Best Practice & Research Clinical Endocrinology & Metabolism 25 (2011) 239–250

Contents lists available at ScienceDirect

Best Practice & Research Clinical Endocrinology & Metabolism

journal homepage: www.elsevier.com/locate/beem

2

# Klinefelter syndrome

Anne M. Wikström, Chief Physician [a,b,e],
Leo Dunkel, Professor of Pediatrics [c,d,*]

[a] HUCH, Hospital for Children and Adolescents, Helsinki University Central Hospital, P.O. Box 281, FI-00029 Helsinki, Finland
[b] University of Helsinki, Helsinki, Finland
[c] Department of Pediatrics, Kuopio University Hospital, P.O. Box 1777, FI-70211 Kuopio, Finland
[d] University of Eastern Finland, Kuopio, Finland

*Keywords:*
Klinefelter syndrome
47,XXY
testicular degeneration
hypogonadism
androgen
androgen deficiency
infertility

Klinefelter syndrome (KS) is the most common genetic form of male hypogonadism, but overt phenotype becomes evident only after puberty. During childhood, and even during early puberty, pituitary-gonadal function in 47,XXY subjects is relatively normal, but from midpuberty onwards, FSH and LH levels increase to hypergonadotropic levels, inhibin B decreases to undetectable levels, and testosterone levels after some increase plateau at low-normal levels for healthy adult men. Hence, most adult KS males display a clear hypergonadotropism with a varying degree of androgen deficiency; subsequently testosterone substitution therapy is widely used to prevent symptoms and sequels of androgen deficiency. Testicular biopsies of prepubertal KS boys have shown preservation of seminiferous tubules with reduced numbers of germ cells, but Sertoli and Leydig cells have appeared normal. The testes in the adult KS male are characterized by extensive fibrosis and hyalinization of the seminiferous tubules, and hyperplasia of the interstitium. However, the tubules may show residual foci of spermatogenesis. Introduction of testicular sperm extraction (TESE) in combination with intracytoplasmic sperm injection (ICSI) techniques has allowed non-mosaic KS males to father children.



* Corresponding author. Department of Pediatrics, Kuopio University Hospital, P.O. Box 1777, FI-70211 Kuopio, Finland. Tel.: +358 17 173311; fax: +358 17 172410.
*E-mail addresses:* anne.wikstrom@hus.fi (A.M. Wikström), leo.dunkel@kuh.fi (L. Dunkel).
[e] Tel.: +358 50 4272854; fax: +358 9 47175888.

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doi:10.1016/j.beem.2010.09.006

## Introduction

Klinefelter syndrome (KS) was first described by Harry F. Klinefelter in 1942 as a clinical entity characterized by gynecomastia, small testes, absent spermatogenesis, normal to moderately reduced Leydig cell function, and increased secretion of FSH.[1] The disorder in 1959 was found to be caused by a supernumerary X chromosome.[2] Today, studies indicate that some 80% of KS males have the karyotype 47,XXY and 20%, higher-grade chromosome aneuploidies, 46,XY/47,XXY mosaicism, or structurally abnormal X chromosomes.[3] With an estimated prevalence of about 1 in 600 newborn males, KS is the most common sex-chromosome abnormality.[4] It is among the most frequent genetic causes of human infertility, occurring in 11% of azoospermic men and 4% of infertile men.[5] The classical phenotype of KS is widely recognized, but many affected subjects present only with very discrete symptoms (Table 1). Consequently, the disorder is underdiagnosed; only approximately one-fourth of adult males with KS receive diagnoses, and fewer than 10% of the expected number are diagnosed before puberty.[4,6]

