# Endocrine aspects of Klinefelter syndrome

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**Purpose of review**
Klinefelter syndrome is the most common sex chromosome abnormality in men. Hypogonadism and testicular degeneration are almost universal. Truncal adiposity, metabolic syndrome and low bone mass occur frequently. This review summarizes the most recent advances in the pathogenesis and management of the endocrine abnormalities in Klinefelter syndrome. It is expected that optimal endocrine management will improve outcomes and quality of life in Klinefelter syndrome.

**Recent findings**
In Klinefelter syndrome, testosterone replacement is routinely prescribed despite lack of evidence on the optimal dose and time for initiation of therapy. Cross-sectional studies have linked hypogonadism to the development of metabolic abnormalities and low bone mass. Testosterone therapy, however, is not consistently associated with improved metabolic and bone outcomes. Increased truncal adiposity and high rates of metabolic syndrome are present in prepubertal children. A randomized trial of oxandrolone in prepubertal boys showed improvement in visual-motor function, socialization and cardiometabolic health. Testicular sperm extraction (TESE) has success rates similar to other causes of nonobstructive azoospermia when performed between 16 and 35 years of age.

**Summary**
Endocrine care in Klinefelter syndrome should start in childhood and include evaluation of metabolic risk factors and bone health. Further research to guide evidence-based endocrine care is very much needed.

**Keywords**
hypogonadism, Klinefelter syndrome, metabolic syndrome, osteoporosis, testicular degeneration, testicular sperm extraction

## INTRODUCTION

Klinefelter syndrome is characterized by the presence of an extra X chromosome in male individuals leading to a 47XXY karyotype and rarely to 46XY/47XXY mosaicism [1,2▪]. It is the most common sex chromosomal aneuploidy with an estimated prevalence of approximately 1 : 650 male births. Individuals with Klinefelter syndrome have frequent developmental deficits, behavioural problems and social difficulties [1,2▪]. From an endocrine perspective, primary testicular insufficiency is the hallmark of the syndrome. In addition, abnormalities in body composition and high rates of metabolic syndrome, diabetes and low bone mass have been described. In this review, we summarize the most recent advances in our understanding and management of endocrine abnormalities in Klinefelter syndrome including an update on fertility preservation.

## TESTICULAR DYSFUNCTION AND HYPOGONADISM

Testicular dysfunction in Klinefelter syndrome involves both Leydig and Sertoli cells. Hypogonadism typically presents during adolescence. Onset of puberty is age appropriate. Testes enlarge to approximately 6–8 ml and then become firm and decrease in size. Serum testosterone concentrations rise, followed by an increase in both luteinizing hormone and follicle-stimulating hormone (FSH) concentrations and then plateau [1,2▪].Testosterone levels are usually sufficient for appropriate virilization. In a cohort of 281 adolescent and young men with Klinefelter syndrome, 62% maintained serum testosterone concentrations above 280 ng/ml despite markedly elevated serum gonadotropins, indicating a state of relative androgen insufficiency [3]. Approximately 50% of adolescent boys and 30% of adults develop persistent gynecomastia [2▪]. Hypogonadism tends to worsen with age, and

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## KEY POINTS

- Increased truncal adiposity and high rates of metabolic syndrome are found in both children and adults with Klinefelter syndrome.
- The effects of hypogonadism and testosterone replacement on the metabolic syndrome and bone mass in Klinefelter syndrome are not fully elucidated because of lack of clinical trials.
- Oxandrolone, a weak androgen, improves visual-motor function, socialization and cardiometabolic health in prepubertal boys with Klinefelter syndrome, according to a recent randomized trial.
- Germ cell loss starts in foetal life and progresses during childhood, while significant testicular fibrosis is already present during peri-pubertal years. Fibrosis worsens in adulthood, but foci of normal spermatogenesis usually remain to allow for fertility preservation.
- Testicular sperm extraction (TESE), when performed at the age between 16 and 35 years, offers fertility options to Klinefelter syndrome men with success rates similar to other causes of nonobstructive azoospermia.

although normal testosterone levels can occasionally be seen in adults, circulating testosterone concentrations fall below normal in most individuals [1].

