Endocrine CONNECTIONS

REVIEW

Sex chromosome aneuploidies and fertility: 47,XXY, 47,XYY, 47,XXX and 45,X/47,XXX

Alan D Rogol

Department of Pediatrics, University of Virginia, Charlottesville, Virginia, USA

Correspondence should be addressed to A D Rogol: adrogol@comcast.net

Abstract

The overall incidence of sex chromosome aneuploidies is approximately 1 per 500 live-born infants, but far more common at conception. I shall review the fertility aspects of the sex chromosome trisomies, XXY, XYY, and XXX, with special reference to the karyotype 45,X/47,XXX. Each has a 'specific' (but variable) phenotype but may be modified by mosaicism. Although the alterations in the hypothalamic–pituitary–gonadal axis are important (and discussed), the emphasis here is on potential fertility and if one might predict that at various epochs within an individual's life span: fetal, 'mini'-puberty, childhood, puberty, and adulthood. The reproductive axis is often affected in females with the 47,XXX karyotype with diminished ovarian reserve and accelerated loss of ovarian function. Fewer than 5% of females with Turner syndrome have the 45,X/47,XXX karyotype. They have taller stature and less severe fertility issues compared to females with the 45,X or other forms of Turner syndrome mosaicism. For the 47,XXY karyotype, non-obstructive azoospermia is almost universal with sperm retrieval by micro-testicular sperm extraction possible in slightly fewer than half of the men. Men with the 47,XYY karyotype have normal to large testes and much less testicular dysfunction than those with the 47,XXY karyotype. They do have a slight increase in infertility compared to the reference population but not nearly as severe as those with the 47,XXY karyotype. Assisted reproductive technology, especially micro-testicular sperm extraction, has an important role, especially for those with 47,XXY; however, more recent data show promising techniques for the *in vitro* maturation of spermatogonial stem cells and 3D organoids in culture. Assisted reproductive technology is more complex for the female, but vitrification of oocytes has shown promising advances.

Key Words

- sex chromosome
- fertility
- XXY
- XYY
- XXX
- *in vitro* spermatogenesis
- assisted reproductive technology

Endocrine Connections
(2023) **12**, **e220440**

Introduction

Overall the incidence of sex chromosome aneuploidy is quite common, approximately 1 per 500 live births, but far more common at conception. Held and colleagues (1) estimated that less than 1% of conceptions with the 45,X karyotype were live born. In fact, the 45,X karyotype is the single most common chromosomal aneuploidy noted for fetal loss (2).

The purpose of this review is to explore the fertility aspects of the sex chromosome trisomies XXY, XYY, and XXX, with specific reference to the mosaic karyotype 45,X/47,XXX. All have distinct phenotypes with altered hypothalamic–pituitary–gonadal (HPG) axis, as recently reviewed (3). However, these phenotypes and states of the HPG axis are modified by mosaicism often expressed differently among the many tissues and organs of the individual. This report is specifically crafted to be included as part of a single issue of *Endocrine Connections* that gathered papers from the 3rd International Workshop on Klinefelter syndrome, trisomy X, and XYY that was held in Leiden, the Netherlands, on September 12–14, 2022.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0440

It is derived from one of the keynote presentations: *Sex Chromosome Aneuploidies and Fertility: 47,XXY, 47,XYY, 47,XXX, and 45,X/47,XXX*. The purpose of the review is to explore the fertility aspects of the above-noted trisomies with special attention to women with 45,X/47,XXX. The endocrine aspects have been previously reviewed (3). A summary table (Table 1) has been added in which the trisomy sex chromosome aneuploidies are compared and contrasted with reference to the phenotype, karyotype, HPG axis hormonal levels, and fertility preservation options. The data set includes: (1) the specific haryotype with the caveat re: determined or occult mosaicism; (2) circulating levels of hormones of the HPG axis; (3) antral follicle content, when available; and (4) methods of oocyte, ovarian tissue or embryo cryoprecipitation (4). Additional data on the cardiovascular contraindications to fertility preservation are noted in summary (5).

It should be noted that the greatest amount of data are available for men with Klinefelter syndrome and women with Turner syndrome. Many fewer are available for the other trisomies, consisting of case reports and reviews of other case reports. The Turner syndrome is not a sex chromosome trisomy but is relevant to the trisomies because of the 45,X/47,XXX mosaic karyotype. Early primary ovarian failure is common in many girls with Turner syndrome; however, up to one-third will have some degree of spontaneous pubertal maturation, but a much smaller percentage complete puberty spontaneously and even fewer have a spontaneous pregnancy (6, 7). If fertility can be preserved, either spontaneous or through oocyte, ovarian tissue, or embryo cryopreservation (including oocyte vitrification), then one must be concerned that the resulting pregnancy will be high risk with mortality from aortic dissection and morbidity from cardiac (hypertensive) and other associated medical conditions (8, 9). The risk of mortality from aortic dissection may be as high as 150-fold above the general population (10). Those factors more favorable to spontaneous fertility or the greater probability of retrieving oocytes include a karyotype with a second or third cell line, a cell line with more than one X chromosome and spontaneous menarche (11) or low follicle-stimulating hormone (FSH) and high anti-Müllerian hormone (AMH) levels to go along with spontaneous puberty and menarche (6, 7, 8, 9). Those with a lymphocyte (and some other tissues) karyotype, 45,X, and spontaneous puberty or fertility are likely to carry cryptic mosaicism in the ovary (12). By contrast, Goldstein and co-investigators reported a girl who phenotypically had multiple stigmata of Turner syndrome and the biochemical signature of hypergonadotropic hypogonadism but multiple tissues with a 46,XX karyotype. The only tissue that had a 45,X karyotype was in the streak gonad (13).

The emphasis here will be on fertility and how one might predict that at various epochs within an individual's lifespan: fetal, 'mini'-puberty,

Table 1 Phenotype and HPG axis function in sex chromosome trisomies.