Many of the clinical findings in KS may be attributed to the hypogonadism typical for this syndrome, but some are instead caused directly by the chromosome abnormality. KS is diagnosed prenatally by routine amnioscentesis quite rarely, because the association with advanced maternal age is weak[3,7], and at birth most 47,XXY neonates appear normal.[8,9] During childhood, the KS boy often presents with speech development delay, learning disabilities or behavioral problems.[6,10] Consequently, child neurologists or child psychiatrists, who perform chromosome analysis, along with fragile X screening, often make the diagnosis. The tall stature typical of KS results from a notable increase in height velocity between ages 5 and 8 years owing to a greater leg-growth, but otherwise identifying any differences between KS boys and normal boys in physical appearance is very difficult.[11] Furthermore, neither magnitude nor timing of the pubertal growth spurt differs from that of normal boys.[11–13] Only after puberty do small, firm testes and variable symptoms of androgen deficiency characterize the KS males most often detected among patients with azoospermia visiting infertility clinics.[6]

## Function of hypothalamic-pituitary-testicular axis during development

### *Fetal and neonatal periods*

When prenatal testosterone was investigated in amniotic fluid obtained at antenatal diagnoses between 16 and 20 weeks of gestation from 20,XXY fetuses and from XY and XX controls of the same age[14], no significant difference was evident between the two male groups; both had significantly higher levels than the XX fetuses.

At birth there already may be some impairment of Leydig cell function. Cord-blood testosterone was significantly lower in two 47,XXY infants and in one 46,XY/XXY than in three control infants.[15]

**Table 1**
Clinical features (%) of adult patients with Klinefelter syndrome.[3,38,81]

| | |
|---|---|
| Small testes (<4–6 mL) | >95 |
| Infertility | >99 |
| Azoospermia | >95 |
| Decreased facial hair | 60–80 |
| Decreased pubic hair | 30–60 |
| Abdominal adiposity | 50 |
| Gynecomastia | 38–75 |
| Varicose veins | 40 |
| Decreased libido and potency | 70 |
| Decreased muscle strength | 70 |
| The metabolic syndrome | 46 |
| Type 2 diabetes | 10–39 |
| Osteopenia and osteoporosis | 40 + 10 |
| Mitral valve prolapse | ≤55 |

However, in another study comparing testosterone levels of six KS infants to levels in a large cohort of normal infants, no significant difference appeared.[8] Lahlou et al. compared reproductive hormone levels during the postnatal hormonal activation of the reproductive axis (i.e. minipuberty) in 18 prenatally diagnosed 47,XXY boys to those in 215 healthy boys.[16] The KS infants' timing of peak serum testosterone was similar to healthy infants', but levels in the KS boys, were significantly lower from birth to 8 months. However, their serum LH, FSH, inhibin B and anti-Müllerian hormone (AMH) levels were normal. Another study found, in 11 of 12 KS boys under age 6 months, lower than normal serum testosterone levels but normal gonadotropin levels.[9] In contrast, Aksglaede et al. found high normal concentrations of testosterone and elevated levels LH and FSH in 10 KS infants aged 3.1 months when compared to healthy controls.[17] Hence, no indisputable hypoandrogenism appears during infancy in KS subjects.

*Childhood and adolescence*

Prepubertal 47,XXY boys are characterized by normal serum levels of testosterone, FSH, LH, and inhibin B until onset of puberty[12,18–23], and their serum testosterone responses to human chorionic gonadotropin (hCG) stimulation are normal.[21,22] During puberty, after an initial normal adolescent increase, serum testosterone concentrations plateau and remain subsequently within the low-normal range throughout puberty.[18,20–22,24] Such testosterone levels seem sufficient to allow in KS boys normal onset and progression of puberty (Fig. 1) and development of satisfactory secondary sexual characteristics.[11,18,21,22]

Insulin-like factor 3 (INSL3) is a peptide hormone secreted in an LH-dependent manner by fetal and fully differentiated Leydig cells.[25–27] It is only weakly expressed in immature prepubertal Leydig cells and in Leydig cells that have become hypertrophic or transformed.[27] Hence, INSL3 is suggested to be more sensitive than testosterone to Leydig cell dysfunction and differentiation status. In healthy boys, puberty is associated with a marked increase in INSL3 levels that occurs concomitantly with significant increases in LH levels (Fig. 2).[20,28] In KS boys, no significant difference in comparison with healthy boys in INSL3 levels emerges in assessment by bone age or Tanner pubertal stages, but from midpuberty onwards, despite stimulation by high LH levels, a leveling-off in INSL3 concentrations occurs (Fig. 2).

