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Primary gonadal failure

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The term primary gonadal failure encompasses not only testicular insufficiency in 46,XY males and ovarian insufficiency in 46,XX females, but also those disorders of sex development (DSD) which result in gender assignment that is at variance with the genotype and gonadal type. In boys, causes of gonadal failure include Klinefelter and other aneuploidy syndromes, bilateral cryptorchidism, testicular torsion, and forms of 46,XY DSD such as partial androgen insensitivity. Causes in girls include Turner syndrome and other aneuploidies, galactosemia, and autoimmune ovarian failure. Iatrogenic causes in both boys and girls include the late effects of childhood cancer treatment, total body irradiation prior to bone marrow transplantation, and iron overload in transfusion-dependent thalassaemia.

In this paper, a brief description of the physiology of testicular and ovarian development is followed by a section on the causes and practical management of gonadal impairment in boys and girls. Protocols for pubertal induction and post-pubertal hormone replacement - intramuscular, oral and transdermal testosterone in boys; oral and transdermal oestrogen in girls - are then given. Finally, current and future strategies for assisted conception and fertility preservation are discussed.

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Definition of primary gonadal failure

The term primary gonadal failure includes impairment of the Leydig cell, Sertoli cell and germ cell systems in the testis, and impairment of the theca/granulosa cell unit and germ cell systems in the ovaries. In this paper, the definition is extended to include subjects with gonadal insufficiency caused by disorders of sex development (DSD) resulting in some individuals being raised as either male or female in contrast to their gonadal and/or genetic type. The terms primary gonadal failure in males and primary gonadal failure in females will therefore be applied to individuals with either testicular or ovarian insufficiency, or gonadal problems leading to male or female gender assignment. The term primary ovarian insufficiency (POI) will be applied to situations where there is ovarian impairment.

Physiology of primary sexual differentiation (see Fig. 1)

Sex determination - testis

Following fertilisation, development of the intermediate mesoderm of the embryo is controlled by genes such as *empty spiracles homeobox 2 (EMX2)*, *LIM Homeobox 1 (LHX1)*, and *PAX2*. Development of the bi-potential gonad requires *Steroidogenic factor 1 (SF1)* and *Wilms' tumour-associated gene 1 (WT1)* [1]. Differentiation of the testis requires expression of the *Sex-determining Region of the Y chromosome (SRY)* gene which activates *SRY-box containing gene 9 (SOX9)* and *fibroblast growth factor 9 (FGF9)* [1–3]. In addition, the *wingless-type MMTV integration site family member 4 (WTN4), respondin-1 (RSPO1)* and *β-catenin (CTNNB1)* genes, which play an important role in ovarian development, are suppressed by *SOX9* in the male determination pathway [4]. Other repressive genes in testis differentiation include *forkhead box L-2 (FOXL2)* and *dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX1)*.

Primary sexual differentiation - male

Internal genitalia. The testicular cords develop from week 6 and contain the gonocytes, the Sertoli cells which secrete anti-Müllerian hormone (AMH), and the Leydig cells which produce testosterone from week 8 [5]. Testosterone stabilizes and develops the Wolffian ducts while AMH causes regression of the Müllerian ducts.

External genitalia. The enzyme 5-α reductase converts testosterone into dihydrotestosterone (DHT) which causes virilisation of the urogenital sinus and external genitalia between 10 and 14 weeks. From week 18 to the final two months of pregnancy, various factors including foetal luteinising hormone (LH), testosterone, insulin-like hormone 3 (INSL3), AMH and other factors play a role in the descent of the testis from the abdomen to the scrotum [6,7].

The testis after birth

During the few months after birth testosterone levels increase, Sertoli cells proliferate and germ cells develop, although meiosis does not start until puberty. This so-called "mini-puberty" may be a significant time for male gender identity development [7]. Until activation of the hypothalamo-pituitary-gonadal (HPG) axis at adolescence, the Leydig cells produce little testosterone, so that AMH is the preferred marker for assessing testicular function during childhood [8].

During puberty, secondary sexual development is testosterone-rather than DHT-dependent. Increased testosterone production results in the germ cells now entering meiosis. The increase in testicular volume at puberty is due mostly to onset of spermatogenesis, with only a modest contribution from Leydig cells. Testosterone induces maturation of Sertoli cells with decreased AMH production, while FSH stimulation increases inhibin B which becomes the dominant hormone produced by the Sertoli cells [9].

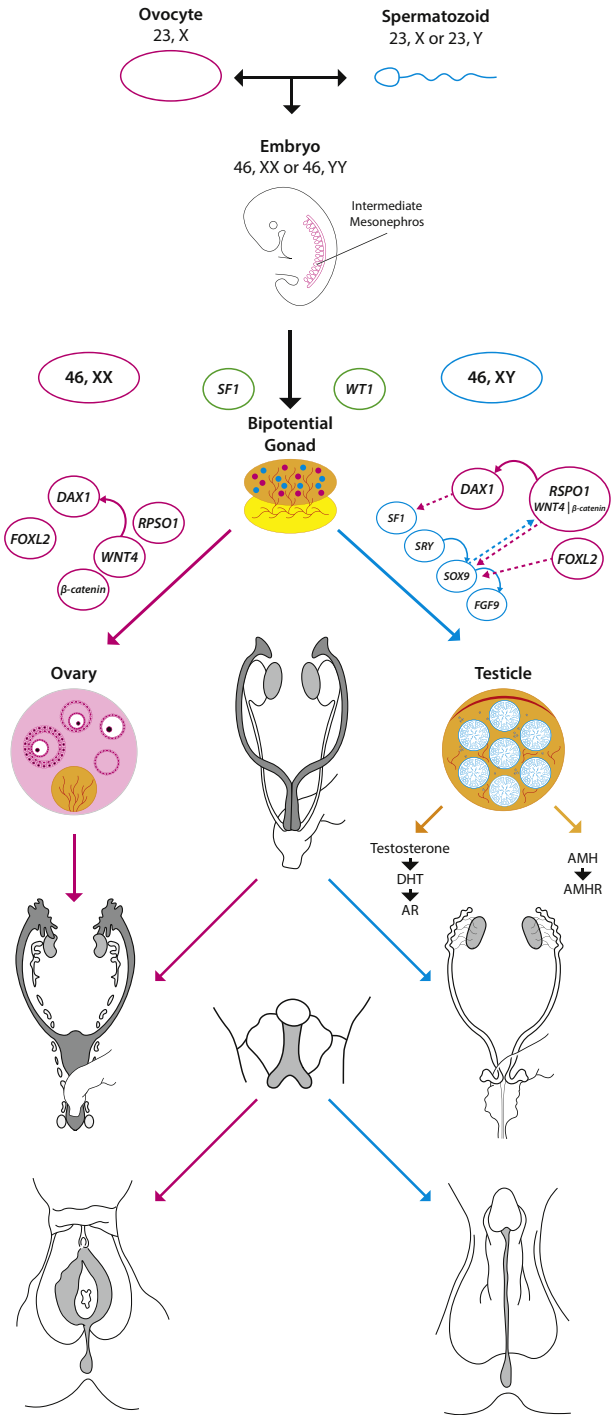

Fig. 1. Schematic representation of male and female sex determination and primary differentiation. Please see text for abbreviations. For the pathways between bipotential gonad and ovary (left side); and testis (right side), blue arrows represent activation/stimulation while pink bars represent inhibition/suppression.

Aromatisation of testosterone to estrogen stimulates the growth hormone axis, thus causing the growth spurt. Larger amounts of estrogen secreted towards the end of puberty then result in epiphyseal fusion and cessation of growth.