Sexual function is considered normal [2▪]. More recently, however, increased rates of erectile dysfunction were found compared with healthy men [4▪▪,5▪]. Androgen deficiency and treatment are not consistently associated with erectile dysfunction in Klinefelter syndrome [2▪]. Men with Klinefelter syndrome may experience decreased libido, and psychological factors, such as depression, have been linked to sexual dysfunction [5▪,6]. Few recent reports of gender dysphoria and increased homosexual or bisexual behaviour have emerged, but these findings need further confirmation [4▪▪,7▪,8].

Whether endocrine insufficiency is present before adolescence remains a topic of debate. Both low and normal serum testosterone concentrations have been described during the mini-puberty of infancy [2▪,9]. Congenital urogenital abnormalities, such as micropenis or hypospadias, can be the result of androgen insufficiency during foetal life. Indeed, certain investigators have observed increased frequency of microphallus, cryptorchism and small testis, and perhaps hypospadias, in Klinefelter syndrome [9,10]. Nonetheless, amniotic fluid testosterone concentrations are not different from that of 46 XY male foetuses [2▪].

Sertoli cell dysfunction leading to azoospermia or oligospermia affects almost all patients with nonmosaic Klinefelter syndrome, albeit the underlying mechanisms remain unclear. By utilizing testicular biopsies and transcriptome analysis from foetuses and individuals with Klinefelter syndrome at different ages, recent studies support that germ cell loss is initiated in foetal life [11▪▪,12▪,13,14▪]. Foetuses with Klinefelter syndrome had normal testicular architecture and number of germ cells [11▪▪], but gonocyte maturation into prespermatogonia was impaired, a process that may be linked to an aberrant testicular expression of long noncoding RNAs [12▪]. Prepubertal boys with Klinefelter syndrome demonstrated reduced number of spermatogonia, while the testicular architecture was still preserved and fibrosis was absent. Further reduction in germ cells was seen in peri-pubertal boys, and at this age, a variable degree of testicular fibrosis was already present [11▪▪]. In adults, typical histological findings include absence of germ cells, hyalinization of the seminiferous tubules and Leydig cell hyperplasia, while transcriptome analysis revealed disturbed differentiation of Sertoli and Leydig cells [14▪]. Despite extensive fibrosis, foci of normal spermatogenesis usually remain, which allows for fertility preservation by applying sperm retrieval techniques.

## TESTOSTERONE THERAPY

Testosterone replacement is typically initiated when there are clinical signs of hypogonadism or biochemical evidence of gonadal insufficiency [1,2▪]. However, practices vary and certain physicians prefer to start testosterone therapy at the beginning of puberty when LH and FSH concentrations start rising with the rationale that signs and symptoms of hypogonadism can be subtle and easily missed [15]. These differences in management stem from the fact that only few studies examine the effects of testosterone replacement on various metabolic, bone and mental health outcomes in Klinefelter syndrome, while randomized testosterone trials are missing [16].

Early androgen replacement has been proposed in Klinefelter syndrome to improve motor function, behaviour and cognition. In a retrospective study, Samango-Sprouce *et al.* [17] reported on a group of 34 children with Klinefelter syndrome who were treated with testosterone 25 mg monthly for 3 months for correction of microphallus at ages 4–15 months. Neurocognitive assessment was performed at 36–72 months of age and results were significant for improved performance in multiple neurocognitive domains, including language and neuromotor skills, in treated children compared with untreated controls [17]. In a follow-up study of the same cohort, when the children had reached the ages of 9–11 years, boys treated with testosterone were found to have fewer behavioural problems

and improved social skills [18]. Finally, the effect of low-dose androgens in prepubertal children was examined in a randomized trial by Ross *et al.* [19▪▪]. Eighty-four boys with Klinefelter syndrome were randomized to oxandrolone at 0.06 mg/kg daily vs. placebo. After 24 months, children treated with oxandrolone fared better than controls in visual-motor function and anxiety/depression and social assessment scales. The therapy had no effect on cognitive function, or hyperactive or aggressive behaviours [19▪▪].