Sex chromosome aneuploidy (trisomies)	**Cardinal manifestations**	**HPG axis**
45,X/47,XXX	Spontaneous puberty (all >10 years) Menarche (all >12 years) Pelvic ultrasound – most with normal ovaries n.b. some with premature ovarian failure increased incidence of autoimmunity	Basal FSH within normal limits or slight ↑ Rest of HPG axis mainly within normal limits
47,XXX	Tall stature Hypotonia Spontaneous puberty and menarche (on time) Fertility is likely Increased incidence of autoimmunity	FSH, LH slightly ↑compared to controls ↑LH, FSH to GnRH stimulation E2 INH B, ovarian volume ↓ (All indicative of dysregulated HPG axis at level of ovary) ↑LH, FSH to GnRH stimulation
47,XXY	Tall stature (onset may be in mid-childhood) Accelerated germ cell loss at puberty Small testes (onset mid-adolescence) Speech and behavioral problems	Mini-puberty-contradictory results Childhood-↑INH B; ↑AMH Adolescent usually normal testosterone, but ↑ FSH, LH Adult primary hypogonadism
47,XYY	Tall stature normal or large testes Puberty usually within normal limits (limited data)	FSH ↑ but not to levels in XXY LH ↑but less than FSH Testosterone near bottom of normal range

Details and original references in reference (3).
AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; HPG, hypothalamic–pituitary–gonadal; LH, luteinizing hormone.

childhood, puberty, adulthood, and aging (summarized in Table 1).

Physiology

Fetal

Masculinization of the fetus is a very time-sensitive process depending on androgen action at specific time windows during fetal development. The masculinization programming window (late first to early second trimester) (14) depends on earlier in gestation androgen action (15, 16). It involves the gonadotrophin-releasing hormone neurons developing and moving from their origin (epithelial tissue of the olfactory placode) migrating along nerve fibers through the cribriform plate to the preoptic area of the medial basal hypothalamus (17). These neurons become active to permit the pituitary to secrete luteinizing hormone (LH) and FSH at approximately 9 weeks' gestation in both sexes; however, due to sex hormone negative feedback, the gonadotropin levels decline until birth.

Fetal Leydig cells produce testosterone and the Sertoli cells secrete AMH that leads to the regression of the Müllerian structures (18). The fetal testosterone fosters the development of the male urogenital system. The levels of circulating testosterone are high, and it is assumed that the levels of intra-testicular testosterone are (much) higher. However, there will be no spermatogenesis (capacity for reproduction), just as there is none at 'mini'-puberty because the expression of the androgen receptor on the Sertoli cells remains very low until early childhood (19). The anogenital distance may be useful as a marker of intrauterine androgen action (20). The Leydig cell also produces insulin-like factor 3, an important component acting in concert with testosterone to promote testicular descent (21).

The female fetus lacks AMH, permitting the Müllerian structures to remain, and lacks the testosterone to stimulate the male ducts. The development of primordial follicles precedes the 13th week of gestation, but the bulk of follicular development occurs after the 14th and 15th weeks, with a peak approximating 7×10^6 germ cells by the fifth month of gestation (22, 23). During the third trimester, the pool of oocytes is being established. Then begins a physiological decline until menopause. It should be noted that follicular growth and atresia occur during all stages of development – fetal to menopause. At birth, there are somewhat fewer than 1×10^6 immature follicles, each with an oocyte arrested in prophase of the first meiotic division (24).

Mini-puberty

After birth, the newborn is no longer susceptible to the gonadotropin-inhibiting effects of placental estrogens. The HPG axis becomes progressively more active but with different kinetics in boys and girls (reviewed in (3)). The LH levels are higher in boys than girls and the opposite is true for FSH. LH levels peak within the first 10 weeks in boys resulting in the testosterone peak. The LH levels then decline to prepubertal levels by the sixth month to remain low until the onset of puberty. The FSH levels remain elevated for up to 4 years in girls, although the LH levels decrease by the sixth month.

Normal mini-puberty is likely important, perhaps critical, for future fertility, as there are increases in the numbers of Sertoli cells (25) and germ cells (26). Despite low adult normal values for testosterone with perhaps much higher levels of intra-testicular testosterone, there is no spermatogenesis, since the Sertoli cells do not have active androgen receptors (19).

During mini-puberty, there is augmented linear growth and less fat accumulation in boys compared to girls as well as increased penile growth in the boys (27). Testosterone treatment within the first 4 months of life of boys with Klinefelter syndrome increased body mass, especially the fat-free component as well as increased body length and stretched penile length (28). For those with gonadotropin deficiency, it may be prudent to treat with gonadotropins rather than testosterone to induce mini-puberty, for that should stimulate the Leydig cell (endogenous testosterone) and Sertoli cell (eventual spermatogenesis) functions (29).

There is greater gain in fat mass and lesser gain in fat-free mass in the girls during the first 6 months of life (27) with a greater increase in length in the boys during this time frame, likely due to the effects of testosterone. There appears to be no difference in the length velocity of boys and girls beyond 6 months (30).

The re-activated HPG axis also affects follicular development in mini-puberty and beyond. After approximately 4 months, the gonadotropin and estradiol concentrations decline reaching their nadir (childhood levels) at 1–2 years and remain very low until mid-to-late childhood (see later).

Childhood

Following mini-puberty, one notes relative gonadal quiescence determined by a centrally active suppression of the gonadal axis. This occurs whether the child had primary hypogonadism or is eugonadal and has been studied in girls with Turner syndrome (31) and boys with testicular regression (vanishing testis) syndrome (32). One is aware of some very low-level follicular activity during the pre-pubertal hiatus between mini-puberty and pubertal maturation because the levels of estradiol are significantly higher in girls than boys when estradiol is measured by an appropriately sensitive assay (33).

Puberty

Clinically, puberty is heralded by breast development in girls and testicular enlargement in boys. However, there is evidence for HPG activity and pulsatile release of LH, albeit at very low levels, well before the external signs of pubertal maturation. Pulsatile secretion of LH begins at night and is virtually at low basal levels as the morning begins. Gonadal steroid secretion remains low and then begins to appear early in the morning after the nighttime pulses of LH. As puberty unfolds the secretion of LH continues further into the day, although still showing a nighttime predominance, especially at mid-puberty. Steroid hormone secretion becomes greater and lasts for a greater period of the day before demonstrating the adult pattern very late in pubertal maturation (34, 35, 36, 37).

In boys, intra-testicular concentrations of testosterone up to 100 times those in the circulation and FSH stimulation of the Sertoli cells drive spermatogenesis since the Sertoli cells now have functional androgen receptors (38). In a similar manner, the early stages of follicular development are mainly caused by local factors, but both gonadotropins are necessary for further follicular maturation (39). However, the AMH levels remain rather constant and are an indication of follicular maturation at least through the pre-antral stage.