Sex determination - ovary

Development of the ovary begins at week 7. Ovarian determination seems to result from an interplay of genes including *RSPO1* which interacts with *WNT4* by binding to the LGR4/5/6 receptors to activate and stabilize *β-Catenin* which in turn upregulates the transcription of WNT-dependant genes [1]. *WNT4* also suppresses *SOX9* as well as upregulating *DAX1*, which antagonises *SF1*. Moreover in the absence of *SRY, SOX9* cannot drive Sertoli cell formation and thus testis differentiation. *FOXL2*, apart from possessing an anti-testis action by repressing *SOX9*, is also important in ovarian development and maintenance, preventing trans-differentiation of the adult ovary to a testis [10]. Genes not directly involved in the ovarian determination but essential for ovarian function and oocyte survival include *factor in the germline α* (*FIGLA*) and *newborn ovary homeoBOX-encoding gene* (*NOBOX*). Bone morphogenic protein 15 (*BMP15*) and growth differentiation factor 9 (*GDF9*) are also crucial for ovarian follicle physiology, probably by controlling its metabolism [11].

Female development

The bipotential gonad is colonized by primordial germ cells of epiblastic origin during week 5. In the 46,XX embryo the different cell lineages of the bipotential gonads differentiate into granulosa (supporting cells), theca (steroidogenic cells), stromal cells (connective cells) and oogonia (germ cells). Ovarian development is characterized by three steps: the entry of the oogonia into meiosis to become primary oocytes during weeks 11 and 12; surrounding of the oocytes by granulosa cells to form follicles; and differentiation of the theca cells to produce steroids. *RSPO1*, *WNT4* and *WNT5* promote the proliferation and the initiation of meiosis of oogonia. Folliculogenesis starts after weeks 11–12 and is controlled by several genes including *FOXL2* whose mutations lead to ovarian insufficiency [12].

In the absence of a testis and hence of AMH production, the Müllerian ducts develop to form the Fallopian tubes, uterus and upper third of the vagina. In the absence of testosterone and DHT, the urogenital sinus develops in to the lower 2/3 of the vagina, the genital and urethral folds remain unfused and develop into the labia minora (homologous with the penile shaft) and labia majora (homologous with the scrotum); and the genital tubercle develops into the clitoris (homologous with the glans penis).

The ovary after birth

At 20 weeks' gestation there are up to seven million germ cells in the foetal ovary. Around the time of birth, the ovary contains its maximum quota of around 400,000 primordial follicles. These contain the primary oocytes, which do not undergo further mitotic division and remain arrested in the prophase stage of the first meiotic division until sexual maturity. The growth of the ovary from the time of birth until menarche is due to an increase of its stroma. Simultaneously there is a decrease in the number of oocytes, mostly occurring during the first year of life [13].

From mid-childhood, pulsatile secretion of hypothalamic GnRH results in increase in both frequency and amplitude of pulses of LH and FSH secretion by the anterior pituitary gland, culminating in the physical features or puberty and gametogenesis. FSH stimulates the growth of ovarian follicles and, with LH, stimulates production of estradiol by the ovaries. Estradiol stimulates breast development as well as the pubertal growth spurt, followed by eventual fusion of the epiphyses. At the onset of puberty, FSH and LH stimulate development of the primordial follicles. In each ovarian cycle, about 20 primordial follicles are activated to begin maturation. Of these, only one fully matures, the rest contributing to the endocrine function of the ovary. AMH, secreted by the granulosa cells is an important marker of ovarian reserve but also plays a role in the selection of the dominant follicle [14,15]. When activated, the oocyte completes its first meiotic division and the primary follicle matures into a secondary follicle. The second division then starts, and a Graafian follicle is formed, containing a secondary oocyte. This second division is not completed, unless the ovum is fertilised. A detailed description of the menstrual cycle can be found elsewhere [16].

History, examination and investigation in suspected primary gonadal insufficiency

A sound history and a focused, appropriate physical examination are prerequisites for clinical diagnosis. Only after this process should investigations be considered.

History

This includes:

- Prenatal history (including any maternal medications)
- Birthweight and gestation (NB the association between low birth weight/smallness-for-gestational age and DSD)
- Noting any history of cryptorchidism, hypospadias and surgical intervention
- General health including middle ear infections (suggestive of Turner syndrome in short girls)
- Nature and treatment of childhood malignancy
- Pubertal milestones (voice-deepening, and onset of growth spurt in boys; onset of breast development and age at menarche in girls)
- Family history: name, age, health and pubertal history of each family member; details of consanguinity; pubertal development, genital anomaly and fertility in the wider family. A history of absent menarche and infertility on one side of the family raises the possibility of an X-linked disorder such as complete androgen insensitivity syndrome.
- Social and educational history including the need for extra support at school. This is relevant to management and may aid diagnosis of syndromic disorders affecting the gonads (e.g. Noonan syndrome).

Examination

This comprises:

- Meticulous auxology with measurement of standing height, weight and - in selected cases - sitting height. Measured rather than reported parental heights should always be sought [17].
- Search for dysmorphic features
- General examination with attention to the cardiovascular system including blood pressure measurement and femoral artery palpation (relevant to Turner syndrome).
- Tanner staging: examination of the penis with testicular palpation and volume measurement in boys; breast development in girls; and axillary and pubic hair in both sexes.

Genital/breast examination requires clear and sensitive explanation *before* any examination is performed [18]; and appropriate chaperoning using either parents or nursing staff.

In boys it is important to palpate the testes to assess not only volume but also texture and consistency, noting the firm texture and smooth capsule of the normal testis, and either excessive firmness or softness (with 'squashy' texture) in dysgenetic testes.

Investigation

This depends on the clinical scenario and may include:

- Biochemical parameters - gonadotrophins, testosterone and estradiol; AMH and Inhibin B
- Diagnostic imaging - abdominal and pelvic ultrasound, computerised tomography (CT) and magnetic resonance imaging (MRI) to demonstrate internal genitalia including gonads and Müllerian structures
- Laparoscopy with biopsy of relevant tissues
- Molecular genetic testing
- Sperm count and analysis in selected patients

Pelvic ultrasound is an observer-dependant technique. Specialist centres should consider developing their own normative data for uterine length/volume and shape, and ovarian volume/follicular activity [19].

Primary gonadal failure in males

Presentation and investigation of male primary gonadal failure according to age

Birth and early infancy

According to the cause and severity of gonadal failure, and the testicular compartments involved - Leydig or Sertoli cells, or both – the spectrum of presentation will range from normal male phenotype ± cryptorchidism, through complex genital anomaly, to normal female phenotype. The state of mini-puberty at this age facilitates investigation [20]. Thus, low testosterone and elevated gonadotrophins indicate impaired Leydig cell function while AMH levels are also informative with very low/unrecordable values indicating absence of testicular tissue; low levels indicating dysgenetic testes; normal levels indicating normal Sertoli cell function; and high levels indicating androgen insensitivity syndrome [21–24].

Childhood

Small or non-palpable testes, with or without genital anomaly can be an indication of gonadal impairment. Because the hypothalamic-pituitary-gonadal axis is quiescent during childhood, measuring AMH is more informative than testosterone and gonadotrophin determination. Measurement of inhibin B as well as testicular volume may also be helpful in assessing testicular function in prepubertal children [25]. In selected cases, testosterone measurement after stimulation with human chorionic gonadotrophin (hCG) is useful. Administration of extractive human chorionic gonadotrophin (hCG), given as between 3 and 6 daily intramuscular injections of 500 international units (IU) is traditional. However, availability of this preparation is problematic in some countries, and a single dose of recombinant hCG (hCG, Ovitrelle® 250 μg) may replace standard protocols. When genetic analysis is unavailable [26–28], post-hCG levels of testosterone and DHT, together with AMH, help to distinguish between:

- Gonadal dysgenesis (↓testosterone and ↓AMH)
- Biosynthetic defects (↓testosterone, normal AMH)
- 5α-reductase deficiency (↑testosterone, ↓ DHT, normal AMH)
- Androgen receptor mutation (↑testosterone, normal DHT, ↑/normal AMH)

Adolescence

Adolescents with primary gonadal failure may present with absent or delayed puberty, or with pubertal arrest in which puberty starts but then fails to progress. In severe deficiency the adolescent growth spurt is absent so that patients generally remain short in relation to the parental heights, although boys with Klinefelter syndrome are often above average in height. Gynecomastia is a frequent finding [29]. At this age, AMH is a less useful index of gonadal failure since levels decrease at the onset of normal puberty, but FSH is now a sensitive measure of gonadal failure. Inhibin B levels, which are of value in discriminating between constitutional delay in growth and adolescence and congenital hypogonadotropic hypogonadism [30,31], are also reduced in primary testicular failure [32].