## FERTILITY PRESERVATION

Approximately 8% of Klinefelter syndrome men have enough spermatogenic activity that sperm can be found in the semen [1,2▪,20]. For those with azoospermia, fertility preservation is possible using urology techniques such as testicular sperm extraction (TESE) along with intracytoplasmic sperm injection (ICSI). During TESE, the surgeon identifies and targets the foci of normal spermatogenesis that exist within the testis in Klinefelter syndrome. The retrieval rate per TESE cycle is approximately 44% [21▪▪]. Pregnancy and live birth rates are the same, in the order of 40% [21▪▪]. These results are similar to men with nonobstructive azoospermia who have a normal karyotype. Of importance, the risk of chromosomal abnormalities is not increased in the offspring of men with Klinefelter syndrome [21▪▪]. This is likely due to the fact that the extra X is lost from the normal foci of spermatogenesis that are used for sperm retrieval [22]. Preimplantation genetic diagnosis is not currently recommended.

A number of studies have looked at predictors of a successful TESE. Initial studies supported that higher circulating testosterone levels combined with lower LH values result in higher retrieval rates [23]. However, this was not confirmed in a recent meta-analysis [21▪▪]. Other hormonal values, such as FSH and inhibin B, testicular size or surgical technique (i.e. conventional TESE vs. microdissection TESE) do not appear to make a difference [21▪▪]. As testicular fibrosis and germ cell loss increase in Klinefelter syndrome during adult life, it has been proposed that TESE performed at a younger age will improve retrieval rates. Indeed, a number of studies have shown that success rates are higher in individuals younger than 35 [24]. However, performing TESE in adolescents or young men in their early twenties does not further increase retrieval rates [25,26]. A recent meta-analysis of 1248 patients with a mean age $30.9 \pm 5.6$ years failed to document an effect of age on sperm recovery [21▪▪]. Furthermore, TESE retrieval rates in adolescents younger than 16 years are much lower (0–20%), and therefore, the procedure is not recommended at this age [27,28]. Banking of testicular tissue from prepubertal children with Klinefelter syndrome is considered experimental at this point and it may even reduce overall fertility rates, as the procedure may remove functional immature germ cells that may develop into spermatozoa after puberty [27,28]. On the basis of this set of data, the optimal age for TESE in Klinefelter syndrome is a time window between 16 and 35 years.

As sperm recovery rates by TESE are similar between late adolescence and adults, the timing of the procedure can be chosen according to the wishes of the individual. Many teenagers with Klinefelter syndrome are not interested in fertility [29], and the procedure may increase their psychological stress. In contrast, parents and older individuals with Klinefelter syndrome are more eager to pursue a fertility treatment [29,30], and the current data support allowing the individual to reach his own decision on the matter.

Whether previous testosterone therapy affects retrieval rates remains a topic of debate that needs to be clarified with further studies [31]. Moreover, the impact of treatments such as human chorionic gonadotropin or aromatase inhibitors that increase intratesticular testosterone secretion is not well understood in Klinefelter syndrome [31].

## ANTHROPOMETRY AND BODY COMPOSITION

Individuals with Klinefelter syndrome typically have tall stature, which is thought the result of *SHOX* duplication [2▪]. They also have specific anthropometric findings, such as increased leg length, bi-iliac width and hip circumference [32]. Increased BMI has been described in both children and adults, with rates of obesity rising up to 42% among men [33,34]. Frequently described changes in body composition include an increase in fat mass with truncal adiposity and a decrease in lean mass [35▪]. Similar changes have been observed in other men with hypogonadism. However, testosterone replacement has not consistently been found to reverse the body composition changes in Klinefelter syndrome [32,36]. Furthermore, findings of increased visceral fat and unfavourable metabolic profile are seen in children with Klinefelter syndrome compared with their healthy peers [36,37], suggesting that hypogonadism alone may not be solely responsible for these outcomes. Pertinent animal data reveal that X chromosome dosage influences food intake, adiposity and metabolic parameters, further supporting that chromosomal abnormalities may play a role in the unfavourable

body composition and metabolic profile of Klinefelter syndrome [38▪].

