Human Reproduction Update, Vol.12, No.1 pp. 39–48, 2006 doi:10.1093/humupd/dmi039
Advance Access publication September 19, 2005

Natural history of seminiferous tubule degeneration in Klinefelter syndrome

Lise Aksglæde[1], Anne M.Wikström[2], Ewa Rajpert-De Meyts[1], Leo Dunkel[2,3], Niels E.Skakkebæk[1] and Anders Juul[1,4]

[1]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Hospital for Children and Adolescents, Helsinki University Central Hospital, University of Helsinki, Helsinki and [3]Department of Pediatrics, Kuopio University Hospital, University of Kuopio, Kuopio, Finland

[4]To whom correspondence should be addressed at: Department of Growth and Reproduction, Rigshospitalet Section 5064, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. E-mail: ajuul@rh.dk

Klinefelter syndrome (47,XXY) is characterized by small, firm testis, gynaecomastia, azoospermia and hypergonadotropic hypogonadism. Degeneration of the seminiferous tubules in 47,XXY males is a well-described phenomenon. It begins in the fetus, progresses through infancy and accelerates dramatically at the time of puberty with complete hyalinization of the seminiferous tubules, although a few tubules with spermatogenesis may be present in adult life. Activation of the pituitary-gonadal axis at 3 months of age is seen in Klinefelter boys similar to healthy boys. However, the level of testosterone in Klinefelter boys is significantly lower than in controls. After this 'minipuberty', the hormone levels decline to normal prepubertal levels until puberty. In puberty, an initial rise in testosterone, inhibin B, LH and FSH occurs in Klinefelter boys. However, the rise in testosterone levels off and ends at a low-normal level in young adults. Likewise, serum concentration of inhibin B exhibits a dramatic decline to a low, often undetectable level, concomitantly with a rise in FSH, reflecting the degeneration of the seminiferous tubules. Many hypotheses about the underlying mechanism of the depletion of the germ cells in Klinefelter males have been reported and include insufficient supranumerary X-chromosome inactivation, Leydig cell insufficiency and disturbed regulation of apoptosis of Sertoli and Leydig cells. However, at present, the exact mechanism remains unclear. In this article, we summarize current knowledge on the development of the classical endocrinological and histological features of 47,XXY males from fetus to adulthood and review the literature concerning the degeneration of the seminiferous tubules in this syndrome.

Key words: azoospermia/infertility/Klinefelter syndrome/Sertoli cells/testes

Introduction

Klinefelter syndrome was first described as a clinical entity by Harry F. Klinefelter in 1942 (Klinefelter, 1942), and the disorder was subsequently found to be caused by the presence of an extra X chromosome (Jacobs and Strong, 1959). Today, it is known that approximately 80% of the cases are because of the numerical chromosome aberration 47,XXY; the remaining 20% have higher-grade chromosome aneuploidies (e.g. 48,XXXY) or mosaicisms (Foresta *et al*., 1998). The syndrome is the most common sex-chromosome abnormality occurring in approximately 1 in 600 newborn males (Bojesen *et al*., 2003), and it is the most frequent genetic cause of infertility occurring in 11% of azoospermic men (Van Assche *et al*., 1996; Foresta *et al*., 1999). Phenotypically, the Klinefelter male is characterized by small firm testes, gynaecomastia, eunuchoid body proportions, azoospermia, high levels of gonadotrophins (FSH and LH) and low normal levels of testosterone (Paulsen *et al*., 1968). It is known that the phenotype of Klinefelter males progressively deviates from normal with the increasing number of extra X chromosomes present, whereas the Klinefelter males with mosaicism most often are less severely affected (Lanfranco *et al*., 2004). The presence of a few cells with normal karyotypes (low-grade mosaicism) may be related to a preservation of some germ cells (Lenz *et al*., 2005), hereby influencing the fertility potential.

Klinefelter syndrome may be associated with an increased risk of certain systemic diseases including malignancies, autoimmune diseases (e.g. diabetes mellitus, hypothyroidism and rheumatic diseases), osteoporosis and venous thromboembolism (Campbell and Price, 1981; Kubler *et al*., 1992; Oktenli *et al*., 2002), although controversy exists. In a nationwide Danish registry study of 781 Klinefelter males, the syndrome was found to be associated with an increased mortality risk of 40% and a reduction in median survival of 2.1 years compared with controls (Bojesen *et al*.,

2004). The increased mortality was mainly because of infectious, neurological, circulatory, pulmonary and urinary tract diseases (Bojesen *et al*., 2004).

Because of the substantial variation in clinical presentation and the relatively discrete symptoms, especially before puberty, most of the patients are never diagnosed. Less than 10% of all subjects with Klinefelter syndrome are diagnosed before puberty, and only approximately one fourth of adult males with the syndrome are diagnosed (Bojesen *et al*., 2003).

The extra X chromosome in Klinefelter syndrome causes infertility because of the degeneration of the germ cells. When and why the germ cells degenerate are important but not yet fully answered questions. To date, various studies have tried to address this subject. However, the results have been difficult to interpret owing to problems of late diagnosis and tissue sampling resulting in small sample sizes. Thus, most of the available data on Klinefelter syndrome are based on studies of less than 20 patients. The increasing use of amniocentesis, however, has made it possible to examine fetuses with the Klinefelter karyotype and to follow boys with Klinefelter syndrome from birth onwards and, thereby, to describe in more detail the testicular degeneration process in Klinefelter males. Some reports suggest that the degeneration of germ cells starts in early infancy, leading to the absence of or to a significantly reduced number of germ cells even before puberty (Mikamo *et al*., 1968; Murken *et al*., 1974; Ratcliffe, 1982; Coerdt *et al*., 1985; Muller *et al*., 1995). Complete absence of germ cells is, however, not always the rule. Even azoospermic patients may have focal spermatogenesis in the testis and may therefore benefit from assisted reproductive techniques to father a child (Denschlag *et al*., 2004).

The aim of this article is to review the existing knowledge on testicular development in subjects with Klinefelter syndrome with particular emphasis on the ontogeny of histological and hormonal changes associated with germ cell demise.

Histopathology of Klinefelter testis during development

The degeneration of the germ cells in Klinefelter syndrome, which is a well-documented phenomenon may be because of a primary effect of the extra X chromosome on the development and function of the germ cells or adverse influence on the supporting somatic cells including the Leydig and Sertoli cells.

Testosterone, produced by Leydig cells, plays an indispensable role in spermatogenesis (Rey, 2003). High intratesticular, rather than circulating levels of testosterone, and an adequate expression of androgen receptors in Sertoli cells are necessary for the onset of puberty (Rey, 2003).

The Sertoli cells, possibly together with the adjacent basement membrane, create a particular microenvironment which controls the renewal and differentiation of the germ cells by providing nutrition, adhesion and several transport functions (Kerr, 1992; Print and Loveland, 2000; Spradling *et al*., 2001).

In the following section, available data on the age-specific changes in histology (Figure 1) and hormone levels in individuals with Klinefelter syndrome are described.

The fetal period

From the studies of fetuses aborted at a gestational age of 18–22 weeks, we know that the degenerative process has already started at this early stage (Murken *et al*., 1974; Autio-Harmainen *et al*., 1980; Coerdt *et al*., 1985). Thus, a significantly reduced number of germ cells were seen in studies of testicular biopsies from 47,XXY mid-term fetuses, whereas the density and number of testicular tubules and mesenchymal structures appeared normal (Coerdt *et al*., 1985). The number of germ cells was even further reduced in a fetus with undescended testes aborted at 20 weeks of gestation (Murken *et al*., 1974) and in an infant with inguinal hernia, who underwent surgery at 4 weeks of age (Ratcliffe, 1982). Leydig cells have appeared morphologically normal in all but one of the studies (Murken *et al*., 1974). However, the testicular biopsy in the latter study was taken from a Klinefelter fetus with testes not yet descended, and similar histological changes are seen in cryptorchid males with a normal karyotype (Nistal, 1982; Regadera *et al*., 1999).