Serum estradiol (E2) levels are already high in early pubertal 47,XXY boys and remain high, irrespective of the presence or absence of gynecomastia.[12,18,21] A tendency for higher E2/testosterone ratios also occur in pubertal KS boys, but their serum SHBG levels decrease normally.[18]

Serum levels of inhibin B are considered to reflect Sertoli cell function during prepuberty and to become germ cell-dependent during midpuberty.[29] Onset of male puberty is normally associated with increasing serum concentration of inhibin B, and already by pubertal stage 2, is the adult serum level of serum inhibin B reached.[30,31] In patients with KS, inhibin B similarly showed progressive increase before the clinical onset of puberty, but this initial rise is followed by a rapid suppression accompanied by a simultaneous increase in serum testosterone.[19,23] Thus, a strong, inverse non-linear correlation appear in KS boys between serum inhibin B and testosterone levels.[19] In healthy subjects, serum concentrations of AMH, another Sertoli cell marker, remain high throughout childhood and wane during normal male puberty concomitantly with rising testosterone levels and onset of meiosis in spermatogenesis.[32–34] Despite their lack of active spermatogenesis, in KS subjects this decrease in AMH levels also occurs.[16,19] From midpuberty (at about age 13) onwards, KS subjects show a gradual increase in FSH and LH concentrations to hypergonadotropic levels; FSH levels increasing somewhat earlier and more markedly than do LH levels.[18,19,21,22,35] At the same time, the responses of both FSH and LH to gonadotropin-releasing hormone (GnRH) stimulation become exaggerated.[18,21,22,36,37] These observations coincide with decreasing inhibin B and AMH levels, and leveling-offs in testosterone and INSL3 levels, and thus indicate a diminished testicular inhibition of gonadotropin secretion.

*Adulthood*

Adult KS patients are characterized by hypergonadotropic hypogonadism. Concentrations of LH and FSH are high; FSH is usually higher, and little overlap occurs with normal individuals.[3,38] In 65–85% of adult KS patients, serum testosterone concentrations are below normal, but some may show levels