## METABOLIC AND CARBOHYDRATE ABNORMALITIES AND EFFECT OF TESTOSTERONE THERAPY

Klinefelter syndrome is associated with an increased risk of insulin resistance, impaired carbohydrate metabolism, dyslipidaemia, diabetes mellitus and metabolic syndrome [32,33,35▪,39]. Metabolic syndrome has been reported in adults with Klinefelter syndrome at rates four to five times greater than controls [1,35▪]. Hypogonadism has been identified in a number of studies as an independent predictor of truncal adiposity, insulin resistance and metabolic syndrome in Klinefelter syndrome [33,35▪]. Although it is well known that testosterone replacement in hypogonadal men improves their metabolic and cardiovascular profile, the data in Klinefelter syndrome indicate that testosterone therapy may improve but not fully restore the cardiometabolic abnormalities of the syndrome [2▪,32,35▪]. Beyond some cross-sectional studies, however, there is lack of randomized trials to fully elucidate the outcomes of testosterone therapy in Klinefelter syndrome and guide clinical practice.

An unfavourable cardiometabolic profile and high rates of truncal adiposity have also been described in children, suggesting that these changes are intrinsic to Klinefelter syndrome and unrelated to hypogonadism [35▪]. However, certain investigators proposed that subtle androgen deficiency exists even in prepubertal children with Klinefelter syndrome, which may also contribute to their increased metabolic risk. In a recent cross-sectional study in 93 boys with Klinefelter syndrome aged 4–12 years, markers of prepubertal gonadal dysfunction, such as inhibin B and antimullerian hormone (AMH), were associated with an unfavourable metabolic profile [37]. To further examine this association, the same cohort was treated with oxandrolone, a weak androgen, in a randomized, placebo-controlled trial. A modest decrease not only in percentage body fat, total cholesterol, triglycerides and SBP, but also in serum high-density lipoprotein (HDL) cholesterol, were found in the oxandrolone-treated group [40▪▪].

Individuals with Klinefelter syndrome have an increased risk for type 2 diabetes mellitus. Diabetes occurs at rates approximately 10% and is one of the causes of increased mortality in Klinefelter syndrome [41,42]. In a recent study of 375 men aged 18–53 years and with a mean BMI of 24.7 $kg/m^2$, prediabetes was observed in 36% and diabetes in 13%, suggesting that carbohydrate abnormalities occur at a younger age and at a lower associated BMI in Klinefelter syndrome compared with the general population [33]. In addition, type I diabetes and presence of diabetes auto-antibodies were recently documented to occur more frequently in Klinefelter syndrome [35▪,43]. These findings are not surprising, as autoimmunity, in general, is increased in Klinefelter syndrome.

## BONE HEALTH

Low bone mass at rates around 25–48% has been reported in Klinefelter syndrome [44]. However, the results vary among reports, and normal bone mass is documented by some investigators [36,44]. The exact fracture rates are unknown. Nonetheless, epidemiologic studies have linked increased morbidity and mortality to fractures in Klinefelter syndrome [41,42], which suggests that bone health is compromised in this population.

Presence of low bone mass is observed in early adolescence [45] and the disease worsens during adulthood [44]. Hypogonadism is frequently considered the underlying culprit and testosterone replacement to improve or prevent bone loss is routinely recommended in hypogonadal individuals with KS. However, the exact effect of hypogonadism and testosterone therapy on skeletal health in Klinefelter syndrome is unclear. Positive correlations between bone mass and serum testosterone levels have been reported, but not consistently [36,44,46]. Furthermore, testosterone therapy may improve but does not fully reverse the low bone mass [44,46,47▪], indicating that additional factors, beyond hypogonadism, may affect bone mass in Klinefelter syndrome. Others have suggested that early therapy starting in adolescence is important to normalize bone mass [44,48].