Neonatal period and 'minipuberty'

There may be some impairment of the Leydig cell function at birth, even though this has not been confirmed histologically (Sorensen *et al*., 1981; Ratcliffe, 1982; Lahlou *et al*., 2004). Sorensen *et al*. (1981) measured cord-blood testosterone in two 47,XXY infants and one with mosaicism (46,XX/47,XXY) and found significantly lower levels compared with three control infants. This study was only based on three patients and three controls. However, when the testosterone levels of the three Klinefelter

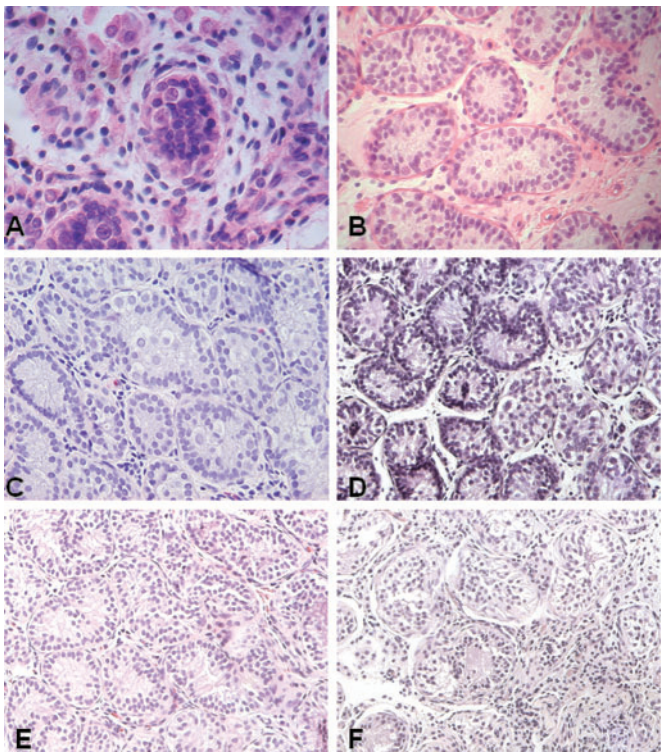

Figure 1. Testicular histology in Klinefelter syndrome during development. **A**, fetal testis; **B**, a 4-year-old boy with a relatively large number of germ cells; **C–E**, three prepubertal 10- to 12-year-old patients with variable numbers of remaining germ cells, note a few focally grouped tubules with germ cells, visible on the right side of the figure in **D**, and no germ cells remaining in **E**; and **F**, a 14-year-old without germ cells present, note degeneration of tubules and Leydig-cell nodules.

infants were compared with the levels of testosterone in a larger cohort of control infants, there was no difference (Ratcliffe, 1982).

The pituitary–gonadal axis in healthy boys is strongly activated after birth—which is manifested by pubertal or even adult levels of serum FSH, LH, testosterone and inhibin B at the age of 3 months—the so called 'minipuberty' (Andersson *et al*., 1998b). Hereafter, the hormone levels decline to normal prepubertal levels until the pubertal reactivation of the axis occurs. The occurrence of the 'minipuberty' represents a window, where it is possible to study the function of the pituitary–gonadal axis by measuring the spontaneous, basal hormone levels (Main *et al*., 2002). Lahlou *et al*. published a study in 2004, in which they compared the gonadotropin and reproductive hormone levels in 215 healthy infants with the hormone levels of 18 infants with prenatally diagnosed nonmosaic Klinefelter syndrome. The infants with Klinefelter had a testosterone peak during the first months of life similar to that of control infants, but the testosterone levels were significantly lower from birth to 8 months in the infants with Klinefelter syndrome, suggesting an impaired Leydig cell function already at this early age. By contrast, LH, FSH, inhibin B and Anti-Mullerian Hormone (AMH) levels were normal (Lahlou *et al*., 2004). In accordance with the normal histology of the Sertoli cells as reported in earlier studies, this finding suggests that the Sertoli cells are qualitatively and quantitatively normal in 47,XXY children in infancy (Mikamo *et al*., 1968; Ratcliffe, 1982; Lahlou *et al*., 2004).

The childhood and adolescence

Several studies of prepubertal Klinefelter boys have revealed a similar testicular histology as already described in the Klinefelter fetus. All studies have shown preservation of seminiferous tubules with a reduced number of germ cells, whereas the Sertoli cells and Leydig cells have appeared (when described) normal and of juvenile type (Ferguson-Smith, 1959; Mikamo *et al*., 1968; Muller *et al*., 1995; Wikstrom *et al*., 2004). Wikstrom *et al*. (2004) published a study of 14 nonmosaic Klinefelter boys aged 10–14 years. Importantly, none of the boys enrolled were cryptorchid or undergoing androgen substitution. The biopsies of pre- and peripubertal boys showed germ cells, but the number of spermatogonia present was markedly reduced and no meiotically dividing germ cells or postmeiotic spermatids appeared in any of the biopsies (Wikstrom *et al*., 2004). The presence of germ cells in peripubertal Klinefelter boys contrasts with the results of Muller *et al*. (1995), who found no germ cells in biopsies from Klinefelter boys aged 2 years or more. However, in the latter study, all boys were cryptorchid, a condition, which has also a detrimental effect on the seminiferous epithelium.

The prepubertal 47,XXY boys were characterized by normal levels of testosterone, FSH, LH and inhibin B until the onset of puberty (Topper *et al*., 1982; Salbenblatt *et al*., 1985; Christiansen *et al*., 2003; Wikstrom *et al*., 2004).

Major histological changes in the testes coincided with the pubertal activation of the pituitary–gonadal axis. As the Klinefelter boys entered puberty, their testis initially grew up to a volume of 6 ml. However, as serum-testosterone levels increased, the depletion of germ cells, the hyalinization of the tubules, the degeneration of the Sertoli cells and the hyperplasia of the Leydig cells accelerated (Wikstrom *et al*., 2004). This was associated with a decrease in the testis volumes to a prepubertal size of 2–4 ml (Ratcliffe *et al*., 1986; Robinson *et al*., 1986). The degeneration process was accompanied by a relative Leydig-cell insufficiency reflected by the impaired serum testosterone levels and increasing LH levels. The initial adolescent rise in testosterone was relatively normal, but from the age of about 14 years serum concentrations of testosterone levelled off and remained in the low-normal range through puberty (Topper *et al*., 1982; Salbenblatt *et al*., 1985; Wikstrom *et al*., 2004). It remains uncertain whether the rise in serum or intratesticular testosterone concentrations in puberty is associated with the accelerated destruction of the seminiferous tubules in puberty. The level of inhibin B is known to increase before puberty, but as the testosterone level increased the inhibin B level was very rapidly suppressed with a concomitant rise in serum FSH levels (Christiansen *et al*., 2003; Wikstrom *et al*., 2004). Thus, inhibin B was most often undetectable at the end of puberty in Klinefelter patients (Christiansen *et al*., 2003; Wikstrom *et al*., 2004). In pre- and peripubertal boys, the production of inhibin B is known to be regulated by Sertoli cells. After the onset of puberty and in adult males, however, the inhibin B production becomes germ-cell dependent, and serum inhibin B can thus no longer serve as an exclusive marker of Sertoli-cell function (Andersson *et al*., 1998a) (Figure 2).