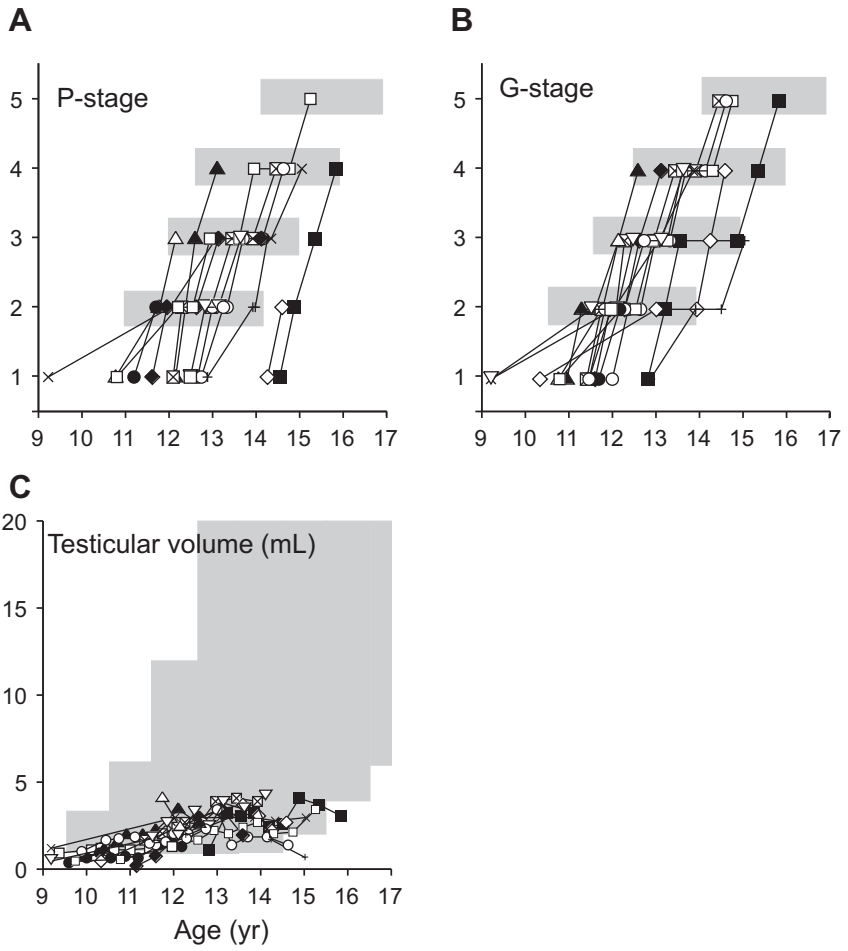


**Fig. 1.** Pubic hair (A) and genital stage (B) development according to Tanner, and testicular volumes (C) by age in boys with Klinefelter syndrome, for details see.[18] Shaded rectangles = mean age ± 2 SD for healthy Finnish boys.[82] Gray area = range of the volume of the right testis in healthy pubertal Swiss boys.[83]

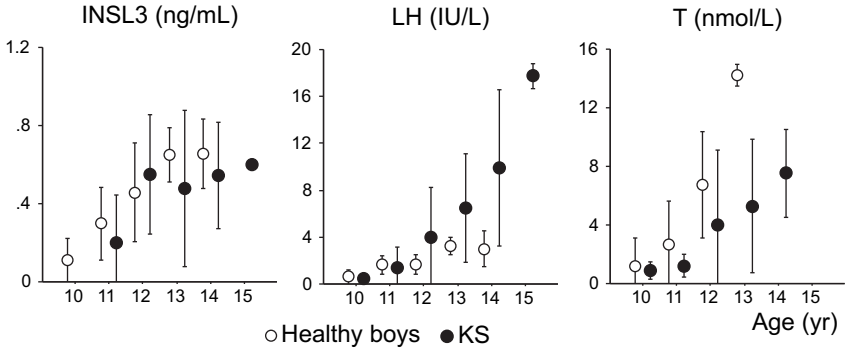


**Fig. 2.** Mean (±SD) plasma insulin-like factor 3 (INSL3), LH, and testosterone (T) concentrations by age in boys with Klinefelter syndrome compared to healthy boys, for details see Ref. [20].

within the normal range.[3,38] On average, serum concentrations of E2 and SHBG are higher than normal.[3] Serum inhibin B levels in most adult KS subjects are undetectable[23,39,40], and adult KS patients have serum INSL3 concentrations significantly below normal.[25,26]

## Morphological degeneration of the testis

### *Fetal and neonatal periods*

The degenerative process may start even during fetal life, as studies of aborted fetuses at gestational ages 18–22 weeks have shown.[41,42] A reduced number of germ cells and an increased proportion of tubules devoid of germ cells are visible in the testicular biopsies of midterm 47,XXY fetuses, whereas the density and number of seminiferous tubules and mesenchymal structures appear normal.[41] Two case reports presented normal testicular histology in 47,XXY fetuses aborted at 17 and 20 weeks.[43,44]

Mikamo et al.[45] showed, over the first year of life, a progressive diminution in the number of spermatogonia from 24 to 0.1% of control value. The number and appearance of immature Sertoli cells appeared normal, as did interstitial tissue. In one 13-day-old 47,XXY infant, germ cells appeared in only 23% of seminiferous tubules, and the number of spermatogonia was reduced.[46] Numerous germ cells and immature Sertoli cells, and Leydig cells that appeared normal were evident in a testicular biopsy of a 4-week-old KS infant undergoing surgery for inguinal hernia[8], but a quantitative assay indicated a reduced number of spermatogonia. The one-month-old 47,XXY infant in the group of KS boys studied by Müller et al. showed a normal germ cell count in his biopsy despite bilateral undescended testes.[47]

### *Childhood and adolescence*

Ferguson-Smith, reporting in 1959 on eight mentally retarded prepubertal chromatin-positive KS boys, aged 7–12[48], noted reduced size of the seminiferous tubules and a reduced number or complete absence of spermatogonia. A minority of the tubules was normal, containing a normal amount of spermatogonia; the majority was smaller tubules with undifferentiated Sertoli cells.[48] Müller et al., studying testicular biopsies of 11 KS boys between the neonatal period and 13 years of age[47], found no germ cells in nine KS boys older than two years. It should, however, be noted in that study all boys were cryptorchid[47], a condition which also reduces germ cell number.[49]