Late adolescence and adulthood

Patients with primary gonadal hypogonadism developing in adulthood may present with specific features of testosterone deficiency - loss of body hair, reduced testicular volume (<6 ml), libido, sexual activity, erectile dysfunction; and with less specific features such as hot flushes and sweats, psychological disturbances and poor sleep pattern.

Investigation comprises measurement of LH, FSH and serial fasting morning testosterone – either total with free testosterone measurement in borderline cases or when sex-hormone-binding globulin (SHBG) levels are abnormal. Semen analysis should also be performed, particularly when there are concerns about fertility [33,34].

Primary gonadal failure in males (see Table 1)

Congenital causes

Chromosomal anomalies

Klinefelter syndrome (47,XXY). This is the most common chromosomal anomaly with an estimated frequency of 1:500 to 1:1000 men [35]. Karyotype is 47,XXY in 90% of cases, with mosaic karyotypes (e.g. 46,XY/47,XXY) and higher grade aneuploidy (e.g. 48,XXXY; 49,XXXXY) accounting for the remaining 10%.

Presentation. Most cases present in adulthood with infertility associated with small testes, azoospermia and gynaecomastia. Only a minority of patients are diagnosed in childhood, representing the 'tip of the iceberg', often presenting with learning difficulties and psychological disorders so that the prevalence of these problems in the childhood Klinefelter population is skewed, estimated at 77% [36].

Table 1
Aetiologies of primary gonadal failure in males.

Congenital	
Chromosomal	- 47,XXY (Klinefelter Syndrome) and variants - 47,XYY syndrome and variants e.g. 48,XXYY - 45X,46,XY DSD - Y chromosome microdeletions - Chromosomal anomalies: deletions (9p), translocations
Genetic (DSD)	- 46,XX DSD with complete sex reversal (SRY+); and with testicular dysgenesis - 46,XY DSD with: - Partial gonadal dysgenesis (mutations of SRY, SF1, WT1, SOX9) - Disorders of androgen synthesis ((StAR, POR deficiency, 17-α OH-ase, 3 β-HSD, 17β-HSD and 5-α-reductase deficiency) - Gonadotrophin resistance (Leydig cell hypoplasia with LH-receptor mutation; and FSH-receptor mutation - Disorders of androgen action (partial androgen insensitivity) - AMH and AMH receptor mutations - INSL3 mutations
Malformative causes	- Congenital anorchidism and 'Vanishing Testis Syndrome' - Other polymalformative syndromes
Genetic causes (non DSD)	- Myotonic Dystrophy
Non-genetic causes	- Syndromic causes (e.g. Noonan, Down, Prader-Willi syndrome) - Syndrome with IUGR (IMAGe, Silver-Russel Syndrome) - Bilateral cryptorchidism
Acquired	
Testicular abnormality/injury	- Varicocele - Testicular torsion (perinatal and childhood) - Testicular trauma
Infectious	- Mumps
Post infectious orchitis	- Tuberculosis
Auto-immune orchitis	- Isolated auto-immune or part of polyglandular syndrome
Chronic illness	- Granulomatous diseases - Amyloidosis - Cystic fibrosis (with absence of vas deferens causing infertility) - Chronic pulmonary disease - Renal failure - Neurologic disorders (adolescents and adults)
Iatrogenic	- Total body irradiation for bone marrow transplantation for treatment of childhood cancer or non-malignant conditions (thalassemia) - Abdominal irradiation as a consequence of cranio-spinal irradiation or direct irradiation for intra-abdominal cancer - Chemotherapy - Surgery (bilateral castration) - Iron overload secondary to treatment of thalassemia
Environmental	- Environmental toxins

Klinefelter syndrome may also be diagnosed prenatally, for example during antenatal screening in older mothers.

Although patients presenting in childhood may have relatively tall stature with long legs, reduced testicular size and micropenis [36,37], examination is usually unremarkable. Delay in early language development, attention deficit disorder, and behavioural problems [38] sometimes requiring special education may occur. However, cognitive ability is usually in the normal range, with IQ 88–110 [36]. Early treatment with testosterone is reported to improve neurodevelopment [39].

Puberty usually proceeds normally in terms of penile development and other sexual characteristic but testicular volume increases only marginally (usually <4 ml) [40], although temporary increases to 10–13 ml can occur at this time. Patients are usually taller than their peers and pubertal gynecomastia may be present.

In adulthood the classic phenotype comprises tall stature, small testes, bilateral gynecomastia and infertility. Up to 3.1% of infertile men are found to have Klinefelter syndrome [35,37]. There is a higher risk of autoimmune disease (comparable with 46,XX females), osteoporosis, and breast and germ cell tumours [41–43]. Body composition may be altered and there is a higher risk of metabolic syndrome and cardiovascular disease compared with normal men [44,45]. Hypogonadism in adults is variable. FSH and LH levels are elevated while testosterone levels are low in 65–85% of the cases [37].

Diagnosis. The diagnosis of Klinefelter syndrome should be considered in men with small testes and infertility, in children with relative tall stature, behaviour and learning difficulties, and in adolescents with gynaecomastia and small testes. Standard karyotype from peripheral lymphocytes is now being replaced with microarray analysis [46]. Patients with small degrees of mosaicism for a 47,XXY cell line may require analysis using fluorescent *in situ* hybridization (FISH) [47].

Management. Following antenatal diagnosis, counselling should be reasonably optimistic, the family being made aware that a normal childhood can generally be anticipated since only a minority of subjects present in childhood with behaviour and learning problems [48]. Testosterone replacement is not usually required during puberty, but testosterone levels and clinical features of hypogonadism should be monitored, giving replacement if mid-pubertal arrest occurs and/or testosterone levels are low. With advances in fertility management, affected boys and men are should be told that they will require assistance with conception, rather than that they will be infertile.

47,XYY syndrome. The 47,XYY karyotype is present in 1/1000 newborn boys but since the phenotype is unremarkable, only a minority of patients are diagnosed after birth. Although some patients have been described with smaller-than-normal testes, decreased spermatogenesis, spermatogenic arrest, subfertility and sterility, most have normal sexual development and fertility [49]. The prevalence of behavioural problems in 47,XYY males is controversial. Violent behaviour has been described in some [50–52]. However, after adjusting for socio-economic factors, criminal convictions in 161 men with 47,XYY syndrome were similar to controls although some types of criminal activity including sexual abuse and arson remained increased [52].

45,X/46,XY DSD. Phenotype in 45,X/46,XY mosaicism is dependent on the proportion of monosomic and 46,XY cells, which influence the gonadal type and hence the degree of testosterone and AMH underproduction during foetal life. Gonadal type ranges from normal testes, dysgenetic testes, a dysgenetic gonad on one side with a contralateral streak gonad, and bilateral streak gonads. The phenotypic spectrum ranges accordingly from normal male sexual differentiation, through genital anomaly with varying degrees of ambiguity, to normal female differentiation with presence of Müllerian structures. Depending on the proportion of 45,X cells, features of Turner syndrome including short stature and aortic stenosis or coarctation may be present. Some patients have sufficient hormone production to enter puberty spontaneously [53,54], but testicular function may decline at the end of the puberty [55]. 45,X/4-, XY patients are at high risk of infertility, and because of the increased risk of gonadoblastoma, prepubertal gonadectomy should be carried out in those raised as females [54–56].