Adult life

As described by Klinefelter in 1942, the histology of the testes is characterized by extensive fibrosis and hyalinization of the seminiferous tubules, and hyperplasia of interstitium in the adult

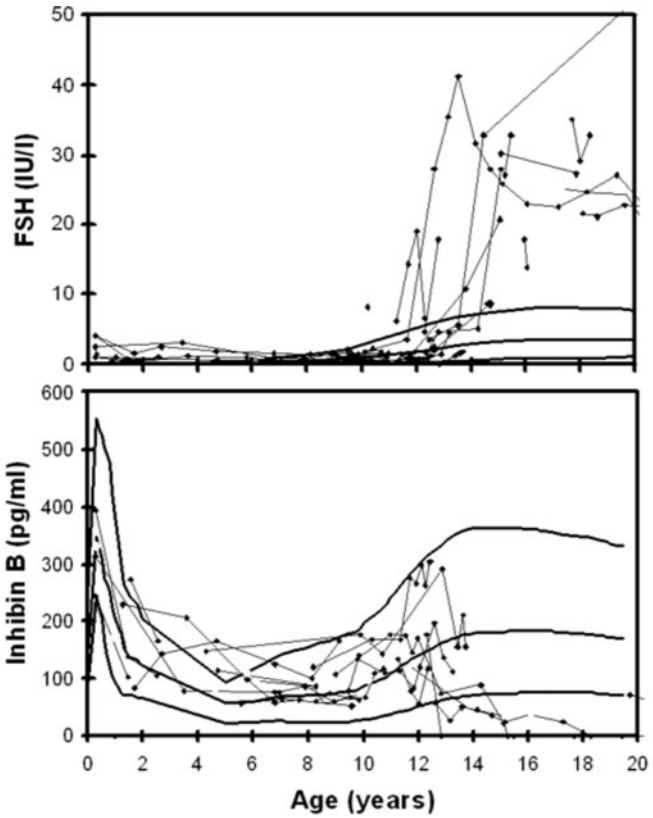

Figure 2. Longitudinal assessment of serum FSH (**top** panel) and inhibin B (**lower** panel) in 36 untreated children and adolescents with Klinefelter syndrome (Part of data previously published by Christiansen *et al*., 2003). The solid lines represent mean ± 2 SD for healthy boys (Andersson *et al*., 1997).

patient (Klinefelter, 1942). Many studies have since then evidenced the patchy nature of the testicular histology with more- and less-affected areas (Heller, 1945; Steinberger, 1965; Skakkebaek, 1969; Skakkebaek *et al*., 1969; Nistal, 1982; Foresta *et al*., 1999; Wikstrom *et al*., 2004). Foresta and colleagues studied ten 47,XXY males aged 28–37 years and found Sertoli-cell-only pattern in eight of ten biopsies, whereas the remaining two showed Sertoli cells and few spermatogenic cells (Foresta *et al*., 1999). In 1969, Skakkebaek described two types of tubules in relation to Sertoli-cell morphology containing either small immature Sertoli cells (chromatin positive) or larger and more differentiated Sertoli cells (chromatin negative) (Figure 3). Later, these immature Sertoli cells were studied further and were found to have an impaired physiological activity resulting in a compromised protein and steroid hormone synthesis (Nistal, 1982). Thus, the adult Klinefelter patients are characterized by hypergonadotropic hypogonadism as evidenced by low to low-normal levels of testosterone, high FSH and LH levels, and undetectable levels of serum inhibin B in most of the patients (Salbenblatt *et al*., 1985; Anawalt *et al*., 1996; Foresta *et al*., 1999; Christiansen *et al*., 2003; Lahlou *et al*., 2004; Lanfranco *et al*., 2004; Wikstrom *et al*., 2004).

Fertility in subjects with Klinefelter syndrome

The Klinefelter subjects are traditionally described as infertile because of a complete absence of germ cells. Although semen analysis most often reveals azoospermia, some Klinefelter men may have single-residual foci with spermatogenesis (Heller, 1945; Ferguson-Smith, 1959; Steinberger, 1965; Skakkebaek, 1969; Skakkebaek *et al*., 1969; Froland and Skakkebaek, 1971; Foresta *et al*., 1999). It is believed that some spermatogonia in Klinefelter subjects are capable of completing the spermatogenic process leading to the formation of mature spermatozoa (Foresta *et al*., 1999; Bergere *et al*., 2002) (Figure 3F). However, natural conception rarely occurs for Klinefelter couples, and most often the only hope for biological paternity is testicular sperm extraction (TESE) combined with ICSI. The initial success rate of TESE in adult 47,XXY males in a small series was reported to be 40–50% (Lanfranco *et al*., 2004). The fact that the germ-cell degeneration accelerates dramatically at the onset of puberty makes it tempting to retrieve germ cells at an earlier age for cryopreservation and future utilization (Damani *et al*., 2001; Lin *et al*., 2004). However, Wikstrom *et al*. (2004) found that only 50% of the Klinefelter boys had germ cells in their testes indicating a severely impaired fertility potential even in the peripubertal period.

The levels of FSH, inhibin B and the inhibin B/FSH ratio are known predictive factors for fertility in males with normal karyotype (Andersson *et al*., 1997). In the Klinefelter male, the only predictive factor for a successful sperm recovery seems to be the testicular histopathology (Westlander *et al*., 2001), but even when no sperm is found in a biopsy, TESE has been successful (Vernaeve *et al*., 2004b). The predictive value of testicular volume, testosterone levels and response to hCG test for successful TESE was shown in one study (Madgar *et al*., 2002), but not in others (Westlander *et al*., 2001; Vernaeve *et al*., 2004b). FSH, LH and inhibin B levels were not predictive for successful TESE. Likewise, testicular ultrasonography, intratesticular blood-flow resistance, degree of virilization or extensive chromosome analyses did not seem to predict the outcome of TESE (Westlander *et al*., 2001; Vernaeve *et al*., 2004b). In fact, even patients with inhibin B below the detection limit underwent successful TESE (Westlander *et al*., 2003). Vernaeve *et al*. (2002, 2004a) have confirmed that inhibin B is an established marker of spermatogenesis, but not a predictive factor for the outcome of TESE in males with nonobstructive azoospermia. To date, more than 40 healthy children of Klinefelter fathers have been born following the use of ICSI (Denschlag *et al*., 2004). A triplet gestation, where one 47,XXY fetus was reduced, has been reported (Ron-El *et al*., 2000). Because of the hypothetical risk of producing a sex chromosomal abnormality in the offspring, most investigators recommend professional genetic counselling and standard prenatal diagnostic techniques (Denschlag *et al*., 2004).

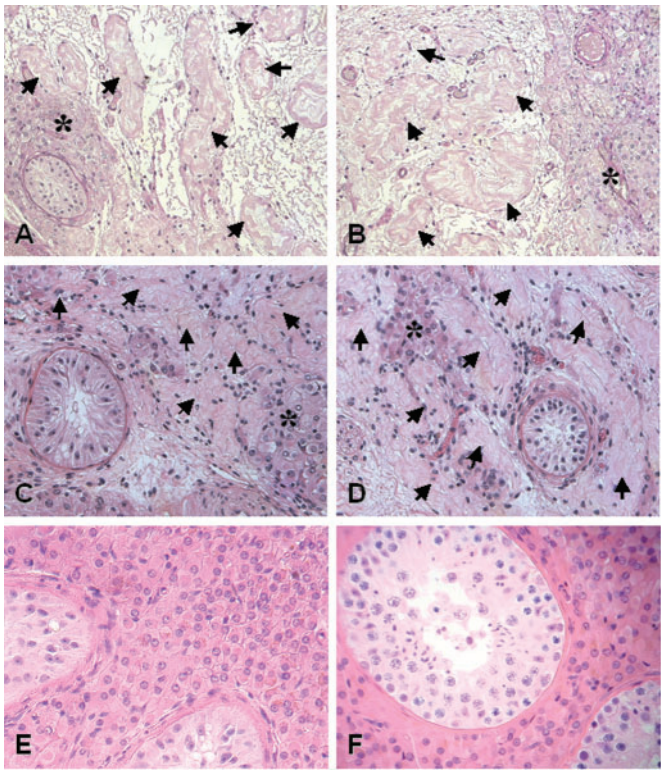

Figure 3. Typical testicular histology in adult Klinefelter patients with two types of Sertoli-cell-only tubules, numerous completely hyalinized 'ghost' tubules (arrows) and nodules of Leydig cells (asterisks). **A–B**, overview in PAS-stained tissues; **C**, a type-A tubule with differentiated Sertoli cells; **D**, a type-B tubule with immature sex-chromatin-positive Sertoli cells; **E**, closer magnification of a large Leydig cell nodule in a patient with Sertoli-cell-only tubules and a massive Leydig hyperplasia; and **F**, a tubule with preserved complete spermatogenesis in a young adult Klinefelter patient.