Karyotype 47,XXX

Trisomy X syndrome occurs in approximately one per thousand live-born females; however, it is estimated that only approximately 10% of these females are ever diagnosed correctly (40). Girls with this syndrome have a wide variety of medical and psychological challenges, most commonly tall stature, hypotonia in infancy, clinodactyly, epicanthal folds, and constipation (41, 42, 43). Multiple psychological difficulties including speech and language deficits, learning disabilities, and attention deficit disorder are common. An unbiased sample of 244,000 women from the UK biobank noted a lower prevalence than previously reported, 45/100,000 (44). The reproductive axis is often affected, but with normal age at menarche, diminished ovarian reserve and an accelerated loss of ovarian function (premature ovarian insufficiency) are prominent. Menopause occurs approximately 5 years earlier (44). Although infertility has been described previously, the UK biobank study noted a similar number of pregnancies and no additional pregnancy loss than 46,XX controls (44). Others have found diminished fertility by approximately 1/3, based on the Danish Cytogenetic Central Registry (45). Osteoporosis and psychological distress are common in women with the 47,XXX karyotype, as are a series of hospitalization-based organ system diagnoses. Those with the mosaic karyotype 46,XX/47,XXX were intermediate between those 47,XXX and those 46,XX (46). Baronchelli and colleagues evaluated 269 women with premature ovarian failure and noted a 5-fold increase of those with 47,XXX karyotype compared to those with 46,XX (47). A similar percentage was noted in a series of 531 Chinese women with premature ovarian failure (~0.6%) (48). In a smaller series of 52 women with premature ovarian failure, 2 were noted to have the 47,XXX karyotype along with autoimmune thyroid disease (49).

Davis and colleagues measured AMH levels, a marker of ovarian reserve for it reflects the primordial follicle pool (43). It is produced by the granulosa cells of the maturing follicles and thus is an index of the remaining number of follicles. The median level for the subjects with trisomy X was significantly below that of control females and all those with the 47,XXX karyotype had serum AMH concentrations below the median level of control subjects with most below the 2.5th percentile. All subjects in both groups had normal levels of gonadotropins and estradiol. Twelve of thirteen girls with the 47,XXX karyotype above the age of 10 years had spontaneous menarche. It is likely in this population that the low AMH level will indicate diminished ovarian reserve and subsequent infertility. Given that the female with trisomy X syndrome is at higher risk for premature ovarian insufficiency, it may be prudent to periodically measure the AMH level and consider fertility preservation options as young adults or as the levels of AMH decrease.

Assisted reproductive technology (ART) for women is more complex than that for men (see later). For women, the steps include ovulatory stimulation, collection of gametes, *in vitro* fertilization including intracytoplasmic sperm injection (ICSI), *in vitro* embryo culture, cryopreservation, embryo transfer, and perhaps trophoblast or blastocyst biopsy (50). More recently oocyte vitrification or fast freezing has replaced the slower method. The oocyte is rapidly supercooled and converted to a glass-like, amorphous solid to specifically prevent ice crystal formation (51). Other applications of this technique include its use on sperm, ovarian tissue, and embryos. There are concerns from animal studies for disordered imprinting of specific genes, but this phenomenon is less well-studied in the human (51). ART is not often required for women with the 47,XXX karyotype or 45,X/47,XXX (see later).

Karyotype 45,X/47,XXX

Special consideration has been given to this variant of the Turner syndrome. This chromosomal mosaic karyotype occurs in less than 5% of females with Turner syndrome (41). Girls with this karyotype have few if any of the common stigmata of Turner syndrome (e.g. cardiovascular and renal anomalies). Short stature may be the only manifestation of a 45,X cell line. Ovarian function in women with the 45,X/47,XXX karyotype ranges from normal to virtually absent with normal ovarian anatomy to streak ovaries, likely depending on the tissue distribution of the two cell lines (52, 53). Follicle number may be low with its attendant premature ovarian insufficiency, but spontaneous (natural) pregnancy is more common than the very low rate in those with 45,X karyotype (52). There are multiple case reports, many with 'a review of the literature' as part of the title. Tang and associates and Lim and associates have described single patients and perhaps have the most robust series of reported cases and case series (54, 55). One might summarize that those with the 45,X/47,XXX mosaic karyotype in general have a mild Turner syndrome phenotype, most, but not all, with short stature, spontaneous pubertal maturation, and menarche without structural abnormalities. Spontaneous pregnancies are far more common in these women than in those with other karyotypes; however, one must consider publication bias, making it likely that the actual pregnancy rate is lower than commonly considered (52, 54). The women are, however, prone to premature ovarian insufficiency, often by age 30 years. That should enter into family planning as an important part of care for those with this mosaic karyotype, encouraging them to start thinking about their fertility at an earlier age. Although oocyte preservation is not so often required compared to women with a 45,X karyotype (noted in the introduction), one should periodically evaluate ovarian reserve and plan for assisted reproduction, if indicated.

Karyotype 47,XYY

The typical man with a 47,XYY karyotype has tall stature (approximately +1 s.d.) and normal sized-to-larger testes, as opposed to the small firm testes of the man with Klinefelter syndrome (56, 57); however, some men do have smaller than normal testicular volume. Although there are limited data, pubertal maturation may be within normal limits and on time (57, 58). For that reason, the genetic diagnosis may be delayed, with a median age of 17.1 years noted by a Danish registry study (59); however, there are many more likely causes of delayed puberty in mid-to-late adolescent males, including the normal variant constitutional delay of growth and puberty and a large number of systemic diseases causing functional hypogonadotropic hypogonadism. Some may be identified in childhood with learning problems and delayed speech and language development, but most are not identified until much later. There are a series of co-morbidities that accompany men with the 47,XYY karyotype: a 2.4-fold increase in hospital-based diagnoses, especially those based on congenital malformations, genetic disorders and disorders of multiple organ systems, compared to a proper control group (60). There was also a 25% increase in the prescription of medications (60).

There is an increased percentage of men with 47,XYY over the general population presenting to infertility clinics (61). Although only approximately 18% of men with the XYY karyotype are eventually diagnosed, most are relatively young (median age at diagnosis is 15.1 years). That is well before issues of infertility present. Diagnosis is often based on behavioral and learning disabilities with mild delays in language and motor development and higher rates of ADHD and autism spectrum disorder (62).

Most published series of diagnosed patients have a bias toward those infertile. Men have an increased incidence of sperm mosaicism and aneuploidy. In addition, sperm maturation may arrest at an immature state (61, 63). Sperm counts may range from within normal limits to frank azoospermia and a low count can contribute to the increased incidence of infertility and require the use of *in vitro* fertilization or ICSI to achieve biological pregnancy (64). Men with the 47,XYY karyotype have a high rate of gonosomal aneuploidy in sperm and pre-implantation embryos (65).