Y chromosome deletions. Short arm deletions which contain the SRY gene result in sex reversal. Microdeletions in specific regions of the long arm of the Y chromosome are relatively frequent causes of primary testicular failure, being found in up to 20% of men with infertility [57]. Most deletions involve the Yq11 region [58–60]. Microdeletions have also been identified in men with cryptorchidism, varicocele, and obstruction of the vas deferens [58,61]. Larger deletions of the Y-chromosome result in complete tubular atrophy, sparing only the Sertoli cells, resulting in normal serum testosterone and LH

but low inhibin B and elevated FSH [62]. These conditions do not present to paediatricians unless genetically detected but genetic counselling for fertility prognosis is needed when this does happen.

Genetic causes of disorders of sex development (DSD)

46,XX testicular DSD patients with male phenotype. Translocation of Y-chromosome material on the tip of the X chromosome is found in 80% of 46,XX patients with male external genitalia and testes [63], of whom 90% are *SRY* positive [64]. Presentation is in adulthood with features of primary gonadal failure and azoospermia, similar to Klinefelter syndrome but without the tall stature and gynaecomastia [65].

Approximately 15% of individuals are more severely affected and present at birth with ambiguous genitalia, typically penoscrotal hypospadias and small/undescended testes [66]. Only a minority of these individuals are SRY positive. Mutations in *SOX9*, *SOX3*, *WnT4* and *RSPO1* have been reported [67].

46,XY DSD. The causes of 46,XY DSD are summarised in Table 1 and comprise: gonadal dysgenesis; defects in androgen biosynthesis and androgen action, with intact Sertoli cell function; and defects in AMH production or action, with intact Leydig cell function. Depending on the severity of the defect in question, subjects may be raised as male or female.

Gonadal dysgenesis results from the under-expression of genes important for the male sex determination (e.g. *SRY*, *WT1*, *NR5A1-SF1*, *SOX9);* or over-expression of genes important for female sex determination such as *DAX1* and *WNT4*) [68,69]. As with 45,X/46,XX DSD, dysgenesis may be complete, leading to a female phenotype with primary gonadal female, or partial, leading to ambiguous genitalia. Biochemical assessment shows low testosterone levels during mini-puberty or after hCG stimulation, and low levels of AMH [23].

Defects in androgen biosynthesis result from inactivating mutations of the LH/hCG receptor; and in mutations encoding enzymes necessary for testosterone biosynthesis.

5α-reductase type 2 deficiency causes undermasculinisation with normal to high levels of testosterone, low DHT levels, and increased testosterone/DHT ratio [70]. Since puberty is testosterone- rather than DHT-dependant, spontaneous virilisation occurs at puberty in this condition. Sexual function in adults may be satisfactory but fertility is affected by retrograde ejaculation, prostatic hypoplasia and previous cryptorchidism [71].

The androgen insensitivity syndrome (AIS) is due to androgen receptor (AR) mutations. Normal or high testosterone and DHT levels are associated with partial or complete undermasculinisation, depending on the severity of AR defect. Most individuals with partial AIS are raised as males.AMH deficiency or resistance due to receptor type II mutations

This results in a male phenotype, but bilateral cryptorchidism may be present [72]. Leydig cell function is preserved and testosterone levels are normal but because of malformation of the vas deferens or agenesis of epididymis, azoospermia is frequent [68].

Management of 46,XX testicular, 45,X/46,XY, and 46,XY DSD. Gender assignment is a crucial and complex decision influenced by many factors, including parental and patient wishes, age at diagnosis, severity of Leydig cell function/androgen resistance, and severity of Sertoli cell impairment.

The following points should be noted.

- In 45,X/46,XY DSD, gender assignment will be influenced by the internal and external genital phenotype resulting from the combined Leydig and Sertoli cell impairment
- In 46,XY DSD with intact AMH secretion, female reconstructive surgery is difficult and sexual function unsatisfactory because Müllerian structures are suppressed. Moreover, female gender assignment with gonadectomy removes any chance of fertility. It is therefore preferable where possible to raise these individuals in the male gender.
- Male assignment is preferred in 5α-reductase deficiency, even when undermasculinisation is severe, because of spontaneous genital development at puberty.

When a decision is made to raise an individual in the male gender, appropriate surgery, e.g. hypospadias repair and orchidopexy and age-appropriate androgen therapy (see below) are indicated.

Malformative causes of primary testicular failure. **The vanishing testis syndrome** is an unexplained condition with absence of testicular tissue in a phenotypically normal 46,XY individual. By implication, onset of testicular tissue regression must have occurred after normal external and internal sexual differentiation took place. Serum AMH and testosterone levels are very low during mini-puberty or after HCG stimulation. FSH is elevated during the neonatal period and at puberty but may be normal during childhood due to the refractory state of the HPG axis [22].

Non DSD genetic causes

Syndromic disorders. Males with Noonan syndrome have an 80% prevalence of cryptorchidism and classically enter puberty late. The testes may have a squashy texture and Sertoli cell dysfunction. Low levels of AMH and high levels of FSH have been reported [73–75].

Although the hypogonadism of Prader-Willi syndrome is mainly hypothalamic in nature, an important primary testicular component has also been recognised [76].

Patients with Alström syndrome may present with hyper or hypogonadotrophic hypogonadism. Male patients may present with small external genitalia and testicular atrophy with obliterative fibrosis of the seminiferous tubules. Sporadic spermatozoa can be found in the seminal fluid. Secondary sex characteristics are normal [77].

Bilateral cryptorchidism. This is usually congenital in onset, with aetiology unknown, as a component of DSD, secondary to hypogonadotropic hypogonadism or as part of a syndromic disorder, e.g. Noonan. AMH levels are lower in bilateral cryptorchidism compared with unilateral cryptorchidism, which are in turn lower than in controls [78]. Cryptorchidism is associated with lower fertility and paternity rates [79], with azoospermia in 13% of men with unilateral and 89% with bilateral untreated cryptorchidism. Cryptorchidism has been also shown to be an independent risk factor for the development of testicular cancer [80].

Acquired causes of primary gonadal failure

Testicular lesions

Varicocele. This is defined as abnormal dilatation of the pampiniform plexus of veins within the scrotum. It is rare in childhood, but an important condition in adults, affecting up to 15% of the general male population and found in 30%–40% of men presenting for evaluation of primary infertility and in up to 85% of those presenting with secondary infertility. In adults, testicular volume and sperm count may improve with surgery or interventional radiology [81]. In children, surgical management may be complicated by size of veins, so that a conservative approach may be preferable [82].

Blunt testicular trauma. This can lead to atrophy and thus to hypogonadism and decreased spermatogenesis [83].

Testicular torsion. Neonatal, or perinatal, testicular torsion may be unilateral or bilateral. It usually occurs prenatally [96], and the testis is dead at the time of presentation. The torsion is extra-vaginal, i.e. the testis and its coverings twist within the scrotum. After 6 weeks of age the tunica vaginalis becomes more firmly attached and extra-vaginal torsions do not occur. In this situation, most surgeons explore the scrotum in daylight hours, remove the necrotic testis and fix the other side.

Torsions beyond the neonatal period are intra-vaginal, the tunica vaginalis being normally inserted at the top and bottom of the epididymis. In 12% of men the tunica inserts in the cord - the 'bell clapper' abnormality where the testis lies horizontally and can twist.

Prompt surgery is required to untwist and fix the affected testis, removing it from the tunica vaginalis and fixing the contralateral testis at the same time. Half of patients with testicular torsion corrected surgically will develop testicular atrophy, even when intraoperatively the testis was assessed as viable [84]. Delay in time to presentation is consistently found to predict poor outcomes [85].

Infectious/post-infectious orchitis. **Mumps orchitis** is rare in prepubertal boys. Males aged 15–29 years are at greatest risk with a prevalence of 15–30%, carrying the risk of testicular atrophy and infertility in 30% of patients [86].

In all cases of testicular impairment growth and pubertal progress, including testicular volume, should be monitored, together with testosterone and gonadotrophin measurement. Although testes may remain small, with inadequate spermatogenesis, testosterone production may remain adequate for pubertal progress and adult virilisation and sexual function.