Other features of the Klinefelter phenotype

There is a wide variation in the Klinefelter phenotype. Skewed X-chromosome inactivation, frequently seen in females, defined as greater than 80% preferential inactivation of one of the two X chromosomes, occurs also in Klinefelter syndrome (Iitsuka *et al*., 2001). It is possible that preferential inactivation of genes may influence the Klinefelter phenotype, but this remains to be elucidated.

One gene that is of physiological importance in the testis is the androgen-receptor gene (AR, mapped to Xq11.2-12). The AR gene contains a polymorphic stretch of CAG repeats in exon 1. The length of this stretch is inversely related to the receptor's basal and ligand-induced activity *in vitro* and may influence physiological response to androgens (Zitzmann and Nieschlag, 2003). In the Klinefelter males, one of the two AR alleles is inactivated (Iitsuka *et al*., 2001; Suzuki *et al*., 2001; Zitzmann *et al*., 2004). Zitzmann *et al*. (2004) studied the CAG polymorphism in 77 Klinefelter males and found evidence for a preferential inactivation of the shorter allele. Conversely Suzuki *et al*. (2001) studied 13 47,XXY males and reported preferential inactivation of the longer allele. Furthermore, Zitzmann *et al*. (2004) demonstrated that Klinefelter males with a longer CAG repeat tend to be more severely affected than those with a shorter CAG stretch in the AR. This association was found in relation to socioeducational status, growth pattern, bone density and occurrence of gynaecomastia. The effect of testosterone treatment was also correlated to the length of the CAG repeat in the AR. Males with short repeats responded to testosterone substitution with a more pronounced suppression of LH and larger increment of testosterone levels than males with longer CAG repeats in the AR.

The variation in the CAG length can, at least partially, explain the variation in the Klinefelter phenotype, as this polymorphism was linked to variability in some androgen-dependent functions in normal healthy 46,XY males (Zitzmann and Nieschlag, 2003). That many Klinefelter males present the classical hypogonadal phenotype even though they have testosterone levels in the low-normal range might reflect some degree of androgen resistance as suggested by the high-normal LH levels as well as from the demonstrated associations between number of the CAG repeats in the AR and phenotypical characteristics in Klinefelter males. In line with these findings, Zinn *et al*. (in press) found an inverse relation between the number of CAG repeats and penile length in 35 Klinefelter boys and men, but did not find any associations with height, BMI, head circumference, testicular volume or presence of gynaecomastia.

Lessons from animal models

The development of the XXY mouse in 1991 provided a tool to investigate the development of germ cells (Hunt and Eicher, 1991). It has initially been suggested that the reduction in germ cells was the result of a difference in the number of germ cells colonizing the genital ridges. However, Hunt *et al*. (1998) counted the primordial germ cells in the genital ridges of the XXY mice at different developmental stages of the gonads and found no significant difference in comparison with XY controls. This suggested that normal numbers of primordial germ cells arrived at the genital ridges of the XXY embryo, and the impairment of the mitotic proliferation became only evident after the differentiation of the testis had begun (Hunt *et al*., 1998). In 1998, Hunt *et al*. found a reduction in the number of germ cells per tubule cross section at all stages from the prenatal period onwards in XXY mice. Furthermore, they did not find any signs of post-natal mitotic proliferation but rather a progressive decline in the number of germ cells during early post-natal period until 12 days post-partum, where they could hardly recognize any remaining germ cells (Hunt *et al*., 1998).

In the same manner, Lue *et al*. (2001) found that germ cells began to degenerate in the XXY mice at 7 days of age with a progressive loss resulting in a total absence of germ cells in the adult animals. Hypertrophy and hyperplasia of the Leydig cells and changes in the Sertoli cells indicating cellular inactivity were furthermore observed.

Another recent study of XXY mice confirmed the abovementioned observations and added new knowledge to the mechanisms of Sertoli-cell degeneration and thereby the mechanism behind germ-cell loss. The investigators found that by the age of 20 days, there was a sporadic loss of AR expression in some of the Sertoli cells of the XXY mice resulting in a complete loss of AR expression in the adult XXY mice. Since Sertoli cells have a supporting influence on spermatogenesis, the resulting dysfunction of the Sertoli cells might be a major factor responsible for the loss of germ cells in early age (Lue *et al*., 2005). Lue *et al*. (2005) furthermore found differences in the intracellular localization of the AR in the Leydig cells of XY and XXY mice. In XXY mice, the AR was only found in the cytoplasm, whereas the AR was present in the nuclei in XY mice representing a possible reason for the impaired Leydig-cell function in adult XXY mice.

Trying to elucidate whether the XXY somatic environment of the testis influenced the degeneration of germ cells, Hunt *et al*. (1998) studied the proliferative potential of the XXY-mice germ cells *in vitro* and found no significant changes. This was indicative of a dysfunction in the communication between the soma and germ cells that could be because of a failure in the proliferative signals from the soma or the ability of the germ cells to respond. Further investigations revealed no apparent abnormalities, neither in the developing Sertoli cells, nor in the production of testosterone in Leydig cells (Hunt and Eicher, 1991).

These studies of animal models helped to describe the germ-cell demise in detail but did not shed much light on the underlying mechanisms.

What are the mechanisms of germ-cell depletion?

One of the fundamental questions is whether the abnormal karyotype affects primarily germ cells or affects primarily somatic cells in the testis, in particular Sertoli cells, which are thought to be main mediators of signals from the outside to the germ-cell compartment. Leydig cells may also be affected, and it is therefore probable that the testicular phenotype is a result of impaired function and interaction of several cell types.

The acceleration of germ-cell demise occurs at the onset of puberty; when in the normal testis, the activation of reproductive hormones triggers the process of gamete production, which requires a switch from mitosis to meiosis. That led to one of earlier hypotheses that aneuploid germ cells cannot efficiently align during meiosis, and the unsynapsed chromosomes would disturb the meiotic checkpoint and trigger apoptosis at the pachytene spermatocyte stage (Miklos, 1974; Burgoyne, 1993). If that hypothesis was true, the predominant histological picture would have been that of the maturation arrest at the level of spermatocyte, which is sometimes seen in patients with large deletions in the Y-chromosome's male-specific region (known also as the azoospermia factor region, *AZF*). On the other hand, numerous reports suggested that aneuploid germ cells sometimes can slip through the meiotic checkpoint and mature to spermatozoa (Skakkebaek *et al*., 1969;

Chevret *et al*., 1996). Some scientists demonstrated by fluorescence in-situ hybridization (FISH) the presence of hyperhaploid (24,XX or 24,XY) spermatids in the vicinity of 47,XXY spermatogonia in presumably nonmosaic Klinefelter-syndrome patients (Yamamoto *et al*., 2002). Others dispute that (Egozcue *et al*., 2002) and claim that only 46,XY spermatogonia can complete meiosis, and their presence in Klinefelter patients is owing to the apparent low percentage mosaicism for sex chromosome aneuploidy (Blanco *et al*., 2001; Ekerhovd and Westlander, 2002; Lenz *et al*., 2005). The sex-chromosome disomy sometimes observed in the sperm of Klinefelter patients may then result from meiosis-II errors caused by impaired cellular microenvironment. The latter opinion is supported by the studies of the mouse XXY model, where only XY spermatogonia survived in the adults (Mroz *et al*., 1999). Discussion on this aspect is not yet over.

Gene dosage and X-chromosome inactivation

As described above in this review, the vast majority of the 47,XXY patients never experience meiosis, and their germ cells disappear at the mitotic stage of spermatogonia or during earlier differentiation. Therefore, a predominant hypothesis is that the altered dosage of some genes on X chromosome may affect the development and/or degeneration of the germ cells in males with 47,XXY (Spatz *et al*., 2004). It is well known that in females, one of the two X chromosomes is randomly inactivated in the somatic cells to obtain a gene dosage, which is equivalent to that in males. Although many genes escape inactivation, the inactivated X chromosome is microscopically visible as the Barr body (sex chromatin) in female cells (Barr, 1949). The inactivation of an extra X chromosome in human somatic cells is mediated primarily by a RNA product from the gene called X-inactive-specific transcript (*XIST*) located on the long arm of the inactive X chromosome (Brown *et al*., 1991; Plath *et al*., 2002). Therefore, the expression of *XIST* is a marker of the presence of the second and any further extra X chromosomes in the somatic cell (Penny *et al*., 1996).