Histomorphometric and immunohistochemical analyses reveal that in early adolescence the majority of boys with KS have germ cells in their testes.[19,50] The number of spermatogonia, especially adult dark spermatogonia is, however, markedly reduced, and the depletion of these cells accelerated with the activation of the pituitary-gonadal axis at the onset of puberty (Fig. 3).[19,50] In prepubertal KS boys, the focal nature of the degeneration process is already evident, since the few seminiferous tubules containing spermatogonia are surrounded by Sertoli cell only (SCO) tubules (Fig. 3D). Germ cell differentiation is not delayed in KS boys; gonocytes mature into spermatogonia without significant delay, but a careful review of serial sections revealed no pachytene spermatocytes.[50] This indicates that in KS, germ cell differentiation is – at least partially – arrested at the spermatogonium or early primary spermatocyte stage. It seems that in KS, spermatogonia have difficulty entering meiosis; instead they proceed at onset of puberty to apoptosis.

In KS immature Sertoli cells are incapable during puberty of transforming into the adult mature cell type.[19] Immunoexpression of inhibin α-subunit indicates degeneration of the Sertoli cells, as also seen in electron microscopy of biopsies.[19,50] Inhibin B synthesis is obviously altered in subjects with KS, since both subunits are expressed in the Sertoli cells, even when serum inhibin B is unmeasurable.[50] With age, fibrosis and hyalinization of the interstitium and peritubular connective tissue increases, and already in 12- to 14-year-old KS boys huge hyperplastic Leydig cells can be visible in testicular biopsies.[19]

In normal males, androgen receptor (AR) expression first appears in Sertoli cell nuclei just before the onset of puberty but before final maturation of the Sertoli cells, concomitant with rising concentrations of FSH and testosterone.[51] In the absence of androgens, AR is located in the cytoplasm.[52] KS boys have, in contrast to age-matched controls, constant AR expression in their Sertoli cell cytoplasm.[50]