Karyotype 47,XXY

Klinefelter syndrome is the leading genetic cause of testicular failure. Its incidence is approximately 1/650 males (66) with an expanded phenotype from the original description by HF Klinefelter in 1942 (67) of tall stature, gynecomastia and small testes and failure of spermatogenesis. The expanded phenotype now includes prepubertal and pubertal boys as well as behavioral and psychological factors (68, 69). Pubertal maturation usually begins on time but only progresses part way. The height increase may not begin until mid-childhood. The testes may increase to 6–8 mL as expected in an early pubertal male, but then stop growing and even regress to 4–6 mL (70) Testicular maturation starts on time, but by mid puberty there is often a marked rise in FSH concentration and a lesser rise in LH level (70). Testosterone levels are often in the lower range of normal for the stage of maturation but may decrease further as destruction of the testis occurs. As the men age, the expanded phenotype includes a higher prevalence of type 2 diabetes mellitus, dyslipidemia, fatty liver disease, hypercoagulability, osteopenia, and osteoporosis (71).

Histologic evaluation shows a near absence of germ cells in the late adolescent/emerging adult (72, 73, 74). It should be noted that when evaluated by the micro-testicular sperm extraction (microTESE) technique, most men will have some spermatic tubules with sperm (72). The process of tubule cell failure begins at the onset of puberty and then rapidly escalates. Nearly all have spermatogonia on testis biopsy before age 10 years (73, 74). The transition to an emerging adult decreases that number to about 50% (72, 73, 74). However, the external genitalia mature normally (70). By mid-puberty, there is often evidence for androgen deficiency. Gynecomastia rarely begins before puberty and is likely due to low androgen concentration, but increased estrogen levels due to a high level of the aromatase enzyme (75, 76). Early neurodevelopmental, behavioral, and language difficulties are often presenting signs in childhood and may be the factor that sets the diagnostic odyssey in motion (77).

Assisted reproductive technology for men

ART had permitted many men with Klinefelter syndrome (and other causes of male infertility) to father biological children. The various techniques available have been reviewed by Bernie and co-workers (78), but the most relevant to men with Klinefelter syndrome has been microTESE (79) (see also a review of this technique with specific reference to men with Klinefelter syndrome, (80)). At present, it is not possible to use the hormonal signature (LH, FSH, testosterone, and Inhibin B) to predict in whom microTESE will be helpful for either predicting who might have the possibility of sperm extraction or the presence of spermatogonial stem cells (SSCs). The closest to that is that the FSH levels are higher in those whose microTESE harvesting has been unsuccessful compared to those successful; however, the median level of FSH is distinctly elevated in both groups (81).

Spermatagonial stem cells and the future of assisted reproduction

A new and exciting field in ART is the use of SSC and the possibility of expanding and differentiating them to more mature sperm (81). Functional SSCs from the seminiferous tubules may either self-renew or mature to become differentiating spermatogonia and then sperm. These SSCs are key to interventions to restore fertility: by transplantation, testicular tissue grafting, and *in vitro* or *ex vivo* spermatogenesis.

Deebel and colleagues have shown that SSCs exist in men with Klinefelter syndrome, even those in whom microTESE was unsuccessful, but as noted earlier in diminishing numbers as the adolescent and emerging adults mature (81). The seminiferous tubules of the prepubertal male seemingly have more SSCs and may be appropriate for biopsy and extraction; however, all the attempts at extraction and proliferation are considered experimental and no clear protocol exists in man, although there is almost 30 years of experience in the rodent (82). Various ‘adjunctive’ therapies have been tried, including stopping exogenous testosterone therapy a few months before planned sperm extraction, adding therapy with human chorionic gonadotropin (hCG), and selective estrogen receptor modulators or aromatase inhibitors with the goal to attain a proper testosterone to estradiol ratio to support spermatogenesis. Others have used intra-nasal testosterone to attempt to preserve some gonadotropin function that will also support spermatogenesis.

Galdon and co-workers successfully propagated immature human SSC for almost 30 weeks with an approximately 10-fold increase in cell number after 11 days of culture (83). They cultured isolated testicular cells and their numbers markedly expanded. XXY spermatogonia were present at the start of the culture, but at the end, there were not only XXY but also XX and XY spermatogonia. However, the difficult step of *in vitro* spermatogenesis had not been accomplished at the time of that report, but it has been in some murine models (84).

Since germ cell loss accelerates in boys with the Klinefelter syndrome as they enter and progress through pubertal maturation, one might consider retrieving viable spermatogonia from peri-pubertal boys with KS. Testicular biopsies could be stored before expanding their cell numbers in *in vitro* culture. As a proof of concept, Galdon and colleagues isolated testicular cells from three biopsies from adolescents with KS. A heterogeneous mix of SSCs and somatic cells was cultured (85). After a few days, qPCR analysis revealed characteristic gene expression from undifferentiated spermatogonia, Leydig, Sertoli, and peritubular cells with at least 1×10^6-fold increase in cell number. As time in culture increased, in addition to XXY cells, XY and XX cells were identified. The investigators considered such expansion could potentially enable SSC transplantation (85). To date, there is no specific technique to return the cultured cells to the recipient, although in animals direct microinjection to the seminiferous tubules or the rete testis has been accomplished (82). The small, often sclerosed testis of the man with Klinefelter syndrome would present an additional barrier compared to one who survived childhood malignancy (86).

A technologically advanced technique is to use three-dimensional testicular organoids to study human spermatogenesis (and perhaps gonadotoxicity) *in vitro*. Pendergraft and colleagues made such preparations and noted the formation and histology that indicated the three primary cell types, SSCs, Sertoli cells, and Leydig cells (87). They produced testosterone with or without added hCG and had the appropriate cell-specific gene expression over time as well as somatic cell functional markers. A proportion of the SSCs demonstrated spermatogenic differentiation. Although many hurdles remain, for example, spatiotemporal micro-environment and essential regulators of the process, optimization of the system, and the ability to culture organoids from men with sex chromosomal aneuploidies to produce sperm *in vitro* with this system may not be too many years into the future.

Ex vivo spermatogenesis has its own set of challenges including the proper micro- and macro-environment for the culture of testicular fragments (not cells). To date, the systems that have been tried are quite inefficient and not translated to primate testicular tissue (88).

The microTESE technique is successful in perhaps half of the men who undergo that procedure. But what about the other half? Here is where the technique of propagating undifferentiated spermatogonia may be employed. Thus, there is hope that this *in vitro* system may be translated to the infertile men with Klinefelter syndrome, especially those in whom no sperm was extracted on microTESE. One of course would have to consider much younger patients with Klinefelter syndrome because as noted earlier there would be near universal availability of SSCs in those below 10 years with continuing loss as emerging adulthood is achieved. This would be another reason to make the diagnosis of Klinefelter syndrome earlier than at present.