Somatic cells in the males with 47,XXY inactivate the supranumerary X chromosomes most probably in the same manner as the somatic cells in females. *XIST* is expressed in blood cells of Klinefelter men but not in the blood of healthy men with normal karyotype (Kleinheinz and Schulze, 1994). The Barr body was also found in Leydig and undifferentiated Sertoli cells in males with Klinefelter syndrome (Froland and Skakkebaek, 1971; Shamsuddin and Tang, 1980). Therefore, we assume that any increase in gene dosage in these cell types will only concern genes that escape inactivation.

As far as the possible role of X-mapped genes that escape inactivation is concerned, very little is known about their expression patterns. It is estimated that approximately 15% of X-linked genes escape inactivation to some degree, but there are many more that show cell-type-specific inactivation pattern (Carrel and Willard, 1999, 2005; Carrel *et al*., 1999). The genes that escape inactivation tend to cluster on the distal part of the short arm (Xp), whereas it is the long arm (Xq) that apparently contains genes, which primarily contribute to the Klinefelter phenotype. This is based on the reports of Klinefelter syndrome in patients with isochromosomes or other structural aberrations of Xq (Arps *et al*., 1996; Nemeth *et al*., 2002).

The situation in germ cells appears to be quite different and much more complex, because the ways of the X-chromosome inactivation in germ cells do not follow the pathways established in female somatic cells (Armstrong *et al*., 1997; Fernandez-Capetillo *et al*., 2003). Earlier studies assumed that the expression of *XIST* was synonymous with the X-chromosome inactivation, therefore it was concluded that the sole X chromosome in male germ cells was inactivated in the adult testis. This conclusion was mainly based on a study of fertile men who did express *XIST* in the testis, whereas males with Sertoli-cell-only syndrome did not (Salido *et al*., 1992). Thus, germ cells were the only cell type expressing *XIST* in the testis. A few years later, it became clear that the X-chromosome inactivation does not fully occur in adult spermatogonia, as it was shown that among the X-chromosome genes, a surprisingly large number was expressed in testicular germ cells (Wang *et al*., 2001). The recent sequencing of the X chromosome revealed that probably as many as 10% of protein-coding genes on the X chromosome may be testis specific and belonging to the so-called 'cancer-testis antigens' family (Ross *et al*., 2005). The name comes from the common transcription activation of these genes in various human cancers, e.g. melanomas, lung cancers (Scanlan *et al*., 2002). A high expression of some of these genes is also seen in fetal germ cells and testicular neoplasms, including preinvasive carcinoma in situ (CIS) and spermatocytic seminomas, but very rarely in nonseminomas, tumours which do not retain germ-cell-like phenotypic features (Aubry *et al*., 2001; Satie *et al*., 2002). Germ-cell cancer may serve to some extent as a model for the Klinefelter syndrome, because neoplastic germ cells in most cases display polyploidy and a significant amplification of the X-chromosome material (Peltomaki *et al*., 1989; Oosterhuis and Looijenga, 2005). The expression of some of the abovementioned 'cancer-testis' antigens and other X-linked genes was reported in germ-cell neoplasms and some derived cell lines, despite the activation of *xist* transcription (Looijenga *et al*., 1997; Kawakami *et al*., 2003; Almstrup *et al*., in press). Studies of the gene inactivation in male germ cells are still in their infancy, so the mechanisms have not been elucidated even in animal models. It is clear that in mice, *XIST* is not required for sex-chromosome inactivation in germ cells in mature testes (Turner *et al*., 2002), but the situation during very early development is not yet known. We expect that the recent progress in genomic analysis of the X chromosome as well as better understanding of epigenetic regulation of gene expression will soon shed some more light on this aspect of the biology of germ cells (Carrel and Willard, 2005; Ross *et al*., 2005).

Klinefelter syndrome and germ-cell neoplasia

It is noteworthy that Klinefelter patients carry an increased risk of extragonadal (mediastinal and intracranial) germ-cell cancer, and some other types of cancer, e.g. breast cancer. It follows, that the supranumerary X chromosome most probably provides a proliferative or survival advantage to germ cells during their migration to the gonadal ridges, but the opposite is true for the germ cells within the testis. The other important point is that the expression of some X-linked genes in male germ cells is developmentally regulated. For example, MAGE-A4 is not expressed in very early gonocytes (and most probably it is not active in primordial germ cells), whereas it is highly expressed in gonocytes from mid-gestation as well as in infantile and mature spermatogonia (Aubry *et al*., 2001). Therefore, it is possible that some testis-specific genes of the X chromosome become activated only when germ cells have completed their migration, or even later at the onset of meiosis.

Moreover, some of the X-chromosome genes are expressed in somatic cells in the testis, and we can speculate that increased expression of those that escape inactivation may affect the germ cells. Among the interesting genes to look at from this perspective, are, e.g. a gene encoding p120, a putative inhibin-binding protein (mapped to Xq24; Chong *et al*., 2000) or the angiotensin type-II receptor gene, AT2 (Xq21.3). The AT2 receptor may be of particular interest with regard to the quickly progressing demise of germ cells in Klinefelter males, because it mediates apoptosis in some cell types and is considered to be involved in the physiological atresia of ovarian follicles (Yamada *et al*., 1996; Kotani *et al*., 1999).

Apoptosis

Apoptosis is a mechanism responsible for the physiological regulation of germ-cell death during differentiation and maturation of normal human germ cells and could contribute to the excessive germ-cell demise in males with 47,XXY. Apoptosis is a prerequisite for continuous spermatogenesis (Print and Loveland, 2000), by selectively removing dysfunctional or damaged germ cells, and by limiting germ cell number (Grootegoed *et al*., 2000).

Gonadotrophins (FSH, LH) and testosterone are important regulators of germ-cell apoptosis (Sinha Hikim and Swerdloff, 1999; Print and Loveland, 2000). Their removal induces apoptosis, which occurs presumably through indirect effects, since hormone receptors are present on somatic cells (Print and Loveland, 2000). FSH is classically considered to be involved in the initiation of the pubertal spermatogenesis. It regulates DNA synthesis, proliferation and differentiation of spermatogonia and spermiogenesis (Rey, 2003). FSH inhibits male germ-cell apoptosis in cultured rat seminiferous tubules partially via stem-cell factor (SCF) produced by Sertoli cells and interacts with the c-kit receptor in the germ cells (Yan *et al*., 2000a,b,c). This mechanism may involve changes in the Bcl-2 family members, since in cultured rat seminiferous tubules, either FSH or Sertoli-cell-derived SCF can regulate antiapoptotic Bcl-w expression (Yan *et al*., 2000a,b,c). It could be hypothesized that the FSH receptor was malfunctioning or down-regulated in XXY Sertoli cells as FSH, which has pro-survival effects on germ cells, is considerably elevated in Klinefelter patients. This would be consistent with the observation that inhibin B is undetectable in Klinefelter males. The hypothetical malfunction of the FSH receptor might lower the SCF expression and thereby influence the ratio of pro- and anti-apoptotic factors early in development forcing the germ cells to undergo apoptosis.

In the human testis, testosterone is able to effectively inhibit *in vitro*-induced apoptosis of spermatocytes and spermatids (Erkkila *et al*., 1997). The anti-apoptotic action of testosterone may also be regulated by some of the testicular metabolites of testosterone, such as dihydrotestosterone and estrogens (Rey, 2003). Estrogens are potential regulators of male reproduction and germ-cell death. Low concentrations of 17β estradiol (10^{-9} and 10^{-10} mol/l) effectively inhibit male germ-cell apoptosis in the cultured human seminiferous tubules (Pentikainen *et al*., 2000). Estrogens can also cause alterations in circulating concentrations of gonadotrophins and testosterone and thus affect apoptosis in germ cells indirectly (O'Donnell *et al*., 2001; Pentikainen *et al*., 2003).