Chronic illnesses. Several chronic illnesses (see Table 1) may cause hypogonadism by direct testicular effect or by the decreased gonadotrophin secretion.

Iatrogenic causes of primary gonadal failure in boys

Late effects of the treatment of childhood cancer [87,88]. Surgery, chemotherapy and radiotherapy given in the course of cancer treatment may affect the gonads, causing impaired spermatogenesis, Leydig and Sertoli cell dysfunction in prepubertal boys, adolescents and boys and young adult cancer survivors [89]. Of note, germ cells are particularly vulnerable to the effects of both chemotherapy and radiotherapy, while the Leydig cells are relatively robust [90].

Chemotherapy. The dominant effect is on the seminiferous tubules, with the risk of azoospermia developing early after the start of treatment [91] particularly after alkylating agents (e.g. cyclophosphamide) and heavy metals (e.g. carboplatin, cisplatin). Depending on the viability of the stem cells, fertility may be conserved [91]. Recovery of sperm production after a cytotoxic insult depends on the survival and ability of stem spermatogonia to resume mitotic activity [92].

Following standard treatment for acute lymphoblastic leukaemia, the outcome is usually favourable for fertility [93]. However, chemotherapy for Hodgkin's disease is gonadotoxic, with azoospermia in virtually all cases after combination treatment with mechlorethamine, vincristine, procarbazine, and prednisone (MOPP), and oligospermia in half of those treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) [94]. Busulphan as conditioning treatment prior to bone marrow transplant is also highly gonadotoxic.

Radiotherapy. The pattern of damage to the testes caused by radiotherapy depends on cell-type and radiation dose [95]. The germ cells are highly sensitive; low doses of radiation (0.1–1.2 Gy) impairing spermatogenesis while >4 Gy results in permanent azoospermia and infertility [96–98]. Direct testicular irradiation, e.g. 24 Gy for treatment of testicular infiltration in relapsed acute lymphoblastic leukaemia, leads to both Leydig cell failure and azoospermia. Fractionated total body irradiation given in the dose of 12 Gy with cyclophosphamide and melphalan conditioning results in germ cell impairment but reasonable preservation of Leydig cell function [99], with pubertal progress in around 50% of boys exposed to ionizing radiation prior to puberty.

Management. This requires:

- Appropriate counselling based on the known effects of the chemotherapy agents and radiotherapy doses which have been used.
- Regular 6-monthly review to monitor height velocity, pubertal progression and testicular volumes, bearing in mind that poor growth may reflect concurrent growth hormone deficiency.
- Timely intervention with testosterone, to avoid undervirlisation, bone and muscle acquisition and psychological sequelae.

In doubtful cases, delaying testosterone treatment risks losing 2–3 years of valuable hormone replacement; it is advisable to treat pre-emptively and to reassess gonadal status in late adolescence/early adulthood.

Transfusion-dependent testicular impairment. Iron overload in transfusion-dependant thalassaemia major causes hypogonadotrophic hypogonadism [100]. However, there is also evidence of lower sperm concentration and higher DNA fragmentation related to ferritin levels [101].

Medications. Other than agents for cancer treatment, certain medications such as supraphysiological doses of glucocorticoids can cause reversible primary hypogonadism, while ketoconazole and spironolactone can also directly and reversibly inhibit testicular steroidogenesis through the alteration of the CYP17 activity.

Environmental causes of primary testicular failure. Environmental toxins may have a negative impact on semen quality, in terms of sperm concentration, motility and/or morphology. Such toxins may exert estrogenic and/or anti-androgenic effects, which in turn may alter the HPG axis, induce sperm DNA damage or cause sperm epigenetic change. Endocrine disrupters such as Polychlorinated Biphenyls, Bisphenol A, Phthalate and Pesticides such as Organophosphates and Dichlorodiphenyl-Dichloroethylene have been incriminated in male infertility, [102].

Hormonal management of primary gonadal failure in males (see Table 2)

Testosterone treatment

Infants. For newborn infants with micropenis, cryptorchidism and scrotal hypoplasia resulting from antenatal disorders, a course of intramuscular testosterone can be given, for example testosterone enanthate 25–50 mg every 3–4 weeks for 3 months (maximum four doses).

If further treatment during infancy is desired transdermal 2.5% DHT gel, applied to the phallus twice daily for 3–4 months is effective, particularly in 5α-reductase deficiency [103]. However, availability of reliable preparations is problematic.

Children. Treatment of severe micropenis during childhood may be requested because of emotional problems affecting child and family. Testosterone preparations should be avoided at this age, owing to the risk of growth acceleration and bone age advancement [104]. However DHT, which cannot be aromatised to estrogen, may be applied as a gel twice daily for up to 6 months.

Pubertal induction

What preparation?. Table 2 shows the types of testosterone preparations available. Most centres prefer intramuscular (IM) testosterone since this route is tried and tested [105]. However, oral or transdermal testosterone can be given to boys who need treatment but either do not wish or cannot tolerate injections in the early stages of puberty.

Who should be treated?. Patients with anorchia or severe DSD conditions with complete gonadal dysgenesis require pubertal induction at around 12–13 years. Boys with Klinefelter Syndrome usually enter in puberty spontaneously but may show mid-pubertal arrest. Careful monitoring of pubertal progression is therefore indicated, adding testosterone at an appropriate mid-pubertal dose (e.g. 100/125 mg monthly) if growth velocity and pubertal progression are unsatisfactory.

In patients with known primary hypogonadism in whom Leydig cell reserve and potential are uncertain it is best to err on the side of giving treatment pre-emptively and reassessing at a later stage.

Androgen replacement for the induction of puberty is started at low doses of 50 mg of IM testosterone enanthate, increasing gradually in 50 mg increments every 6–12 months to full adult replacement (200–250 mg/2–4 weeks) over 2–3 years.

Postpubertal maintenance. Intramuscular depot testosterone undecanoate 1000 mg is effective for long term maintenance therapy [105]. The 4 ml injection may be painful and can be given as 2 ml into each buttock. There is less experience with transdermal testosterone in whom local irritation and virilisation of female contacts has been reported [105].

Fertility options and strategies in males

Fertility and fatherhood have been reported in few patients with 46,XY DSD raised as males with milder forms of gonadal dysgenesis or defects in androgen synthesis or action [106]. New techniques of fertility preservation and stimulation of viable spermatogenesis from preserved spermatogonia should improve the prognosis for fertility, especially since germ cells are present in those patients who have been assessed when young [107].

Fertility options must be discussed with patients with Klinefelter syndrome and their families from the time of diagnosis. Techniques such as testicular sperm extraction and intracytoplasmic sperm injection (ICSI) now offer these patients the opportunity to conceive with their own gametes. Although

Table 2
Formulations and protocols for testosterone treatment in pubertal induction and postpubertal maintenance according to route, dose and adverse effects. Abbreviations: T = testosterone.