In addition to hypogonadism, decreased lean mass and increased adiposity have been associated with low bone mass in Klinefelter syndrome [36,47▪]. Few studies point to low circulating 25-hydroxy vitamin D concentrations in Klinefelter syndrome and vitamin D supplementation was able to improve bone mass [49]. In terms of bone turnover, decreased bone formation was described in adolescence and increased resorption in untreated hypogonadal men [50,51▪▪]. Insulin-like protein 3 (INSL3), a bone-anabolic protein produced by the Leydig cells, is reduced in adolescents and adults with Klinefelter syndrome and is proposed as a factor that regulates the cross-talk between testis and bone in Klinefelter syndrome [50,51▪▪]. INSL3 has been shown to act directly on the osteoblast and a recent study provides evidence that it also regulates osteocyte function, which through sclerostin

secretion, may exert a bone antianabolic effect in Klinefelter syndrome [51▪▪]. Elevated serum sclerostin levels were negatively associated with BMD in Klinefelter syndrome [51▪▪], raising the role of sclerostin as a therapeutic target for the treatment of osteoporosis in Klinefelter syndrome.

## CONCLUSION

Although testosterone therapy is routinely prescribed in Klinefelter syndrome, there is still a lack of understanding about the optimal timing for initiation of therapy, appropriate dosing and its exact effects on body composition, metabolic syndrome and bone health. Pertinent studies are cross-sectional or retrospective and randomized controlled trials are missing. Hypogonadism has been implicated in the development of metabolic and skeletal abnormalities of the syndrome, but testosterone therapy does not consistently improve or restore metabolic and bone health. Prepubertal children manifest increased truncal adiposity and high rates of metabolic syndrome, similar to adults. Emerging evidence suggests that low-dose androgens in prepubertal and peri-pubertal boys may improve visual-motor function, socialization and cardiometabolic health. TESE offers fertility options to Klinefelter syndrome men with results similar to other causes of nonobstructive azoospermia. Data indicate that the optimal age for this procedure is between 16 and 35 years. Overall literature data support that endocrine care should be comprehensive, and not just focused on hypogonadism, and appropriate endocrine evaluation should start in childhood to avoid long-term endocrine-related comorbidities.

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### Conflicts of interest

*There are no conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

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

- ▪ of special interest
- ▪▪ of outstanding interest

1. Kanakis GA, Nieschlag E. Klinefelter syndrome: more than hypogonadism. Metabolism 2018; 86:135–144.
2. ▪ Gravholt CH, Chang S, Wallentin M, *et al.* Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. Endocr Rev 2018; 39:389–423.

A comprehensive literature review of Klinefelter syndrome providing a summary of best evidence and highlighting areas of controversy and gaps in research.

3. Rohayem J, Nieschlag E, Zitzmann M, Kliesch S. Testicular function during puberty and young adulthood in patients with Klinefelter's syndrome with and without spermatozoa in seminal fluid. Andrology 2016; 4:1178–1186.
4. ▪▪ Skakkebaek A, Moore PJ, Chang S, *et al.* Quality of life in men with Klinefelter syndrome: the impact of genotype, health, socioeconomics, and sexual function. Genet Med 2018; 20:214–222.

This study involved 103 Klinefelter syndrome mean and 313 healthy controls and observed that men with Klinefelter syndrome had significantly more erectile dysfunction and lower intercourse satisfaction. The study is the largest on this topic and uses healthy controls for comparison, while most of previous studies used men seeking medical attention for infertility or sexual dysfunction as controls.

5. ▪ Ferlin A, Selice R, Angelini S, *et al.* Endocrine and psychological aspects of sexual dysfunction in Klinefelter patients. Andrology 2018; 6:414–419.