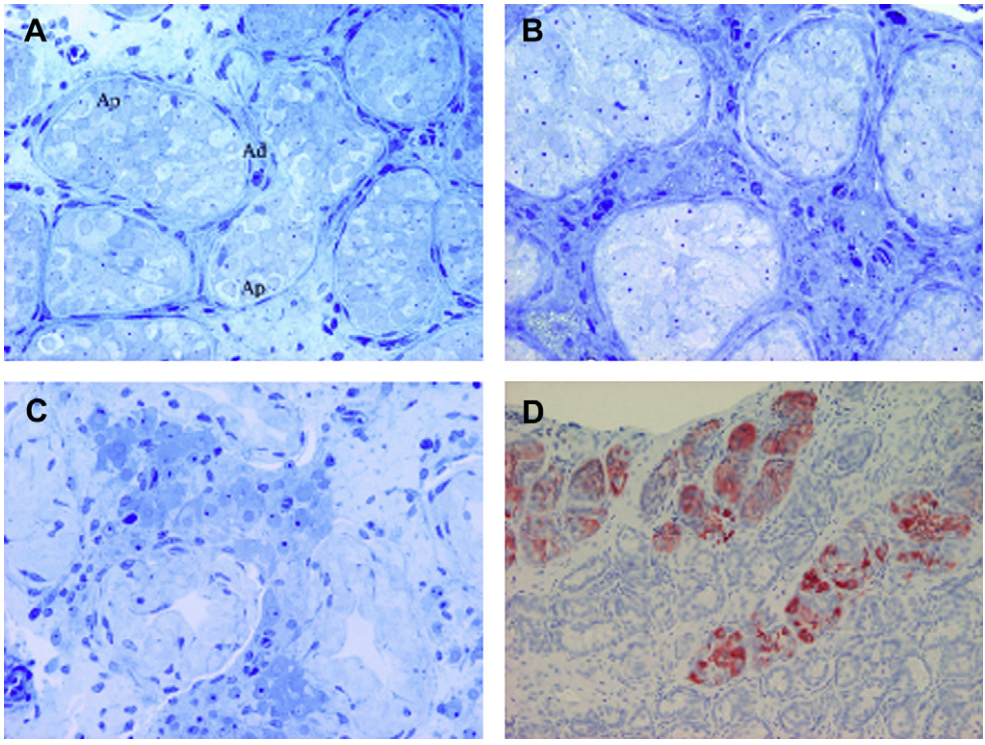


**Fig. 3.** Testicular biopsies of adolescent boys with Klinefelter syndrome displaying the progression of testicular degeneration during puberty. (A) KS boy, age 10.7 years, with spermatogonia; (B) 13.7-year-old with no spermatogonia, and (C) 14-year-old with extensive degeneration. The focal nature of the degeneration is obvious in (D) seminiferous tubules with spermatogonia stained with MAGE-A4 surrounded by Sertoli cell only tubules in a 10-year-old patient. Ap; pale adult spermatogonia; Ad; dark adult spermatogonia. See Refs. [19,50] for details.

Furthermore, they have a smaller proportion of Sertoli cell nuclei expressing AR than do controls.[50] Older KS boys display a strong cytoplasmic AR staining in Leydig cells, which may be a sign of impaired function of hypertrophied Leydig cells, as also suggested by high serum LH levels and low testosterone and INSL3 levels.[18–20,50]

In summary, these results characterizing the testicular degeneration process in the testes of adolescent KS boys confirm that this process accelerates at the onset of puberty.

### *Adulthood*

Histology of the testes in the adult KS patient is characterized by extensive fibrosis and hyalinization of the seminiferous tubules, absence of spermatogenesis, and hyperplasia of Leydig cells and interstitium.[1,53] The patchy nature of the testicular histology, with more – and less-affected areas, has been described.[54,55] The seminiferous tubules can be divided into two types according to Sertoli cell morphology, the first containing small immature Sertoli cells and the second type larger and more differentiated ones.[56] Later studies of the cytological features of these immature Sertoli cells have suggested a lower activity than that of mature Sertoli cells, probably resulting in impaired protein synthesis.[57] Regadera et al. showed in their immunohistochemical and quantitative study that 78.9 $\pm$ 9.1% of the Leydig cells were normal in adult KS males compared to 96.0 $\pm$ 10.0% in control men, and in KS the functional activity of the Leydig cells was reduced.[58]

## Genetic mechanisms of gonadal failure

Apart from normal inter-individual genetic variation, several genetic mechanisms may explain the clinical features and variability of the phenotype in KS. In principal, gene-dosage effects and the parental origin of the supernumerary X chromosome in conjunction with (possibly skewed) X-chromosome inactivation, may play significant roles.

In females, one of the X chromosomes is inactivated to achieve dosage-compensation, and probably likewise in KS. Genes from the pseudoautosomal regions (PARs) and an additional 15% of other genes, however, escape X inactivation and are putatively contributing to the KS phenotype. Therefore, the KS phenotype may reflect increased gene dosage originating either from two active copies of these strictly X linked genes or from three active copies of the X–Y homologous genes of the PAR. For instance, in KS longleggedness is already evident from early childhood despite normal circulating concentrations of IGF-I and IGFBP-3, suggesting that hypogonadism in puberty and young adulthood cannot solely explain tall stature and long extremities. Excessive expression of growth-related genes (e.g. the triplicate of the SHOX gene of the PAR of X chromosome) is one explanation put forward.

The supernumerary X chromosome is paternal in 40–60% and maternal in 40–60% of KS cases.[3,7,59] KS boys with additional paternal X chromosome seem to have later onset and slower progression of puberty.[60] However, some studies have suggested that parental origin of the extra X chromosome has no evident effect on the phenotypes of KS males.[61–63] Data from several small patient series suggest that X-chromosome isodisomy/heterodisomy and X-chromosome inactivation pattern have no impact on the phenotype[60,62,63], although these issues require detailed studies in larger patient series.