Formulation	Preparation and route of delivery	Usual dosing	Puberty induction dose/regime	Adult dose	Adverse effects
All preparations					Erythrocytosis Acne and oily skin Aggravation of prostate cancer Reduced fertility
Injectable preparations (IM)	T Cypionate IM T Enanthate IM	100 200 mg 200 250 mg	100/125 mg every six weeks for one year; every four weeks for second years; 200/250 mg every four weeks for third year	200/250 mg every 2−4 weeks	For all IM preparations: Local side effects (pain, erythema, inflammatory reaction and sterile abscess) Gynecomastia, mood disturbances
	T Undecanoate IM	1000 mg/4 ml		1000 mg every 12 (10−14) weeks	Cough and dyspnoea post injection Risk of pulmonary oil micro-embolism and anaphylaxis
Transdermal preparations	T transdermal Androgel® 1%, 1.62% Testim®1%	1pump= 25 mg 20.25 mg 50 mg/tube	Application site: shoulder, upper arm Dose: 50 mg/d (1%), increasing dose according to testosterone levels	Started when 50% of adult IM dose has been achieved and then 50−100 mg/d	Local irritation Potential transfer to other people Skin irritations
	Transdermal T patch	2 mg/24 h 4 mg/24 h	12.5−15 years: 5 mg/8-12 h 14−16 years: 2.5 mg/12 night hours 17−19 years: 2.5 mg/d	2−4 mg/d	Skin irritation
Intranasal testosterone	Nasal T gel	5.5 mg per actuation		11 mg,X3/d	Frequent administration Rhinorrhoea Epistaxis Sinusitis
Oral testosterone	Oral testosterone undecanoate	40 mg	40 mg alternate days for six months, then daily for six months; 80 mg daily for second year' 120−160 mg daily during third year	120 mg daily	Frequent dosing, low testosterone delivery
Buccal testosterone	Buccal bioadhesive T tablets	30 mg per buccal system		30mgX^2/d	Poor adherence Taste alteration, gingival irritation No experience in adolescence

testicular sperm extraction is reported to allow sperm to be found in 50% of affected adults [40], and adolescents sometimes have increased testicular volumes of up to 12 ml, efforts to extract sperm by biopsy at this age have not been successful [108].

For children, adolescents and young adults diagnosed with cancer the prognosis and management options for fertility should be discussed with the family before the initiation of treatment. Improved survival in cystic fibrosis has rendered male infertility an important issue, requiring appropriate and timely counselling [109].

Fertility preservation techniques. In adults, fertility preservation techniques, such as semen and embryo cryopreservation are established and successful, with cryopreservation of ejaculated semen sample demonstrated to be feasible in patients as young as 13 years. In pubertal boys, sperm can be surgically retrieved from the testicle via testicular sperm extraction (TESE) procedures [110]. The sperm thus obtained is easily cryopreserved and may be used in the future for assisted reproduction procedure.

However, despite being recommended for all pubertal male patients, sperm banking is not universally practised in paediatric oncology centres. There are few adolescent-friendly facilities [111], and national programs are needed to improve access to fertility [112].

Biopsy and cryopreservation of prepubertal germ cells in males is currently experimental [110,113] and limited by difficulties with maturation to adult spermatogenesis and concerns regarding re-implantation of potential malignant cells.

Primary gonadal failure in females (see Table 3)

Congenital causes

Turner syndrome and other aneuploidies

Turner syndrome

Definition and prevalence. Turner syndrome results from loss of the second sex chromosome (X or Y); and/or abnormality of the second X chromosome in at least one major cell line in a phenotypic female [114]. Its relative rarity occurring in 1 in 2500 live female births [115], has important implications for implementing adequately powered studies.

Genetic aspects. Approximately 40% of girls have 45,X monosomy, with mosaicism in 30% and structural abnormalities of the second X in 20% [116]. Complete loss one short arm (p) pair, e.g. with 45,X monosomy; and/or duplication of the long arm (q) to give an isochromosome (e.g. 45,X/46,X,iXq) leads to a more severe phenotype in terms of short stature and ear problems. Phenotype is milder with 45,X/46,XX mosaicism; and (surprisingly) the 45,X/47, XXX genotype - an important consideration for antenatal counselling [117].

The mildness of phenotype in Turner syndrome given that all or part of such a large chromosome is missing or altered remains a mystery. Inactivation of the second X chromosome by the end of the first week of conception is the standard explanation but this is incomplete, with 30% of short arm genes unsilenced.

Phenotypic features. The dominant features are short stature, dysmorphic traits, gonadal dysgenesis and associated anomalies including cardiac, middle ear disease, sensori-neural hearing loss, renal anomalies, and immune dysregulation with increased prevalence of coeliac and inflammatory bowel disease, and autoimmune thyroiditis.

Presentation and diagnosis. Turner syndrome may present.

- **Antenatally -** as an unexpected finding on chorionic villus sampling or amniocentesis; or when foetal ultrasound shows cystic hygroma/nuchal translucency.
- **After birth -** with pedal lymphoedema (Fig. 2) and other dysmorphic features ± aortic coarctation.
- **In infancy and preschool phase -** with failure to thrive, borderline or frank short stature, dysmorphic features, high activity levels, sleeplessness and fearfulness, often accompanied by recurrent middle ear disease.
- **During childhood** - with short stature ± ear problems.
- **During adolescence** - with short stature and delayed puberty.

Table 3
Aetiologies of primary gonadal failure in females.

Congenital causes	
Chromosomal	- Turner syndrome (45,X etc) - 47,XXX - 48,XXXX
46,XX DSD	- 46,XX pure gonadal dysgenesis - Idiopathic - LH/hCG receptor defect - FSH receptor defect - Enzyme disorders (StAR protein deficiency/lipoid congenital adrenal hyperplasia, side chain cleavage (20,22 desmolase), 17α-hydroxylase and aromatase deficiency
46,XY DSD	- 46,XY DSD with complete sex reversal (Swyer's syndrome) - 46,XY DSD with partial gonadal dysgenesis (mutations of SRY,SF1, WT1, SOX9) - 46,XY DSD with disorders of androgen synthesis raised as females (StAR, POR deficiency, 17α-hydroxylase, 3 β-hydroxysteroid-deshydrogenase, 17β-hydroxysteroid-deshydrogenase deficiency and 5-α-reductase deficiency) - Leydig cell hypoplasia (LH-receptor mutation), and FSH-Receptor mutation - 46,XY DSD with disorders of androgen action raised as females (complete and partial androgen insensitivity)
Acquired Causes	
Autoimmune	- Autoimmune oophoritis - isolated or part of polyglandular syndrome type 1 and type 2
Metabolic	- Galactosaemia
Iatrogenic	Iron overload as consequence of treatment for thalassaemia **Consequences of childhood cancer treatment** Surgery Chemotherapy - Acute leukaemia - Hodgkin's disease Radiotherapy - Abdominal irradiation - Craniospinal irradiation - Total body irradiation prior to bone marrow transplantation **Consequences of non-cancer treatment** Bone marrow transplantation for non-malignant conditions

The mainstay of clinical diagnosis is recognition of short stature which is inappropriate for the parental heights [116]. This requires a basic knowledge of normal growth in the lay population – including teachers - which is conspicuously lacking, and in health care professionals including oto-rhino-laryngologists (ORL) [118].

Management. Sensitive and thorough counselling of girls and parents is the foundation of good clinical care. A helpful model is to regard girls with Turner syndrome as normal individuals who have more than their fair share of common problems (Fig. 3). Contact with and information leaflets/accessories from support societies, e.g. Turner Syndrome Support Society in the United Kingdom (UK) (https://tss.org.uk/) are most valuable.

Cardiac management comprises cardiology referral at diagnosis, echocardiography to determine cardiac anatomy (e.g. biscuspid aortic valve), blood pressure surveillance using standard charts [119], and cardiac ultrasound or MRI before and after pubertal induction to assess aortic root size index.

Otological management involves education of families, prompt ORL referral after more than one middle ear infection, suspected hearing loss, and annual audiogram.

The mainstay of growth management is growth hormone (GH), usually given from 4 to 5 years of age [120], The recommended dose of GH is 10 mg/m^2/week - roughly twice that used for classic GH deficiency. This reflects the underlying skeletal dysplasia related to loss of SHOX from Xp.

Although the anabolic steroid oxandrolone has been shown to increase final height in Turner syndrome [121–123] it is no longer manufactured in Europe and is currently reserved for girls whose growth is disappointing despite good adherence to GH therapy.

Fig. 2. Girl with Turner syndrome (45,X), ice-skating.

47,XXX syndrome. Triple X aneuploidy has a prevalence of 1 in 1000 females but most cases are undiagnosed, affected individuals being phenotypically normal. Recognised associations include a tendency to tall stature, and intellectual disability [124]. Pubertal development and fertility are usually normal, but there are sporadic case reports of POI [125–127].