Summary and conclusions

Men and women who have sex chromosome aneuploidies have a number of alterations in their HPG axes (reviewed in (3)) and bio-behavioral development (reviewed in (77)). In addition to relatively wide variation among children and adults with trisomies (XXX, XXY, and XYY), there is the added variability in mosaicism, tissue by tissue. The focus here has been the natural and assisted fertility procedures that may be offered to these patients. Testicular sperm extraction and microTESE have led to a marked rise in possible paternity for men, especially those with Klinefelter syndrome. Newer methods of cryopreservation have made fertility possible for men with trisomies and other conditions that lead to infertility. In addition, cryopreservation (including oocyte vitrification) of follicles or ovarian tissue has changed the fertility landscape for those with mosaic Turner syndrome, perhaps most importantly those with the 45,X/47,XXX mosaic karyotype.

On the horizon are more sophisticated *in vitro* techniques to mature sperm, including co-culture with additional testicular tissue to the point of being able to devise and maintain three-dimensional organoids capable of supporting germ cell differentiation.

Declaration of interest

Dr Rogol is a consultant to: Anteres Pharma, Ascendis Pharma, BioMarin Pharmaceuticals, Lumos Pharma, Tolmar Pharmaceuticals, the United States Anti-doping Agency, and the World Anti-doping Agency. He receives royalties from UpToDate.

Funding

This study did not receive any specific grant from any agency in the public, commercial, or not-for-profit sector.

References

1 Held KR, Kerber S, Kaminsky E, Singh S, Goetz P, Seemanova E & Goedde HW. Mosaicism in 45,X Turner syndrome: does survival in

early pregnancy depend on the presence of two sex chromosomes? *Human Genetics* 1992 **88** 288–294. (https://doi.org/10.1007/BF00197261)

2 Cheng HH, Ou CY, Tsai CC, Chang SD, Hsiao PY, Lau KC & Hsu TY. Chromosome distribution of early miscarriages with present or absent embryos: female predominance. *Journal of Assisted Reproduction and Genetics* 2014 **31** 1059–1064. (https://doi.org/10.1007/s10815-014-0261-9)

3 Rogol AD. Human sex chromosome aneuploidies: the hypothalamic-pituitary-gonadal axis. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 2020 **184** 313–319. (https://doi.org/10.1002/ajmg.c.31782)

4 Cobo A, Garcia-Velasco JA, Remohi J & Pellicer A. Oocyte vitrification for fertility preservation for both medical and nonmedical reasons. *Fertility and Sterility* 2021 **115** 1091–1101. (https://doi.org/10.1016/j.fertnstert.2021.02.006)

5 Wiecek M, Gawlik J, Nowak Z & Gawlik A. Questions concerning fertility preservation during transition in girls with Turner syndrome. Review of the literature. *Endocrine Connections* 2022 **11** e220344. (https://doi.org/10.1530/EC-22-0344)

6 Pasquino AM, Passeri F, Pucarelli I, Segni M & Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1810–1813. (https://doi.org/10.1210/jcem.82.6.3970)

7 Colindres JV, Childress KJ, Axelrad M, McCollough LB, Shao Y, Macias C, Loveless J, Gunn SK, Bercaw-Pratt J, Sutton R, *et al.* A multidisciplinary approach to puberty and fertility in girls with Turner syndrome. *Pediatric Endocrinology Reviews* 2016 **14** 33–47. (https://doi.org/10.17458/PER.2016.CCALM.MultidisciplinaryApproach)

8 Karnis MF. Fertility, pregnancy, and medical management of Turner syndrome in the reproductive years. *Fertility and Sterility* 2012 **98** 787–791. (https://doi.org/10.1016/j.fertnstert.2012.08.022)

9 Oktay K, Bedoschi G, Berkowitz K, Bronson R, Kashani B, McGovern P, Pal L, Quinn G & Rubin K. Fertility preservation in females with Turner syndrome: a comprehensive review and practical guidelines. *Journal of Pediatric and Adolescent Gynecology* 2016 **29** 409–416. (https://doi.org/10.1016/j.jpag.2015.10.011)

10 U.S. Department of Health and Human Services, Health Resources and Services Administration & Maternal and Child Health Bureau. *Women's Health USA*. Rockville, MD, USA: United States Department of Health and Human Services, 2011. (available at: http://www.mchb.hrsa.gov/whusa11)

11 Calanchini M, Aye CYL, Orchard E, Baker K, Child T, Fabbri A, Mackillop L & Turner HE. Fertility issues and pregnancy ouctomes in Turner syndrome. *Fertility and Sterility* 2020 **114** 144–154. (https://doi.org/10.1016/j.fertnstert.2020.03.002)

12 Nadesapillai S, van der Velden J, Smeets D, van de Zande G, Braat D, Fleischer K & Peek R. Why are some patients with 45,X Turner syndrome fertile? A young girl with classical 45,X Turner syndrome and a cryptic mosaicism in the ovary. *Fertility and Sterility* 2021 **115** 1280–1287. (https://doi.org/10.1016/j.fertnstert.2020.11.006)

13 Goldstein DE, Kelly TE, Jojanson AJ & Blizzard RM. Gonadal dysgenesis with 45,XO/46,XX mosaicism demonstrated only in a streak gonad. *Journal of Pediatrics* 1977 **90** 604–605. (https://doi.org/10.1016/s0022-3476(77)80378-8)

14 O'Shaughnessy PJ, Antiognac JP, Le Bizec B, Morvan ML, Svechnokov K, Söder O, Savchuk I, Monteiro A, Saffientini U, Johnston ZC, *et al.* Alternative (back door) androgen production and masculinization in the human fetus. *PLOS Biology* 2019 **17** e3000002. (https://doi.org/10.1371/journal.pbio.3000002)

15 Siiteri PK & Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. *Journal of Clinical Endocrinology and Metabolism* 1974 **38** 113–125. (https://doi.org/10.1210/jcem-38-1-113)

16 Welsh M, Saunders PT, Fisken M, Scott HM, Hutchison GR, Smith LB & Sharp RM. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *Journal of Clinical Investigation* 2008 **118** 1479–1490. (https://doi.org/10.1172/JCI34241)

17 Schwanzel-Fukada M & Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. *Nature* 1989 **338** 161–164. (https://doi.org/10.1038/338161a0)

18 Josso N & Rey RA. What does AMH tell us in pediatric disorders of sex development? *Frontiers in Endocrinology* 2020 **11** 619. (https://doi.org/10.3389/fendo.2020.00619)