To our knowledge, no studies have directly addressed the possible relation between apoptosis and the degeneration of the seminiferous tubules in Klinefelter syndrome.

Conclusions

Severe degeneration of germ cells occurs in the testes of both XXY mice and humans. Descriptive studies of testicular histology clearly show that the disturbances of gonadal development of Klinefelter testes occurs very early in life and progress slowly through infancy with a dramatic acceleration in germ cell and Sertoli-cell degeneration at the onset of puberty. The histological changes found in infancy mainly concern the number of germ cells, whose reduction is highly significant. The endocrinological disturbances of Klinefelter males are likewise noticed already in the neonatal period. Puberty is however initiated with a normal rise in sex hormones but shortly after the level of testosterone levels off and ends at a low-normal level with elevated LH levels (relative hypogonadism). By contrast, inhibin B becomes extremely low or most often undetectable concomitantly with a markedly elevated FSH.

The underlying mechanisms of testicular degeneration are poorly understood. The different hypotheses concerning Leydig-cell insufficiency, impaired somatic environment of the testes, a dysfunctioning communication between the soma and the germ cells, incomplete X-chromosome inactivation as well as disturbed apoptotic activity of Leydig cells and Sertoli cells have been described. We believe that increased expression of genes located on the X chromosome that escape inactivation may play an important role. Existing evidence suggests a role for both germ-cell-specific genes and genes expressed in somatic testicular cells. The findings of the abundant expression of X-chromosome genes in the testis and the recent advances in understanding the genomic organization of the human X chromosome will undoubtedly contribute to a better understanding of the testicular phenotype in Klinefelter patients.

Most of the classical symptoms of Klinefelter syndrome can most probably be ascribed to the relative hypogonadism. It is therefore of utmost importance to detect this syndrome as early as possible to initiate androgen substitution and thereby hopefully prevent or ameliorate those features characterizing Klinefelter adults.

Most Klinefelter males are azoospermic, but some may have residual foci of spermatogenesis. Only few cases of spontaneous conception have been reported, therefore the Klinefelter patients with residual spermatogenesis may benefit from the ICSI treatment combined with the use of TESE. At present, however, we do not have any biochemical parameter for predicting the outcome of TESE. To date, birth of more than 40 healthy children of Klinefelter fathers following the use of ICSI have been reported (Denschlag *et al*., 2004).

Further collaborative studies regarding the efficacy of early androgen substitution as well as the possible fertility options for the Klinefelter patients are strongly needed.

Acknowledgements

The work of the Copenhagen group cited in this review was supported by grants from the Svend Andersen Foundation and the Danish Cancer Society.

References

Almstrup K, Ottesen AM, Sonne SB, Hoei-Hansen CE, Leffers H, Rajpert-De Meyts E and Skakkebaek NE (2005) Genomic and gene expression signature of the pre-invasive testicular carcinoma in situ. Cell Tissue Res.

Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS and Bremner WJ (1996) Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab 81,3341–3345.

Andersson AM, Juul A, Petersen JH, Muller J, Groome NP and Skakkebaek NE (1997) Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. J Clin Endocrinol Metab 82,3976–3981.

Andersson AM, Muller J and Skakkebaek NE (1998a) Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. J Clin Endocrinol Metab 83,4451–4458.

Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O and Skakkebaek NE (1998b) Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metab 83,675–681.

Armstrong SJ, Hulten MA, Keohane AM and Turner BM (1997) Different strategies of X-inactivation in germinal and somatic cells: histone H4 underacetylation does not mark the inactive X chromosome in the mouse male germline. Exp Cell Res 230,399–402.

Arps S, Koske-Westphal T, Meinecke P, Meschede D, Nieschlag E, Harprecht W, Steuber E, Back E, Wolff G, Kerber S et al. (1996) Isochromosome Xq in Klinefelter syndrome: report of 7 new cases. Am J Med Genet 64,580–582.

Aubry F, Satie AP, Rioux-Leclercq N, Rajpert-De Meyts E, Spagnoli GC, Chomez P, De Backer O, Jegou B and Samson M (2001) MAGE-A4, a germ cell specific marker, is expressed differentially in testicular tumors. Cancer 92,2778–2785.

Autio-Harmainen H, Rapola J and Aula P (1980) Fetal gonadal histology in XXXXY, XYY and XXX syndromes. Clin Genet 18,1–5.

Barr ML and Bertram, EG (1949) A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 163,676–677.

Bergere M, Wainer R, Nataf V, Bailly M, Gombault M, Ville Y and Selva J (2002) Biopsied testis cells of four 47,XXY patients: fluorescence in-situ hybridization and ICSI results. Hum Reprod 17,32–37.

Blanco J, Egozcue J and Vidal F (2001) Meiotic behaviour of the sex chromosomes in three patients with sex chromosome anomalies (47,XXY, mosaic 46, XY/47,XXY and 47,XYY) assessed by fluorescence in-situ hybridization. Hum Reprod 16,887–892.

Bojesen A, Juul S and Gravholt CH (2003) Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 88,622–626.

Bojesen A, Juul S, Birkebaek N and Gravholt CH (2004) Increased mortality in Klinefelter syndrome. J Clin Endocrinol Metab 89,3830–3834.

Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R and Willard HF (1991) A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 349,38–44.

Burgoyne PS and Mahadevaiah, SK (1993) Unpaired sex chromosomes and gametogenic failure. Chromosomes Today 11,243–263.

Campbell WA and Price WH (1981) Venous thromboembolic disease in Klinefelter's syndrome. Clin Genet 19,275–280.

Carrel L and Willard HF (1999) Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. Proc Natl Acad Sci USA 96,7364–7369.

Carrel L and Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434,400–404.

Carrel L, Cottle AA, Goglin KC and Willard HF (1999) A first-generation X-inactivation profile of the human X chromosome. Proc Natl Acad Sci USA 96,14440–14444.

Chevret E, Rousseaux S, Monteil M, Usson Y, Cozzi J, Pelletier R and Sele B (1996) Increased incidence of hyperhaploid 24, XY spermatozoa detected by three-colour FISH in a 46,XY/47,XXY male. Hum Genet 97,171–175.

Chong H, Pangas SA, Bernard DJ, Wang E, Gitch J, Chen W, Draper LB, Cox ET and Woodruff TK (2000) Structure and expression of a membrane component of the inhibin receptor system. Endocrinology 141,2600–2607.

Christiansen P, Andersson AM and Skakkebaek NE (2003) Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. J Clin Endocrinol Metab 88,888–891.

Coerdt W, Rehder H, Gausmann I, Johannisson R and Gropp A (1985) Quantitative histology of human fetal testes in chromosomal disease. Pediatr Pathol 3,245–259.

Damani MN, Mittal R and Oates RD (2001) Testicular tissue extraction in a young male with 47,XXY Klinefelter's syndrome: potential strategy for preservation of fertility. Fertil Steril 76,1054–1056.

Denschlag D, Tempfer C, Kunze M, Wolff G and Keck C (2004) Assisted reproductive techniques in patients with Klinefelter syndrome: a critical review. Fertil Steril 82,775–779.

Egozcue J, Blanco J and Vidal F (2002) Meiosis and Klinefelter's syndrome. Hum Reprod 17,3006–3007.

Ekerhovd E and Westlander G (2002) Testicular sonography in men with Klinefelter syndrome shows irregular echogenicity and blood flow of high resistance. J Assist Reprod Genet 19,517–522.

Erkkila K, Henriksen K, Hirvonen V, Rannikko S, Salo J, Parvinen M and Dunkel L (1997) Testosterone regulates apoptosis in adult human seminiferous tubules in vitro. J Clin Endocrinol Metab 82,2314–2321.