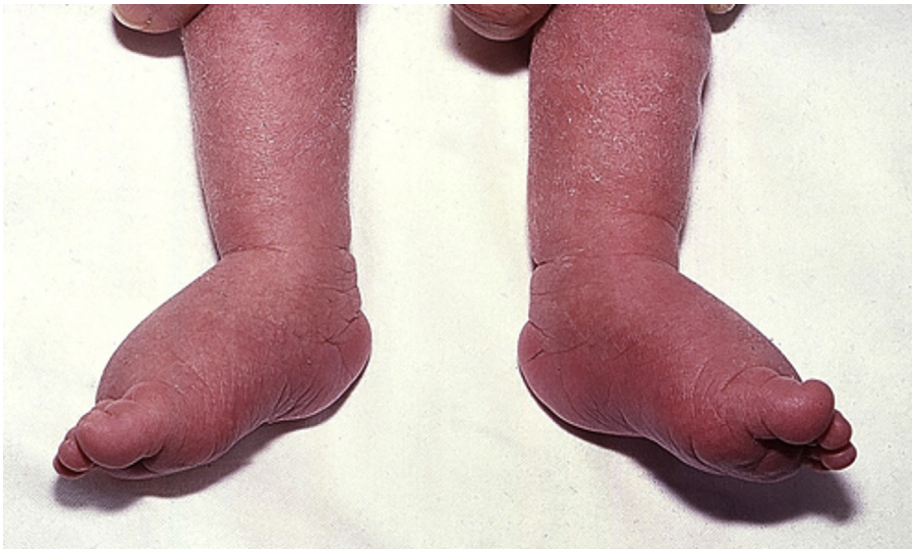

Fig. 3. Pedal oedema in a newborn infant with Turner syndrome.

48,XXXX syndrome. Reported features of this very rare condition include mild to moderate learning difficulties, dysmorphic traits including epicanthic folds and upslanting palpebral fissures, hypotonia and joint laxity. The prevalence of ovarian impairment is uncertain, one paper reporting menstrual dysfunction in half of adult women [128] while POI and secondary amenorrhoea occurred in only 2 and 5 of 39 cases in another study [129].

46,XX DSD with hypogonadism

46,XX gonadal dysgenesis. This cause of primary gonadal failure has been comprehensively reviewed by Joe Leigh Simpson [130].

The term "pure gonadal dysgenesis" applies to phenotypically normal female individuals with streak gonads in whom karyotype is either 46,XX or 46,XY, with no cytogenetic abnormality. Müllerian structures are present although the uterus may be severely hypoplastic. There are usually no associated abnormalities.

No gene abnormality is found in most cases of 46,XX gonadal dysgenesis. However, mutations in **X-linked genes** encoding bone morphogenetic protein 15 (*BMP15*), the premutation of *fragile X mental retardation 1 (FMR1)* and the *FMR2* gene have been implicated [131] and breakpoints in the X chromosome may alter genes responsible for ovarian development. Mutations in **autosomal genes** may occur with either 46,XX and 46,XY karyotypes. These include mutations in the follicle stimulating hormone receptor gene (*FSH-R*) situated on the short arm of chromosome 2 (2p). Most cases have been described in Finland [132], usually showing the cytosine–thymidine transition C566T mutation. Mutations in the luteinising hormone receptor (*LH-R*) gene, which is also situated on 2p, have also been described.

Presentation is in adolescence is with failure to enter puberty normally in a girl with normal stature, although one case report described marked tall stature with 46,XX dysgenesis [133]. Pubertal staging may show some pubic and axillary hair, reflecting adrenal androgen activity, but breast development is absent.

Investigation includes karyotype, gonadotrophin and AMH measurement and a careful search with ultrasound or MRI for the hypoplastic uterus.

46,XX DSD due to enzyme disorders. Severe StAR and side-chain cleavage enzyme deficiency due to mutations in the *StAR* and *CYP11A1* genes affect all three steroidogenic pathways so that 46,XX individuals surviving infancy require hormone replacement at puberty. Less severe forms present with primary adrenal insufficiency and show variable ovarian impairment at puberty. CYP17A1 mutations causing 17-hydroxylase deficiency typically cause primary amenorrhoea, absent breast and pubic hair development, and hypertension [134].

Aromatase deficiency caused by *CYP19* mutations results in impaired estrogen synthesis from androgen with elevated testosterone and decreased estradiol levels, foetal virilization, delayed puberty with primary amenorrhoea, tall stature due to lack of epiphyseal fusion, and osteoporosis. Investigations show low estrogen levels associated with high LH and FSH secretion and polycystic ovaries. Treatment is with high dose estrogen replacement.

46,XY DSD in individuals raised as girls

46,XY pure gonadal dysgenesis. This condition, also known as Swyer's syndrome, presents similarly to pure 46,XX dysgenesis with normal female phenotype and streak gonads. Subjects are at increased risk of gonadoblastoma, with associated dysgerminoma [135], so that prophylactic laparoscopic gonadectomy is recommended.

Complete androgen insensitivity. Androgen insensitivity is caused by mutations in the androgen receptor (*AR*) gene which is located on the long arm of the X chromosome (Xq11-12) and encodes a nuclear receptor activated by binding with testosterone and dihydrotestosterone. Depending on their severity, *AR* mutations result in complete or partial androgen insensitivity (CAIS and PAIS).

Affected individuals with CAIS are phenotypic females with intra-abdominal testes, absent Müllerian structures including uterus and upper third of the vagina, and normal female external genitalia. At puberty, testosterone levels are high with modest LH elevation. Normal conversion of estradiol from testosterone by aromatase and normal estrogen sensitivity leads, to normal or enhanced breast development.

Management of CAIS. This involves the sensitive and honest counselling of patients and families, making it clear that a small minority otherwise normal women have a 46,XY chromosome pattern with internal testes rather than ovaries and hence no uterus or menses; but that the body responds normally to estrogen so that the genital and breast development are normal.

There is an increased risk of developing germ cell neoplasia *in situ* in CAIS, but malignant change in childhood is virtually unknown. This risk increases in adulthood, with reports of seminoma [136], but remains relatively low [137]. Prophylactic laparoscopic gonadectomy should not therefore be performed until after puberty is complete. Women opting for non-intervention post puberty should be screened every two years with ultrasound or MRI of the gonads and measurement of tumour markers [138]. Monitoring of bone health and adequate estrogen replacement are also important in adults who have undergone gonadectomy [139]. In cases where sexual intercourse is uncomfortable due to the short vagina, self-dilation has proved effective [140].

Other forms of 46,XY DSD with female gender assignment. Causes of 46,XY DSD other than CAIS are listed in Table 1. When a decision to raise the individual in the female rather than the male gender is taken, gonadectomy and feminising surgery followed by pubertal induction and maintenance therapy with estrogen will be required.

Acquired causes of primary ovarian failure

Autoimmune oophoritis

Autoimmune oophoritis in childhood and adolescence is seen in association with other autoimmune diseases, particularly autoimmune adrenalitis and usually in the context of one of the autoimmune polyglandular syndrome (APS) complexes [141]. Type 1 APS, also known as APECED (autoimmune polyendocrinopathy—candidiasis—ectodermal dystrophy), is caused by a mutation in the autoimmune regulator (*AIRE*) gene on chromosome 21. Features include hypoparathyroidism, mucocutaneous candidiasis and adrenal insufficiency. Type II APS occurs in older patients and the spectrum includes autoimmune adrenalitis, thyroiditis and type 1 diabetes as well as oophoritis.

Presentation. This is usually with pubertal failure and elevated gonadotrophins in the context of other autoimmune disorders. If POI is the presenting problem, then clinical and biochemical assessment including electrolytes, calcium and phosphate, thyroid and adrenal function and antibodies is indicated.

Galactosemia

POI is a constant late effect of classic galactosemia. Pubertal development is usually normal, although primary amenorrhoea may occur. Secondary amenorrhoea with impaired fertility follows but spontaneous pregnancy can occur. It is important not to misinterpret short stature in late childhood as growth hormone deficiency when caused by delayed puberty compounded by low body mass due to dietary restriction.