This study of 67 men with Klinefelter syndrome and 60 healthy controls showed that testosterone replacement therapy improved sexual desire and overall satisfaction but had no effect on erectile dysfunction, which was increased among Klinefelter syndrome men.

6. El Bardisi H, Majzoub A, Al Said S, *et al.* Sexual dysfunction in Klinefelter's syndrome patients. Andrologia 2017; 49:e12670.
7. ▪ Fernandez R, Guillamon A, Gomez-Gil E, *et al.* Analyses of karyotype by G-banding and high resolution microarrays in the gender dysphoria population. Genes Genomics 2018; 40:465–473.

This study shows an increased frequency of Klinefelter syndrome among 717 individuals with gender dysphoria.

8. Davies GW, Parkinson J. Gender dysphoria in Klinefelter's syndrome: three cases. Australas Psychiatry 2018; 26:313–314.
9. Davis SM, Rogol AD, Ross JL. Testis development and fertility potential in boys with Klinefelter syndrome. Endocrinol Metab Clin North Am 2015; 44:843–865.
10. Akcan N, Poyrazoglu S, Bas F, *et al.* Klinefelter syndrome in childhood; variability in clinical and molecular findings. J Clin Res Pediatr Endocrinol 2018; 10:100–107.
11. ▪▪ Van Saen D, Vloeberghs V, Gies I, *et al.* When does germ cell loss and fibrosis occur in patients with Klinefelter syndrome? Hum Reprod 2018; 33: 1009–1022.

By using immunohistochemistry on testicular biopsies from foetal, prepubertal, peripubertal and adult Klinefelter syndrome patients, the authors describe a significant germ cell loss during childhood. These data question the validity of testicular tissue banking at prepubertal age as a strategy to preserve fertility in Klinefelter syndrome.

12. ▪ Winge SB, Dalgaard MD, Jensen JM, *et al.* Transcriptome profiling of fetal Klinefelter testis tissue reveals a possible involvement of long noncoding RNAs in gonocyte maturation. Hum Mol Genet 2018; 27:430–439.

This study provides evidence that testicular degeneration in Klinefelter syndrome is already initiated in foetal life and examines potential mechanisms.

13. D'Aurora M, Ferlin A, Garolla A, *et al.* Testis transcriptome modulation in Klinefelter patients with hypospermatogenesis. Sci Rep 2017; 7:45729.
14. ▪ Winge SB, Dalgaard MD, Belling KG, *et al.* Transcriptome analysis of the adult human Klinefelter testis and cellularity-matched controls reveals disturbed differentiation of Sertoli- and Leydig cells. Cell Death Dis 2018; 9:586.

The study compares transcriptome data from foetal, prepubertal and adult Klinefelter syndrome testis and indicates that different mechanisms are likely to operate at each developmental stage.

15. Groth KA, Skakkebaek A, Host C, *et al.* Clinical review: Klinefelter syndrome: a clinical update. J Clin Endocrinol Metab 2013; 98:20–30.
16. Nieschlag E, Ferlin A, Gravholt CH, *et al.* The Klinefelter syndrome: current management and research challenges. Andrology 2016; 4:545–549.
17. Samango-Sprouse CA, Sadeghin T, Mitchell FL, *et al.* Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47,XXY syndrome at 36 and 72 months of age. Am J Med Genet A 2013; 161A:501–508.
18. Samango-Sprouse C, Stapleton EJ, Lawson P, *et al.* Positive effects of early androgen therapy on the behavioral phenotype of boys with 47,XXY. Am J Med Genet C Semin Med Genet 2015; 169:150–157.
19. ▪▪ Ross JL, Kushner H, Kowal K, *et al.* Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. J Pediatr 2017; 185:193–199; e4.

This is the first randomized trial of oxandolone vs. placebo in prepubertal and peripubertal boys with Klinefelter syndrome. The study showed positive effects of androgens in visual-motor and psychosocial functions (anxiety/depression and socialization) in treated boys.