Ferguson-Smith MA (1959) The prepubertal testicular lesion in chromatin-positive Klinefelter's syndrome (primary micro-orchidism) as seen in mentally handicapped children. Lancet 1,219–222.

Fernandez-Capetillo O, Mahadevaiah SK, Celeste A, Romanienko PJ, Camerini-Otero RD, Bonner WM, Manova K, Burgoyne P and Nussenzweig A (2003) H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. Dev Cell 4,497–508.

Foresta C, Galeazzi C, Bettella A, Stella M and Scandellari C (1998) High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome. J Clin Endocrinol Metab 83,203–205.

Foresta C, Galeazzi C, Bettella A, Marin P, Rossato M, Garolla A and Ferlin A (1999) Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. J Clin Endocrinol Metab 84,3807–3810.

Froland A and Skakkebaek NE (1971) Dimorphism in sex chromatin pattern of Sertoli cells in adults with Klinefelter's syndrome: correlation with 2 types of "Sertoli-cell-only" tubules. J Clin Endocrinol Metab 33,683–687.

Grootegoed JA, Siep M and Baarends WM (2000) Molecular and cellular mechanisms in spermatogenesis. Baillieres Best Pract Res Clin Endocrinol Metab 14,331–343.

Heller CG and Nelson WO (1945) Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Microscopic picture in the testis and associated changes in the breast. J Clin Endocrinol 5,1–33.

Hunt PA and Eicher EM (1991) Fertile male mice with three sex chromosomes: evidence that infertility in XYY male mice is an effect of two Y chromosomes. Chromosoma 100,293–299.

Hunt PA, Worthman C, Levinson H, Stallings J, LeMaire R, Mroz K, Park C and Handel MA (1998) Germ cell loss in the XXY male mouse: altered X-chromosome dosage affects prenatal development. Mol Reprod Dev 49,101–111.

Iitsuka Y, Bock A, Nguyen DD, Samango-Sprouse CA, Simpson JL and Bischoff FZ (2001) Evidence of skewed X-chromosome inactivation in 47, XXY and 48, XXYY Klinefelter patients. Am J Med Genet 98,25–31.

Jacobs PA and Strong JA (1959) A case of human intersexuality having a possible XXY sex-determining mechanism. Nature 183,302–303.

Kawakami T, Okamoto K, Sugihara H, Hattori T, Reeve AE, Ogawa O and Okada Y (2003) The roles of supernumerical X chromosomes and XIST expression in testicular germ cell tumors. J Urol 169,1546–1552.

Kerr JB (1992) Spontaneous degeneration of germ cells in normal rat testis: assessment of cell types and frequency during the spermatogenic cycle. J Reprod Fertil 95,825–830.

Kleinheinz A and Schulze W (1994) Klinefelter's syndrome: new and rapid diagnosis by PCR analysis of XIST gene expression. Andrologia 26,127–129.

Klinefelter HF, Reifenstein EC and Albright F (1942) Syndrome characterized by gynecomastia, aspermatogenesis without A-Leydigism, and increased excretion of follicle-stimulating hormone. J Clin Endocrinol 2,615–627.

Kotani E, Sugimoto M, Kamata H, Fujii N, Saitoh M, Usuki S, Kubo T, Song K, Miyazaki M, Murakami K et al. (1999) Biological roles of angiotensin II via its type 2 receptor during rat follicle atresia. Am J Physiol 276, E25–E33.

Kubler A, Schulz G, Cordes U, Beyer J and Krause U (1992) The influence of testosterone substitution on bone mineral density in patients with Klinefelter's syndrome. Exp Clin Endocrinol 100,129–132.

Lahlou N, Fennoy I, Carel JC and Roger M (2004) Inhibin B and anti-Mullerian hormone, but not testosterone levels, are normal in infants with non-mosaic Klinefelter syndrome. J Clin Endocrinol Metab 89,1864–1868.

Lanfranco F, Kamischke A, Zitzmann M and Nieschlag E (2004) Klinefelter's syndrome. Lancet 364,273–283.

Lenz P, Luetjens CM, Kamischke A, Kuhnert B, Kennerknecht I and Nieschlag E (2005) Mosaic status in lymphocytes of infertile men with or without Klinefelter syndrome. Hum Reprod 20,1248–1255.

Lin YM, Huang WJ, Lin JS and Kuo PL (2004) Progressive depletion of germ cells in a man with nonmosaic Klinefelter's syndrome: optimal time for sperm recovery. Urology 63,380–381.

Looijenga LH, Gillis AJ, van Gurp RJ, Verkerk AJ and Oosterhuis JW (1997) X inactivation in human testicular tumors. XIST expression and androgen receptor methylation status. Am J Pathol 151,581–590.

Lue Y, Rao PN, Sinha Hikim AP, Im M, Salameh WA, Yen PH, Wang C and Swerdloff RS (2001) XXY male mice: an experimental model for Klinefelter syndrome. Endocrinology 142,1461–1470.

Lue Y, Jentsch JD, Wang C, Rao PN, Hikim AP, Salameh W and Swerdloff RS (2005) XXY mice exhibit gonadal and behavioral phenotypes similar to Klinefelter syndrome. Endocrinology 146,4148–4154.

Madgar I, Dor J, Weissenberg R, Raviv G, Menashe Y and Levron J (2002) Prognostic value of the clinical and laboratory evaluation in patients with nonmosaic Klinefelter syndrome who are receiving assisted reproductive therapy. Fertil Steril 77,1167–1169.

Main KM, Schmidt IM, Toppari J and Skakkebaek NE (2002) Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH. Eur J Endocrinol 146,75–79.

Mikamo K, Aguercif M, Hazeghi P and Martin-Du PR (1968) Chromatin-positive Klinefelter's syndrome. A quantitative analysis of spermatogonial deficiency at 3, 4, and 12 months of age. Fertil Steril 19,731–739.

Miklos GL (1974) Sex-chromosome pairing and male fertility. Cytogenet Cell Genet 13,558–577.

Mroz K, Hassold TJ and Hunt PA (1999) Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. Hum Reprod 14,1151–1156.

Muller J, Skakkebaek NE and Ratcliffe SG (1995) Quantified testicular histology in boys with sex chromosome abnormalities. Int J Androl 18,57–62.

Murken JD, Stengel-Rutkowski S, Walther JU, Westenfelder SR, Remberger KH and Zimmer F (1974) Letter: Klinefelter's syndrome in a fetus. Lancet 2,171.

Nemeth AH, Gallen IW, Crocker M, Levy E and Maher E (2002) Klinefelter-like phenotype and primary infertility in a male with a paracentric Xq inversion. J Med Genet 39, E28.

Nistal M, Paniagua R, Abaurra MA and Santamaria L (1982) Hyperplasia and the immature appearance of Sertoli cells in primary testicular disorders. Hum Pathol 13,3–12.

O'Donnell L, Robertson KM, Jones ME and Simpson ER (2001) Estrogen and spermatogenesis. Endocr Rev 22,289–318.

Oktenli C, Yesilova Z, Kocar IH, Musabak U, Ozata M, Inal A, Gul D and Sanisoglu Y (2002) Study of autoimmunity in Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism. J Clin Immunol 22,137–143.

Oosterhuis JW and Looijenga LH (2005) Testicular germ-cell tumours in a broader perspective. Nat Rev Cancer 5,210–222.

Paulsen CA, Gordon DL, Carpenter RW, Gandy HM and Drucker WD (1968) Klinefelter's syndrome and its variants: a hormonal and chromosomal study. Recent Prog Horm Res 24,321–363.

Peltomaki P, Halme A, and de la Chapelle A (1989) Molecular studies of the sex chromosomes in human testicular cancer: pronounced changes in X and Y chromosome dosage in some tumors. Genes Chromosomes Cancer 1,42–47.

Penny GD, Kay GF, Sheardown SA, Rastan S and Brockdorff N (1996) Requirement for Xist in X chromosome inactivation. Nature 379,131–137.

Pentikainen V, Erkkila K, Suomalainen L, Parvinen M and Dunkel L (2000) Estradiol acts as a germ cell survival factor in the human testis in vitro. J Clin Endocrinol Metab 85,2057–2067.