19 Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, Gonzalez-Peramato P & Serrano A. Physiological androgen insensitivity of the fetal, neonatal and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4408–4412. (https://doi.org/10.1210/jc.2008-0915)

20 Thankamony A, Pasterski V, Ong KK, Acerini CL & Hughes IA. Anogenital distance as a marker of androgen exposure in humans. *Andrology* 2016 **4** 616–625. (https://doi.org/10.1111/andr.12156)

21 Ivell R, Mamsen LS, Andersen CY & Anand-Ivell R. Expression and role of INSL3 in the fetal testis. *Frontiers in Endocrinology* 2022 **13** 868313. (https://doi.org/10.3389/fendo.2022.868313)

22 Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 1963 **158** 417–433. (https://doi.org/10.1098/rspb.1963.0055)

23 Macklon NS & Fauser BCJM. Aspects of ovarian follicle development throughout life. *Hormone Research* 1999 **52** 161–170. (https://doi.org/10.1159/000023456)

24 Forabosco A, Sforza C, de Pol A, Vizzotto L, Marzona L & Ferrario VF. Morphometric study of the human neonatal ovary. *Anatomical Record* 1991 **231** 201–208. (https://doi.org/10.1002/ar.1092310208)

25 Cortes D, Müller J & Skakkebaek NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *International Journal of Andrology* 1987 **10** 589–596. (https://doi.org/10.1111/j.1365-2605.1987.tb00358.x)

26 Müller J & Skakkebaek NE. Fluctuations in the number of germ cells during the late foetal and early postnatal periods in boys. *Acta Endocrinologica* 1984 **105** 271–274. (https://doi.org/10.1530/acta.0.1050271)

27 Davis SM, Kaar JL, Ringham BM, Hockett CW, Glueck DH & Dabelea D. Sex differences in infant body composition emerge in the first 5 months of life. *Journal of Pediatric Endocrinology and Metabolism* 2019 **32** 1235–1239. (https://doi.org/10.1515/jpem-2019-0243)

28 Davis SM, Reynolds RM, Dabelea DM, Zeitler PS & Tartaglia NR. Testosterone treatment in infants with 47,XXY: effects on body composition. *Journal of the Endocrine Society* 2019 **3** 2276–2285. (https://doi.org/10.1210/js.2019-00274)

29 Main KM, Schmidt IM, Toppari J & Skakkebaek NE. Early postnatal treatment of hypogonadotropic hypogonadism with recombinant FSH and LH. *European Journal of Endocrinology* 2002 **46** 75–79. (https://doi.org/10.153/eje.0.1460075)

30 Kiviranta P, Kuiri-Hännienin T, Saati A, Lamidi ML, Dunkel L & Sankilampi U. Transient postnatal gonadal activation and growth velocity in infancy. *Pediatrics* 2016 **138** e20153561. (https://doi.org/10.1542/peds.2015-3561)

31 Conte FA, Grumbach MM & Kaplan SL. A diphasic pattern of gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. *Journal of Clinical Endocrinology and Metabolism* 1975 **40** 670–674. (https://doi.org/10.1210/jcem-40-4-670)

32 Rey RA. Mini-puberty and true puberty: differences in testicular function. *Annales d'Endocrinologie* 2014 **75** 58–63. (https://doi.org/10.1016/j.ando.2014.03.001)

33 Kline KO, Baron J, Colli MJ, McDonnell DP & Cutler GB, Jr. Estrogen levels in childhood determined by an ultrasensitive recombinant cell

bioassay. *Journal of Clinical Investigation* 1994 **94** 2475–2480. (https://doi.org/10.1172/JCI117616)

34 Abreu AP & Kaiser UB. Pubertal development and regulation. *Lancet. Diabetes and Endocrinology* 2016 **4** 254–264. (https://doi.org/10.1016/S2213-8587(15)00418-0)

35 Grumbach MM. The neuroendocrinology of human puberty revisited. *Hormone Research* 2002 **57**(Supplement 2) 2–14. (https://doi.org/10.1159/000058094)

36 Ropeleto MG, Escobar ME, Gottlieb S & Bergada C. Gonadotropin secretion in prepubertal normal and agonadal children evaluated by ultrasensitive time-resolved immunofluorometric assays. *Hormone Research* 1997 **48** 164–172. (https://doi.org/10.1159/000185508)

37 Wu FC, Butler GE, Kelnar CJ, Huhtaniemi I & Veldhuis JD. Ontogeny of pulsatile releasing hormone secrtion from mid-childhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive innunofluorometric assay. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1798–1805. (https://doi.org/10.1210/jcem.81.5.8626838)

38 Salonia A, Rastrelli G, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, Hellstron WJG, Palmert MR, Corona G, Dohle GR, *et al.* Pediatric and adult-onset male hypogonadism. *Nature Reviews. Disease Primers* 2019 **5** 38. (https://doi.org/10.1038/s41572-019-0087-y)

39 Peters H, Byskov AG & Grinsted J. Follicular growth in fetal and pre-pubertal ovaries of humans and other primates. *Clinics in Endocrinology and Metabolism* 1978 **7** 469–485. (https://doi.org/10.1016/s0300-595x(78)80005-x)

40 Berglund A, Viuff MH, Skakkebaek A, Chang S, Stochholm K & Gravholt CH. Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXY and 47,XYY syndrome: a nationwide cohort study. *Orphanet Journal of Rare Diseases* 2019 **14** 16. (https://doi.org/10.1186/s13023-018-0976-2)

41 Sybert VP. Phenotypic effects of mosaicism for a 47,XXX cell line in Turner syndrome. *Journal of Medical Genetics* 2002 **39** 217–220. (https://doi.org/10.1136/jmg.39.3.217)

42 Tartaglia NR, Howell S, Sutherland A, Wilson R & Wilson L. A review of trisomy X (47,XXX). *Orphanet Journal of Rare Diseases* 2010 **5** 8. (https://doi.org/10.1186/1750-1172-5-8)

43 Davis SM, Soares K, Howell S, Cree-Green M, Buyers E, Johnson J & Tartaglia NR. Diminished ovarian reserve in girls and adolescents with trisomy X syndrome. *Reproductive Sciences* 2020 **27** 1985–1991. (https://doi.org/10.1007/s43032-020-00216-4)

44 Tuke MA, Ruth KS, Wood AR, Beaumont RN, Tyrell J, Jones SE, Yaghootkar H, Turner CLS, Donohoe ME, Brooke AM, *et al.* Mosaic Turner syndrome shows reduced penetrance in an adult population study. *Genetics in Medicine* 2019 **21** 877–886. (https://doi.org/10.1038/s41436-018-0271-6)