20. Kitamura M, Matsumiya K, Koga M, *et al.* Ejaculated spermatozoa in patients with nonmosaic Klinefelter's syndrome. Int J Urol 2000; 7:88–92; discussion 3–4.
21. ▪▪ Corona G, Pizzocaro A, Lanfranco F, *et al.* Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. Hum Reprod Update 2017; 23:265–275.

This meta-analysis reports on the current success rates and predictors of successful TESE in Klinefelter syndrome. Retrieval rates were independent of age, hormonal parameters such as LH, FSH or testosterone, and clinical parameters such as TESE technique or testicular size.

22. Sciurano RB, Luna Hisano CV, Rahn MI, *et al.* Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. Hum Reprod 2009; 24:2353–2360.

**23.** Rohayem J, Fricke R, Czeloth K, *et al.* Age and markers of Leydig cell function, but not of Sertoli cell function predict the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome. Andrology 2015; 3:868–875.

**24.** Aksglaede L, Juul A. Testicular function and fertility in men with Klinefelter syndrome: a review. Eur J Endocrinol 2013; 168:R67–R76.

**25.** Plotton I, Giscard d'Estaing S, Cuzin B, *et al.* Preliminary results of a prospective study of testicular sperm extraction in young versus adult patients with nonmosaic 47,XXY Klinefelter syndrome. J Clin Endocrinol Metab 2015; 100:961–967.

**26.** Nahata L, Yu RN, Cohen LE. Klinefelter syndrome: fertility considerations and gaps in knowledge. Transl Pediatr 2016; 5:183–184.

**27.** Franik S, Hoeijmakers Y, D'Hauwers K, *et al.* Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome. Hum Reprod 2016; 31:1952–1959.

**28.** Gies I, Oates R, De Schepper J, Tournaye H. Testicular biopsy and cryopreservation for fertility preservation of prepubertal boys with Klinefelter syndrome: a pro/con debate. Fertil Steril 2016; 105:249–255.

**29.** Rives N, Milazzo JP, Perdrix A, *et al.* The feasibility of fertility preservation in adolescents with Klinefelter syndrome. Hum Reprod 2013; 28:1468–1479.

**30.** Maiburg MC, Hoppenbrouwers AC, van Stel HF, Giltay JC. Attitudes of Klinefelter men and their relatives towards TESE-ICSI. J Assist Reprod Genet 2011; 28:809–814.

**31.** Rogol AD, Skakkebaek NE. Sperm retrieval in adolescent males with Klinefelter syndrome: medical and ethical issues. Transl Pediatr 2016; 5:104–106.

**32.** Chang S, Skakkebaek A, Trolle C, *et al.* Anthropometry in Klinefelter syndrome: multifactorial influences due to CAG length, testosterone treatment and possibly intrauterine hypogonadism. J Clin Endocrinol Metab 2015; 100:E508–E517.

**33.** Han SJ, Kim KS, Kim W, *et al.* Obesity and hyperglycemia in Korean men with Klinefelter syndrome: the Korean Endocrine Society Registry. Endocrinol Metab (Seoul) 2016; 31:598–603.

**34.** Zeger MP, Zinn AR, Lahlou N, *et al.* Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. J Pediatr 2008; 152:716–722.

**35.** ■ Salzano A, D'Assante R, Heaney LM, *et al.* Klinefelter syndrome, insulin resistance, metabolic syndrome, and diabetes: review of literature and clinical perspectives. Endocrine 2018; 61:194–203.

Broad review of the literature around insulin resistance, metabolic syndrome and carbohydrate abnormalities in KS

**36.** Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. Arch Dis Child 2008; 93:30–34.

**37.** Davis S, Lahlou N, Bardsley M, *et al.* Gonadal function is associated with cardiometabolic health in prepubertal boys with Klinefelter syndrome. Andrology 2016; 4:1169–1177.

**38.** ■ Zore T, Palafox M, Reue K. Sex differences in obesity, lipid metabolism, and inflammation: a role for the sex chromosomes? Mol Metab 2018; 15:35–44.

A review of human data and mouse models with engineered alterations in sex chromosomes providing evidence that sex chromosomes exert independent and interactive effects on obesity and metabolism.