The *AR* gene on the X chromosome may play a particular role in differences in the KS phenotype. The N-terminal domain of exon 1 of the *AR* gene contains a highly polymorphic CAG repeat, the length of which is inversely associated with activity of the receptor.[64] A positive correlation exists with body height and presence of gynecomastia, but an inverse association with bone density, social status, testicular volume, and even with response to androgen substitution.[65] In another study, however, the only parameter associated with CAG repeat length was penile length; the correlation was inverse.[63] In addition, KS boys with a longer CAG repeat show later onset and slower progression of puberty and slower testicular degeneration process.[60] These findings are in agreement with diminished AR response to androgens when the *AR* gene has a longer CAG repeat. Recently, in healthy elderly men increased estrogen rather than decreased androgen action was associated with longer androgen receptor CAG repeats.[66]Thus also increased estrogen action can play a role in the variation of KS phenotype.

Among genes on the X chromosome, a large number belong to the cancer-testis antigen family and are expressed in testicular germs cells.[67,68] Mroz et al. showed that X-reactivation occurs during germ cell development in the XXY mouse, and it is assumed that for the survival of germ cells in the mature testis the proper X-chromosome dose is crucial.[69] Hence, molecular mechanisms induced by an altered dose of X-encoded genes in testicular cells may, during puberty, initiate the degeneration process in the testes of boys with KS.

## Testosterone substitution therapy in KS

When serum testosterone concentrations in KS patients become low, lifelong substitution therapy is indicated to prevent symptoms and consequences of androgen deficiency, and subsequently to improve quality of life. Beneficial effects of testosterone therapy in hypogonadal men have been demonstrated in several studies.[3,38]

KS subjects might benefit from testosterone supplementation during the first 2–3 months of life[7], although we still lack evaluation of the role of the minipuberty as a predictor of testicular insufficiency in KS. Any such treatment should be performed only in controlled, randomized clinical trials. The KS boys have sufficient testosterone levels to allow normal onset and progression of puberty[11,18,21,22], but development of a relative testosterone deficiency from midpuberty onwards is obvious. For instance, leveling-off of INSL3 levels and exaggerated responses to GnRH stimulation indicate Leydig cell dysfunction.[18,20] This is also in agreement with histomorphometric and immunohistochemical analyses: Leydig cell hyperplasia and fibrosis of the interstitium develop with age, and immunoexpression

of AR indicate diminished androgen action.[19,50] Consequently, although it seems that androgen supplementation in KS boys from midpuberty onwards is necessary, to date, placebo-controlled studies showing the benefits of early testosterone substitution are lacking, especially regarding the positive effects of early testosterone therapy on cognitive and behavioral parameters.

That sperm retrieval rate appeared to be lower in KS men who previously received exogenous testosterone causes an argument against the routine treatment of KS males with testosterone.[70] Actually, during spermatogenesis testosterone causes a marked inhibition of spermatogonial maturation.[71] Concern for the maintenance of fertility potential in young KS men must be balanced against the potential benefits of testosterone replacement.

## Fertility in KS

KS subjects are traditionally described as infertile. Semen analysis most often reveals azoospermia; in a cohort of 131 KS males, only 8.4% had spermatozoa in their ejaculate.[3] Some spermatogonia in KS subjects are capable of completing the spermatogenic process leading to formation of mature spermatozoa, but with an increased risk for genetically imbalanced spermatozoa.[72] Consequently, because of the risk for producing a chromosomal abnormality both on sex chromosomes and autosomes (chromosomes 18 and 21) in the offspring, most investigators recommend professional genetic counseling and standard prenatal diagnosis techniques.[72,73]