45 Stochholm K, Juul S & Gravholt CH. Poor socio-economic status in 47,XXX—an unexpected effect of an extra X chromosome. *European Journal of Medical Genetics* 2013 **56** 286–291. (https://doi.org/10.1016/j.ejmg.2013.03.008)

46 Berglund A, Stochholm K & Gravholt CH. The comorbidity lancscape of 47,XXX syndrome: a nationwide epidemiologic study. *Genetics in Medicine* 2022 **24** 475–487. (https://doi.org/10.1016/j.gim.2021.10.012)

47 Baronchelli S, Conconi D, Panzeri E, Bentivegna A, Radaelli S, Lissoni S, Saccheri F, Villa N, Crosti F, Sala E, *et al.* Cytogenetics or premature ovarian failure: an investigation on 269 affected women. *Journal of Biomedicine and Biotechnology* 2011 **2011** Article ID 370195. (https://doi.org/10.1155/2011/370195)

48 Jiao X, Qin C, Li J, Qin Y, Gao X, Zhang B, Shen X, Feng Y, Simpson JL & Chen ZJ. Cytogenetic analysis of 531 Chinese women with premature ovarian failure. *Human Reproduction* 2012 **27** 2201–2207. (https://doi.org/10.1093/humrep/des104)

49 Goswami R, Goswami D, Kabra M, Gupta N, Dubey S & Dadhwai V. Prevalence of Triple X syndrome in phenotypically normal women with premature ovarian failure and its association with autoimmune thyroid disorders. *Fertility and Sterility* 2003 **80** 1052–1054. (https://doi.org/10.1016/s0015-0282(03)01121-x)

50 Grahan ME, Jelin A, Hoon, Jr AH, Wilms Floet AM, Levey E & Graham EM. Assisted reproductive technology: short- and long-term outcomes. *Developmental Medicine and Child Neurology* 2023 **65** 38–49. (https://doi.org/10.1111/dmcn.15332)

51 Chen H, Zhang L, Meng L, Liang L & Zhang C. Advantages of vitrification preservation in assisted reproduction and potential influences on imprinted genes. *Clinical Epigenetics* 2022 **14** 141. (https://doi.org/10.1186/s13148-022-01355-y)

52 Bouchlariotou S, Tsikouras P, Dimitraki M, Athanasiadis A, Papoulidis I, Maroulis G, Liberis A & Liberis V. Turner's syndrome and pregnancy: has the 45,X/47,XXX mosaicism a different prognosis? Own clinical experience and literature review. *Journal of Maternal-Fetal and Neonatal Medicine* 2011 **24** 668–672. (https://doi.org/10.3109/14767058.2010.520769)

53 Nazarenko SA, Timoshevsky VA & Sukhanova NN. High frequency of tissue-specific mosaicism in Turner syndrome patients. *Clinical Genetics* 1999 **56** 59–65. (https://doi.org/10.1034/j.1399-0004.1999.560108.x)

54 Tang R, Lin L, Guo Z, Hou H & Yu Q. Ovarian reserve evaluation in women with 45,X/47,XXX mosaicism: a case report and a review of the literature. *Molecular Genetics and Genomic Medicine* 2019 **7** e732. (https://doi.org/10.1002/mgg3.732)

55 Lim HH, Kil HR & Koo SH. Incidence, puberty, and fertility in a 45,X/47. *American Journal of Medical Genetics. Part A* 2017 **173** 1961–1964. (https://doi.org/10.1002/ajmg.a.38276)

56 Bardsley MZ, Kowalk K, Levy C, Gosek A, Ayari N, Tartaglia N, Lahlou N, Winder B, Grimes S & Ross JL. 47,XYY syndrome: clinical phenotype and timing of ascertainment. *Journal of Pediatrics* 2013 **163** 1085–1094. (https://doi.org/10.1016/j.jpeds.2013.05.037)

57 Zhao Y, Gradner EJ, Tuke MA, Shang H, Peitzner M, Koprulu M, Jia RY, Ruth KS, Wood AR, Beaumont RN, *et al.* Detection and characterization of male sex chromosome abnormalities in the UK Biobank study. *Genetics in Medicine* 2022 **24** 1909–1919. (https://doi.org/10.1016/j.gim.2022.05.011)

58 Davis SM, Bloy L, Roberts TPL, Kowal K, Alston A, Tahsin A, Truxon A & Ross JL. Testicular function in boys with 47,XYY and relationship to phenotype. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 2020 **184** 371–385. (https://doi.org/10.1002/ajmg.c.31790)

59 Stochholm K, Juul S & Gravholt CH. Diagnosis and mortality in *47,XYY* persons: a registry study. *Orphanet Journal of Rare Diseases* 2010 **5** 15. (https://doi.org/10.1186/1750-1172-5-15)

60 Berglund A, Stochholm K & Gravholt CH. Morbidity in 47,XYY syndrome: a nationwide epidemiological study of hospital diagnoses and medication use. *Genetics in Medicine* 2020 **22** 1542–1551. (https://doi.org/10.1038/s41436-020-0837-y)

61 Borjian Boroujeni P, Sabbaghian M, Vosough Dizaji A, Zarei Moradi S, Almadani N, Mohammadpour Lashkari F, Zamanian MR & Mohseni Meybodi A. Clinical aspects of infertile 47,XYY patients: a retrospective study. *Human Fertility* 2019 **22** 88–93. (https://doi.org/10.1080/14647273.2017.1353143)

62 Ross JL, Roeltgen DP, Kushner H, Zinn AR, Reiss A, Bardsley MZ, McCauley E & Tartaglia N. Behavioral and social phenotypes in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics* 2012 **129** 769–778. (https://doi.org/10.1542/peds.2011-0719)

63 Lim AST, Fong Y & Yu SL. Analysis of sex chromosome constitution of sperm in men with a 47,XYY mosaic karyotype by fluorescence in situ hybridization. *Fertility and Sterility* 1999 **72** 121–123. (https://doi.org/10.1016/s0015-0282(99)00194-6)

64 Kim IW, Khadilkar AC, Ko EY & Sabanegh ES. 47,XYY syndrome and male infertility. *Reviews in Urology* 2013 **15** 188–196.

