnosis; however, the test is

Figure 2 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. Thick black line represents 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 the 2-year window at early puberty and by 16 years of age most boys have similar levels of FSH as adult men who presented with infertility. (■) FSH; (□) LH; (▒) T (nmol/l).

expensive, labor intensive and has relatively low sensitivity for 47,XXY/46,XY mosaicism.

Laboratory and auxiliary evaluation

All men with Klinefelter syndrome should have a full hormonal evaluation including FSH, LH, testosterone, estradiol, prolactin and IGF-1. Cortisol levels should be routinely measured, because there is growing evidence that adrenal steroidogenic deficiency may be seen in 47% of men with Klinefelter syndrome [33]. Because decreased testosterone significantly increases the risk of osteopenia and osteoporosis, bone density is routinely performed to diagnose these conditions. Men with Klinefelter syndrome have an increased risk of deep vein thrombosis, and in 85 adult men we have seen over the last 3 years, three had pulmonary embolism and deep vein thrombosis. At this point, it is unclear whether screening for mutations leading to hypercoagulability is indicated in all men with Klinefelter syndrome. Regardless, all patients with Klinefelter syndrome should be informed about the increased risk of deep vein thrombosis and have their hematocrit checked to avoid increased viscosity [34].

Patients with Klinefelter syndrome have an increased risk of extratesticular germ cell tumors and possibly increased risk of breast cancer [35].

The physiological approach to the management of men with Klinefelter syndrome

Management of men with Klinefelter syndrome is challenging because one's reproductive goals have to be included in optimal medical treatment. Most men present with infertility and seek therapeutic interventions, specifically microsurgical TESE with IVF. Treatment options in adolescents and adults differ, and especially in younger adolescents, fertility preservation should be discussed with parents. Currently, fertility preservation in adolescents should be reserved to large academic centers, as each team needs to solve complex ethical, legal and logistics issues that arise when a child with a genetic defect is subjected to a surgical procedure. The benefits of sperm retrieval, although very likely, are not certain at this point. Because recovery of sperm through TESE combined with intracytoplasmic sperm injection (ICSI) has led to successful live births of children, it is critical that reproductive endocrinologists and reproductive urologists are familiar with the current literature and success rates. The best success rate of IVF in Klinefelter syndrome seems to be obtained through the use of fresh sperm through testicular biopsy performed the same day as egg retrieval. In the largest study reported so far, Schiff *et al.* [20] reported a retrieval rate of 69% (29/42), much higher than the 42% (5/12) published by Friedler *et al.* [36]. In addition, the fertilization

rate was higher: 85% using fresh sperm in the study by Schiff *et al.* compared with 58% using cryopreserved sperm. Although no statistical difference between fresh and cryopreserved sperm has been reported, there was a trend toward a lower rate of fertilization and implantation in the study by Friedler *et al.* Even with conservative estimates, at least 50% of adult men with Klinefelter syndrome will yield viable sperm that can be used successfully for IVF. This represents a remarkable success in reproductive medicine. So far, the follow-up of boys born from fathers with Klinefelter syndrome has not shown any phenotypic abnormalities or increased risk of Klinefelter syndrome [20,37**]. Optimal timing of sperm retrieval as well as optimal hormonal treatment prior to sperm retrieval has not been established so far. Injectable testosterone may lower sperm recovery rate; however, this may simply reflect that often men with Klinefelter syndrome who had to be treated with testosterone injections early may have more severe testicular failure with delayed puberty and poor development during puberty. Much more data are needed to establish optimal hormonal treatment of men with Klinefelter syndrome. In our practice, we cease injectable testosterone in men with Klinefelter syndrome 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, most commonly AndroGel (Solvay, Marietta, Georgia, USA), which achieves physiological levels of testosterone and does not suppress FSH and LH as much as injectable testosterone does. An aromatase inhibitor like Arimidex (AstraZeneca, Wilmington, Detroit, USA) 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 [29]. Some practitioners use 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 [38].

In patients who are not interested in fertility treatment, the focus is on testosterone replacement therapy, health maintenance, adequate bone health and decreasing the risk of deep vein thrombosis.

Because sterility is often a main concern of parents and the adolescent patient, several centers including our own have developed programs for the preservation of fertility in boys with chromosomal aberrations using similar principals of practice that are used for children and adolescents who will undergo chemotherapy or radiation treatment [39,40].

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

in many oncological centers and is becoming a standard of care in adolescents and young adults undergoing chemotherapy or radiation despite the fact that not all forms of chemotherapy result in sterility. Klinefelter syndrome results in infertility in over 97% of men and thus every effort should be considered for preservation of fertility in children diagnosed with Klinefelter syndrome [41].

Our own experience, together with published reports, indicate that the loss of spermatogonial cells in men with Klinefelter syndrome occurs progressively and that most boys with Klinefelter syndrome are born with spermatogonia, which undergo massive apoptosis most likely during early puberty [22,25]. It is highly likely that during early puberty there is a period during the development of the adolescent when spermatogenesis starts to occur and sperm is present in the ejaculate. Preservation of ejaculated sperm not only offers clear benefits to our patients in respect to their biological reproductive options but also might have important positive impact on the psychological development of an adolescent faced with a diagnosis that for years has been synonymous with sterility. This prospect of storing the sperm facilitates the discussion of the impact of fertility in young men with Klinefelter syndrome, who in our practice seem to have an easier time accepting the diagnosis knowing that they are not sterile. Having sperm available simplifies the IVF procedure itself, avoids general anesthesia and reduces the cost to procure associated with procurement of sperm in adult men (typically done by TESE).

There are significant regulatory, logistic and developmental physiology issues faced by the male reproductive specialist offering gamete preservation. For example, 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 that would allow us to decide on the best timing for the cryopreservation. If no sperm are found in ejaculate 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 small volume of testis to be obtained. The sample is examined in the operating room and the testicular tissue is cryopreserved if spermatogonia are found. This approach allows for the highest chance for preservation of fertility.

Several new technologies such as testis xenografting and spermatogonial stem cell transplantation are being investigated to work around the current lack of an in-vitro culture system that would support full spermatogenesis. We currently have an ongoing research program devoted to maturing spermatogonia from boys with Klinefelter syndrome. Optimal time of testicular biopsy would be at 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 are using scrotal ultrasound and magnetic resonance spectroscopy to follow adolescents to determine the optimal timing for testicular biopsy.

Conclusion

Many questions remain to be answered before recommendations about optimal treatment and long-term management of Klinefelter syndrome can be made. Better understanding of molecular mechanisms governing X chromosome inactivation, regulation of meiosis and timing as well as the pathophysiology of loss of spermatogonia 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 Klinefelter syndrome 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, possibly providing 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 these are daunting challenges, we cannot forget that just a decade earlier most of us considered our patients with Klinefelter syndrome sterile with no hope for paternity.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 635).

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