Most often the only hope for biological paternity in KS couples is TESE combined with ICSI. To date, more than 100 healthy children have been reported born after ICSI with testicular sperm from non-mosaic KS men. The initial success rate of TESE in adult 47,XXY males in small series has been 40–50%.[3] A new technique, "microdissection TESE", has shown significantly better sperm recovery rates compared to conventional TESE, sperm recovery rates as high as 70% have been achieved[70], and therefore this technique should be favored instead of TESE[74]. Live birth rates of 20–46% have been reported once sperm are obtained.[70,72] In the KS male, the only predictive factor for successful sperm recovery seems to be the testicular histopathology[75], but even with no sperm in histological sections, TESE has proven successful.[70,77] Neither testicular ultrasonography, extensive chromosome analysis, degree of virilization, testicular volume, nor serum testosterone, FSH, LH, nor inhibin B level is predictive for outcome of TESE[75,76]; thus even patients with unmeasurable inhibin B levels have undergone successful TESE.[77] It is to be noted, however, that results from only few centres have been published thus far, and there is a possibility of a positive publication bias, i.e. centres with less impressive success rates have not been able to report their data.

In boys with KS, the fact that the number of adult dark spermatogonia – of fundamental importance for development of male fertility[49] – is markedly reduced indicates a severely impaired fertility potential even before puberty.[19] Cryopreservation of semen samples containing very low numbers of spermatozoa from KS boys in early puberty is possible and should be offered to appropriate patients before the start of testosterone supplementation. The expected success rate is, however, exceedingly low, since the onset of puberty initiates a marked acceleration in germ cell depletion, and one must also take into account the limited ability of boys to provide semen samples during early puberty. Another option would be TESE, if the biopsy sample contains haploid germ cells. The possible future use for infertility treatments of cryopreserved testicular samples containing spermatogonia but not more mature germ cells would require *in vitro* maturation of spermatogonia into mature spermatozoa or at least into late/elongated spermatids. Recent studies indicate that human testicular tissue can be cultured for at least up to 3 weeks without essential loss of spermatogonia.[78,79] Early results also suggest that meiosis and spermatogenesis may resume under culture conditions, yielding normal spermatids with some fertilization potential.[79] However, at present this option for fertility preservation in boys before spermarche remains entirely experimental.

## Summary

Placebo-controlled studies are vital to determine the role of hypogonadism in aggravating the 47,XXY phenotype, because all the characteristics of the KS phenotype cannot be ascribed to the relative androgen deficiency; other factors such as the excess of X-chromosome genes probably also

have some impact. Whether the defect in the 47,XXY testis is intrinsic to germ cells or is due to inability of the Sertoli cells to support normal germ cell development is not fully resolved, although recent data on mice show that there is intrinsic capability of donor XY germ cells to develop into haploid germ cells in XXY environment.[80] Furthermore, we do not know whether the Leydig cell failure is a consequence of germ cell depletion and Sertoli cell injury or is intrinsic to Leydig cells. Molecular mechanisms behind the testicular degeneration in KS have remained a mystery and require further elucidation.

**Practice points**

- The testicular degeneration process in the testes of KS boys accelerates at the onset of puberty.
- Concern for the maintenance of fertility potential in young KS men must be balanced against the potential benefits of testosterone replacement.
- Cryopreservation of semen samples containing very low numbers of spermatozoa from KS boys in early puberty is possible and should be offered to appropriate patients before the start of testosterone supplementation.
- The only option for biological paternity in most KS couples is TESE combined with ICSI.
- The only predictive factor for successful sperm recovery seems to be the testicular histopathology, but even with no sperm in biopsy specimens, TESE has proven successful.
- Professional genetic counseling and standard prenatal diagnosis techniques are recommended, because of the risk for chromosomal abnormality both on sex chromosomes and autosomes in the offspring.

**Research agenda**

- The role of the postnatal testicular activation as a predictor of testicular insufficiency has remained unresolved.
- Induction of an altered dose of X-encoded genes in testicular cells is a hypothetical mechanism accelerating germ cell loss and testicular degeneration during puberty.
- Studies with larger number of patients are needed to substantiate if X-chromosome isodisomy/heterodisomy and X-chromosome inactivation pattern have an impact on the KS phenotype.
- Placebo-controlled studies showing the benefits of early testosterone substitution are lacking, especially regarding the positive effects of early testosterone therapy on cognitive and behavioral parameters.
- The possible future use for infertility treatments of cryopreserved testicular samples would require development of *in vitro* maturation techniques of immature germ cells into spermatids.

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