**39.** Lee HS, Park CW, Lee JS, Seo JT. Hypogonadism makes dyslipidemia in Klinefelter's syndrome. J Korean Med Sci 2017; 32:1848–1851.

**40.** ■■ Davis SM, Cox-Martin MG, Bardsley MZ, *et al.* Effects of oxandrolone on cardiometabolic health in boys with Klinefelter syndrome: a randomized controlled trial. J Clin Endocrinol Metab 2017; 102:176–184.

This is the first randomized trial of oxandolone vs. placebo in prepubertal and peripubertal boys with Klinefelter syndrome. The study showed that oxandrolone had modest positive effects on several cardiometabolic parameters, such as adiposity and serum triglycerides. A decrease in HDL cholesterol and an advancement in bone age were also found with oxandrolone.

**41.** Swerdlow AJ, Higgins CD, Schoemaker MJ, *et al.* Mortality in patients with Klinefelter syndrome in Britain: a cohort study. J Clin Endocrinol Metab 2005; 90:6516–6522.

**42.** Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. J Clin Endocrinol Metab 2006; 91:1254–1260.

**43.** Panimolle F, Tiberti C, Granato S, *et al.* Screening of endocrine organ-specific humoral autoimmunity in 47,XXY Klinefelter's syndrome reveals a significant increase in diabetes-specific immunoreactivity in comparison with healthy control men. Endocrine 2016; 52:157–164.

**44.** Ferlin A, Schipilliti M, Di Mambro A, *et al.* Osteoporosis in Klinefelter's syndrome. Mol Hum Reprod 2010; 16:402–410.

**45.** Stagi S, Di Tommaso M, Manoni C, *et al.* Bone mineral status in children and adolescents with Klinefelter syndrome. Int J Endocrinol 2016; 2016:3032759.

**46.** Jo DG, Lee HS, Joo YM, Seo JT. Effect of testosterone replacement therapy on bone mineral density in patients with Klinefelter syndrome. Yonsei Med J 2013; 54:1331–1335.

**47.** ■ Tahani N, Nieddu L, Prossomariti G, *et al.* Long-term effect of testosterone replacement therapy on bone in hypogonadal men with Klinefelter syndrome. Endocrine 2018; 61:327–335.

This study finds reduced bone mass by DXA at lumbar and femur areas in untreated Klinefelter syndrome men. Three years of testosterone therapy improved bone mass at the lumbar area only. Increased adiposity was found to negatively affect bone parameters.

**48.** Wong SC, Scott D, Lim A, *et al.* Mild deficits of cortical bone in young adults with Klinefelter syndrome or anorchia treated with testosterone. J Clin Endocrinol Metab 2015; 100:3581–3589.

**49.** Ferlin A, Selice R, Di Mambro A, *et al.* Role of vitamin D levels and vitamin D supplementation on bone mineral density in Klinefelter syndrome. Osteoporos Int 2015; 26:2193–2202.

**50.** Overvad S, Bay K, Bojesen A, Gravholt CH. Low INSL3 in Klinefelter syndrome is related to osteocalcin, testosterone treatment and body composition, as well as measures of the hypothalamic-pituitary-gonadal axis. Andrology 2014; 2:421–427.

**51.** ■■ Di Nisio A, De Toni L, Rocca MS, *et al.* Negative association between sclerostin and INSL3 in isolated human osteocytes and in Klinefelter syndrome: new hints for testis-bone crosstalk. J Clin Endocrinol Metab 2018; 103:2033–2041.

This study describes a negative association between the Leydig-specific marker INSL3 and sclerostin, which is an osteocyte secreted negative regulator of bone formation, in 103 men suffering from Klinefelter syndrome compared with controls. The association was further confirmed with in-vitro experiments. This study strengthens previous data on the cross-talk between testis and bone in Klinefelter syndrome and highlights sclerostin as a potential therapeutic target for the treatment of osteoporosis in Klinefelter syndrome.