Pentikainen V, Dunkel L and Erkkila K (2003) Male germ cell apoptosis. Endocr Dev 5,56–80.

Plath K, Mlynarczyk-Evans S, Nusinow DA and Panning B (2002) Xist RNA and the mechanism of X chromosome inactivation. Annu Rev Genet 36,233–278.

Print CG and Loveland KL (2000) Germ cell suicide: new insights into apoptosis during spermatogenesis. Bioessays 22,423–430.

Ratcliffe SG (1982) The sexual development of boys with the chromosome constitution 47,XXY (Klinefelter's syndrome). Clin Endocrinol Metab 11,703–716.

Ratcliffe SG, Murray L and Teague P (1986) Edinburgh study of growth and development of children with sex chromosome abnormalities. III. Birth Defects Orig Artic Ser 22,73–118.

Regadera J, Martinez-Garcia F, Paniagua R and Nistal M (1999) Androgen insensitivity syndrome: an immunohistochemical, ultrastructural, and morphometric study. Arch Pathol Lab Med 123,225–234.

Rey R (2003) Regulation of spermatogenesis. Endocr Dev 5,38–55.

Robinson A, Bender BG, Borelli JB, Puck MH, Salbenblatt JA and Winter JS (1986) Sex chromosomal aneuploidy: prospective and longitudinal studies. Birth Defects Orig Artic Ser 22,23–71.

Ron-El R, Strassburger D, Gelman-Kohan S, Friedler S, Raziel A and Appelman Z (2000) A 47, XXY fetus conceived after ICSI of spermatozoa from a patient with non–mosaic Klinefelter's syndrome: case report. Hum Reprod 15,1804–1806.

Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burrows C, Bird CP et al. (2005) The DNA sequence of the human X chromosome. Nature 434,325–337.

Salbenblatt JA, Bender BG, Puck MH, Robinson A, Faiman C and Winter JS (1985) Pituitary-gonadal function in Klinefelter syndrome before and during puberty. Pediatr Res 19,82–86.

Salido EC, Yen PH, Mohandas TK and Shapiro LJ (1992) Expression of the X-inactivation-associated gene XIST during spermatogenesis. Nat Genet 2,196–199.

Satie AP, Rajpert-De Meyts E, Spagnoli GC, Henno S, Olivo L, Jacobsen GK, Rioux-Leclercq N, Jegou B and Samson M (2002) The cancer-testis gene, NY-ESO-1, is expressed in normal fetal and adult testes and in spermatocytic seminomas and testicular carcinoma in situ. Lab Invest 82,775–780.

Scanlan MJ, Gure AO, Jungbluth AA, Old LJ and Chen YT (2002) Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev 188,22–32.

Shamsuddin AK and Tang CK (1980) Barr bodies in testis with Klinefelter syndrome. Urology 15,74–76.

Sinha Hikim AP and Swerdloff RS (1999) Hormonal and genetic control of germ cell apoptosis in the testis. Rev Reprod 4,38–47.

Skakkebaek NE (1969) Two types of tubules containing only Sertoli cells in adults with Klinefelter's syndrome. Nature 223,643–645.

Skakkebaek NE, Philip J and Hammen R (1969) Meiotic chromosomes in Klinefelter's syndrome. Nature 221,1075–1076.

Sorensen K, Nielsen J, Wohlert M, Bennett P and Johnsen SG (1981) Serum testosterone of boys with karyotype 47,XXY (Klinefelter's syndrome) at birth. Lancet 2,1112–1113.

Spatz A, Borg C and Feunteun J (2004) X-chromosome genetics and human cancer. Nat Rev Cancer 4,617–629.

Spradling A, Drummond-Barbosa D and Kai T (2001) Stem cells find their niche. Nature 414,98–104.

Steinberger E, Smith KD and Perloff WH (1965) Spermatogenesis in Klinefelter's syndrome. J Clin Endocrinol Metab 25,1325–1330.

Suzuki Y, Sasagawa I, Tateno T, Ashida J, Nakada T, Muroya K and Ogata T (2001) Mutation screening and CAG repeat length analysis of the androgen receptor gene in Klinefelter's syndrome patients with and without spermatogenesis. Hum Reprod 16,1653–1656.

Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z and Laron Z (1982) Puberty in 24 patients with Klinefelter syndrome. Eur J Pediatr 139,8–12.

Turner JM, Mahadevaiah SK, Elliott DJ, Garchon HJ, Pehrson JR, Jaenisch R and Burgoyne PS (2002) Meiotic sex chromosome inactivation in male mice with targeted disruptions of Xist. J Cell Sci 115,4097–4105.

Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, Van Steirteghem A and Liebaers I (1996) Cytogenetics of infertile men. Hum Reprod 11,1–24.

Vernaeve V, Tournaye H, Schiettecatte J, Verheyen G, Van Steirteghem A and Devroey P (2002) Serum inhibin B cannot predict testicular sperm retrieval in patients with non-obstructive azoospermia. Hum Reprod 17,971–976.

Vernaeve V, Brugnon F and Tournaye H (2004a) [Inhibin B, predictive factor for testicular sperm recovery?]. Gynecol Obstet Fertil 32,767–770.

Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P and Tournaye H (2004b) Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? Hum Reprod 19,1135–1139.

Wang PJ, McCarrey JR, Yang F and Page DC (2001) An abundance of X-linked genes expressed in spermatogonia. Nat Genet 27,422–426.

Westlander G, Ekerhovd E, Granberg S, Hanson L, Hanson C and Bergh C (2001) Testicular ultrasonography and extended chromosome analysis in men with nonmosaic Klinefelter syndrome: a prospective study of possible predictive factors for successful sperm recovery. Fertil Steril 75,1102–1105.

Westlander G, Ekerhovd E and Bergh C (2003) Low levels of serum inhibin B do not exclude successful sperm recovery in men with nonmosaic Klinefelter syndrome. Fertil Steril 79,1680–1682.

Wikstrom AM, Raivio T, Hadziselimovic F, Wikstrom S, Tuuri T and Dunkel L (2004) Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. J Clin Endocrinol Metab 89,2263–2270.

Yamada T, Horiuchi M and Dzau VJ (1996) Angiotensin II type 2 receptor mediates programmed cell death. Proc Natl Acad Sci USA 93,156–160.

Yamamoto Y, Sofikitis N, Mio Y, Loutradis D, Kaponis A and Miyagawa I (2002) Morphometric and cytogenetic characteristics of testicular germ cells and Sertoli cell secretory function in men with non-mosaic Klinefelter's syndrome. Hum Reprod 17,886–896.

Yan W, Kero J, Huhtaniemi I and Toppari J (2000a) Stem cell factor functions as a survival factor for mature Leydig cells and a growth factor for precursor Leydig cells after ethylene dimethane sulfonate treatment: implication of a role of the stem cell factor/c-Kit system in Leydig cell development. Dev Biol 227,169–182.

Yan W, Samson M, Jegou B and Toppari J (2000b) Bcl-w forms complexes with Bax and Bak, and elevated ratios of Bax/Bcl-w and Bak/Bcl-w correspond to spermatogonial and spermatocyte apoptosis in the testis. Mol Endocrinol 14,682–699.

Yan W, Suominen J and Toppari J (2000c) Stem cell factor protects germ cells from apoptosis in vitro. J Cell Sci 113,161–168.

Zinn AR, Ramos P, Elder FF, Kowal K, Samango-Sprouse C and Ross JL (in press) Androgen receptor CAGn repeat length influences phenotype of 47, XXY (Klinefelter) syndrome. J Clin Endocrinol Metab.

Zitzmann M and Nieschlag E (2003) The CAG repeat polymorphism within the androgen receptor gene and maleness. Int J Androl 26,76–83.

Zitzmann M, Depenbusch M, Gromoll J and Nieschlag E (2004) X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab 89,6208–6217.

Submitted on June 15, 2005; revised on August 1, 2005; accepted on August 15, 2005