65 Gonzalez-Merino E, Hans C, Abramowicz M, Englert Y & Emiliani S. Aneuploidy study in sperm and preimplantation embryos from

nonmosaic 47,XYY men. *Fertility and Sterility* 2007 **88** 600–606. (https://doi.org/10.1016/j.fertnstert.2006.12.020)

66 Bojesen A, Juul S & Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 622–626. (https://doi.org/10.1210/jc.2002-021491)

67 Klinefelter HF, Jr, Reifenstein EC, Jr & Albright F. Syndrome characterizdd by gynecomastia, aspermatogenesis, without aleydigism and increased excretions of FSH. *Journal of Clinical Endocrinology* **1942** 615–627. (https://doi.org/10.1210/jcem-2-11-615)

68 Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P & Skakkebaek A. Klinefelter syndrome: integrating genetics, neuropsychology and endocrinology. *Endocrine Reviews* 2018 **39** 389–423. (https://doi.org/10.1210/er.2017-00212)

69 Skakkebaek A, Wallentin M & Gravholt CH. Klinefelter syndrome or testicular dysgenesis: genetics, endocrinology and neuropsychology. *Handbook of Clinical Neurology* 2021 445–462. (https://doi.org/10.1016/b978-0-12-820683-6.00032-4)

70 Davis S, Howell S, Wilson R, Tanda T, Ross J, Zeitler P & Tartaglia N. Advances in the interdisciplinary care of children with Klinefelter syndrome. *Advances in Pediatrics* 2016 **63** 15–46. (https://doi.org/10.1016/j.yapd.2016.04.020)

71 Bojeson A, Host C & Gravholt CH. Klinefelter's syndrome, type 2 diabetes and the metabolic syndrome: the impact of body composition. *Molecular Human Reproduction* 2010 **16** 396–401. (https://doi.org/10.1093/molehr/gaq016)

72 Zitzmann M & Rohayem J. Gonadal dysfunction and beyond: clinical challenges in children, adolescents, and adults with 47,XXY Klinefelter syndrome. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 2020 **184** 302–312. (https://doi.org/10.1002/ajmg.c.31786)

73 Willems M, Gies I & Van Saen D. Germ Cell Loss in Klinefelter Syndrome: When and why. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics* 2020 **184C** 356–370. (https://doi.org/10.1002/ajmg.c.31787)

74 Wikstrő̈m AM & Dunkel L. Testicular function in Klinefelter syndrome. *Hormone Research* 2008 **69** 317–326. (https://doi.org/10.1159/000117387)

75 Tanner M, Miettinen PJ, Hero M, Toppari J & Raivio T. Onset and progression of puberty in Klinefelter syndrome. *Clinical Endocrinology* 2022 **96** 363–370. (https://doi.org/10.1111/cen.14588)

76 Wosnitzer MS & Paduch DA. Endocrinological issues and hormonal manipulation in chidren and men with Klinefelter syndrome. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics* 2013 **163C** 16–26. (https://doi.org/10.1002/ajmg.c.31350)

77 Tartaglia N, Howell S, Davis S, Kowal K, Tanda T, Brown M, Boada C, Alston A, Crawford L, *et al.* Early neurodevelopmental and medical profile in children with sex chromosome trisomies: Background for the prospective eXtraordinarY babies study to identify early risk factors and targets for intervention. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics* 2020 **184** 428–443. (https://doi.org/10.1002/ajmg.c.31807)

78 Bernie AM, Mata DA, Ramasamy R & Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis. *Fertility and Sterility* 2015 **104** 1099–103.e1. (https://doi.org/10.1016/j.fertnstert.2015.07.1136)

79 Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Human Reproduction* 1999 **14** 131–135. (https://doi.org/10.1093/humrep/14.1.131)

80 Fainberg J, Hayden RP & Schlegel PN. Fertility management of Klinefelter syndrome. *Expert Review of Endocrinology and Metabolism* 2019 **14** 369–380. (https://doi.org/10.1080/17446651.2019.1671821)

81 Deebel NA, Galdon G, Zarandi NP, Stogner-Underwood K, Howards S, Lovato J, Kogan S, Atala A, Lue Y & Sadri-Ardekani H. Age-related presence of spermatogonia in patients with Klinefelter syndrome: a systematic review and meta-analysis. *Human Reproduction Update* 2020 **26** 58–72. (https://doi.org/10.1093/humupd/dmz038)

82 Brinster RL & Zimmerman JW. Spermatogenesis following male germ cell transplantation. *Proceedings of the National Academy of Sciences of the United States of America* 1994 **91** 11298–11302. (https://doi.org/10.1073/pnas.91.24.11298)

83 Galdon G, Deebel NA, Pourhabibi-Zarandi NP, Pettenati MJ, Kogan S, Wang C, Swerdloff RS, Atala A, Lue Y & Sadri-Ardekani H. *In vitro* propagation of XXY undifferentiated mouse spermatogonia: model for fertility preservation in Klinefelter syndrome Patients. *International Journal of Molecular Sciences* 2021 **23** 173. (https://doi.org/10.3390/ijms23010173)

84 Sato T, Katagiri K, Kojima K, Komeya M, Yao M & Ogawa T. *In vitro* spermatogenesis in explanted adult mouse testis tissues. *PLoS One* 2015 **10** e0130171. (https://doi.org/10.1371/journal.pone.0130171)

85 Galdon G, Deebel NA, Zarandi NP, Teramoto D, Lue Y, Wang C, Swerdloff R, Pettenati MJ, Kearns WG, Howards S, *et al. In vitro* propogation of XXY human Klinefelter spermatogonial stem cells: a step towards new fertility opportunities. *Front Reprod* 2022 epub in advance of print. (https://doi.org/10.3389/fendo.2022.1002279)

86 Eugeni E, Arato I, del Sordo R, Sidoni A, Garolla A, Ferlin A, Calafiore R, Brancorsini S, Mancuso F & Luca G. Fertility preservation and restoration options for pre-pubertal male cancer patients: current approaches. *Frontiers in Endocrinology* 2022 **13** 877537. (https://doi.org/10.3389/fendo.2022.877537)

87 Pendergraft SS, Sadri-Ardekani H, Atala A & Bishop CE. Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity *in vitro*. *Biology of Reproduction* 2017 **96** 720–732. (https://doi.org/10.1095/biolreprod.116.143446)

88 Sanou I, van Maaren J, Eliveld J, Lei Q, Meissner A, de Melker AA, Hamer G, van Pelt AMM & Mulder CL. Spermatogonial stem cell-based therapies: taking preclinical research to the next level. *Frontiers in Endocrinology* 2022 **13** 850219. (https://doi.org/10.3389/fendo.2022.850219)

Received 19 October 2023
Accepted 3 July 2023
Available online 3 July 2023
Version of Record published 1 August 2023