The 2017 guidelines for classic galactosemia recommend FSH screening if puberty is delayed beyond 12 year or regular menses are not established at 14 years, annual post pubertal monitoring with FSH measurement, and reproductive endocrine referral to discuss hormone replacement and fertility management [142,143].

Iatrogenic causes of ovarian failure

Consequence of iron overload in the treatment of thalassaemia

While delayed puberty in iron-overloaded girls with thalassaemia major is predominantly central in nature, an inverse relationship between AMH secretion and non-transferrin-bound iron has been

found. This suggests that the impaired fertility seen in thalassaemia is partly owing to ovarian tissue iron overload [144].

The UK Thalassaemia 2016 guidelines advocate minimising iron loading with effective chelation treatment, regular monitoring of growth and puberty, multidisciplinary and transition clinics encompassing endocrine and bone health management, and appropriate counselling [145]. To ensure normal growth and feminisation before the epiphyses fuse, patients should receive adequate transfusion to prevent chronic anaemia, and timely estrogen induction of puberty when required.

The late effects of chemotherapy and radiotherapy for treatment of childhood cancer

Chemotherapy. The risk of ovarian impairment depends on the agent(s) used, and dosage. Standard chemotherapy for acute lymphoblastic leukaemia is associated with good ovarian function in the medium term [146]. However, the use of alkylating agents such as cyclophosphamide in Hodgkin's disease [147], and busulphan prior to bone marrow transplant [161] is associated with POI.

Radiotherapy. Craniospinal irradiation, for example in the treatment of medulloblastoma, may cause ovarian impairment [148] as well as reducing adult height by 5–10 cm, depending on age at treatment.

Total body irradiation (TBI) is a key strategy in the conditioning treatment prior to BMT, e.g. for relapsed leukaemia and usually given as a fractionated dose of 12 Gy, invariably causes a degree of POI [149,150]. An estimated 50% of adolescents will have sufficient ovarian function to complete puberty but will have early menopause - an important consideration for family planning [151]. Uterine development and blood flow are also affected by TBI with small adult uterus, absent endometrium and reduced vascularity in survivors of TBI in childhood [152].

Clinical assessment and management. The effects of cancer therapy on growth and pubertal development vary according to the combination of damage to pituitary gland, skeleton and ovary sustained. These in turn are influenced by the underlying diagnosis, age, mode and dose of treatment. In order not to miss subtle changes in linear, spinal and limb growth, falling height velocity, and pubertal failure, careful surveillance is required. This involves.

- Regular 3–6 monthly assessment by haematology and oncology staff who are adequately trained in growth and puberty monitoring
- Joint review with an endocrinologist 4–6 monthly.
- Accurate measurement of standing and sitting height, and pubertal stage.
- LH, FSH and AMH measurement, and pelvic ultrasound examination.

Bone marrow transplant for non-malignant conditions

TBI and BMT is now an established strategy for selected patients with non-malignant conditions such as such as severe combined immune deficiency, thalassaemia major and sickle cell disease [153].

Management of premature ovarian failure/gonadal insufficiency in females

Prepubertal children

Estrogen levels during childhood are low in girls, but significantly higher than in boys. It is therefore logical to examine strategies for low-dose estrogen replacement in childhood. A synergistic effect of low dose ethinyl estradiol (25 and 50 ng/kg/day from 5 to 8 and 8–12 years) on growth in Turner syndrome has been suggested [154]. However, at present low dose estrogen should only be given to prepubertal girls within the confines of an approved clinical trial.

Pubertal induction - when, how, which preparations and in whom?

This is a controversial area. Clinical practice guidelines were published recently following the International Turner Syndrome Meeting in Cincinnati [120], while the British Society for Paediatric Endocrinology and Diabetes has agreed guidelines on pubertal induction in POI [155].

When?

Pubertal induction should usually start no later than 12 years, the possible final height advantages of late induction in Turner syndrome being outweighed by the adverse effects on uterine growth and self-esteem [156,157].

How?

Table 4 shows some examples of oral and transdermal regimens recommended for pubertal induction. Superior cardiac, bone and uterine health in women with POI treated with transdermal estradiol versus oral ethinyl estradiol has been reported [158–160]. However, adequately powered studies are needed to confirm these benefits, and also to evaluate compliance with the different routes of delivery.

Recently the Turner Syndrome Working Group (TSWG) of the European Society for Paediatric Endocrinology (ESPE) has agreed oral and transdermal protocols for 3-year pubertal induction [161]. Both protocols use 17β-estradiol and the doses of both are weight-based. This group also proposed that clinicians be encouraged to help families choose one of the recommended protocols; and to give written informed consent for prospectively collected data to be sent to a designated center so that outcomes with oral and transdermal inductions can be compared. This approach represents a departure from the traditional model of the randomised controlled clinical trial which, although desirable in theory, has not proved feasible in practice in terms of securing adequate funding and adequate patient numbers.

What preparations?

17β-estradiol preparations of 0.5 mg are required for giving the initial doses of 0.5, 0.25 and < 0.25 mg recommended by the British, Cincinnati and TSWG recommendations (see Table 4). However, only 1 mg preparations are currently available in most European countries. It is important therefore for clinicians and pharmacists to ensure that appropriate oral preparations are made available for girls with POI.

The transdermal regimen proposed by TSWG involves applying an overnight patch for 10–12 h during the first two years of induction. In the third year the desired dose in halved to give two pieces, applying both overnight and then removing one in the morning, to mimic the diurnal rhythm of estradiol secretion. The stability and uniformity of estradiol distribution in specified patches cut and stored at either 21 °C or 35 °C has recently been shown [162].

Who should receive pubertal induction?

Treatment is mandatory in girls with complete gonadal dysgenesis, or in whom gonadectomy has been performed. However, not all patients with POI require pubertal induction, e.g. 17 of 92 (18%) girls with Turner syndrome in a UK study [123].

The decision to treat is essentially clinical, based on growth rate, pubertal stage and progression. If in doubt, it is better to ensure adequate estrogen provision than to risk inadequate pubertal and uterine development, bone mineralisation and psychological disturbance.

Biochemical markers are of value in identifying patients who will need pubertal induction. During childhood, when FSH levels may be low even in POI, AMH levels can be used [163]. FSH levels of >6.5 IU/L in mid-childhood are predictive of the need for future estrogen replacement [164]. In girls aged ≥11 years, a basal FSH of >10 IU/L is a reasonable threshold for inducing puberty.

When should progestogen be added and what is the optimal regimen?

Standard advice is to add progesterone either when breakthrough bleeding occurs or after 2 years of estrogen treatment [120]. An alternative is to perform ultrasound examination at the time of bleeding. If this shows the uterus to be underdeveloped with a thin endometrium, estradiol treatment should continue unopposed. When the uterus is mature uterus with thick endometrium, progesterone can be introduced.

Table 4
Examples of formulations and protocols for estradiol and progesterone treatment in pubertal induction and postpubertal maintenance.

Formulation	Preparation	Usual dosing	Puberty induction dose/regime	Adult regime	Comment
Oral estrogen	Ethinyl estradiol	2 mcg tablets 10 mcg tablets	2 mcg daily for 1st year; 4 mcg daily for 2nd year; 6,8,10 mcg daily during 3rd year	20 or 30 mcg continuously	Natural, rather than synthetic estrogen preparations are now recommended as more physiological; and enabling serum estradiol measurement
	17β-estradiol as: Micronised (e.g. Estrace) Hemihydrate (e.g. Cetura, Valerate (e.g. Climaval)	0.5 mg 0.5 mg 1 mg	5 mcg/kg/day for 1st year; 7.5 mcg/kg/day for 2nd year; 10 and 15 mcg/kg/day during 3rd year	20 mcg/kg/day - usually 2 (1–4) mg daily	Girls requiring <0.25 mg daily need to have their medication prepared by the pharmacy. The 1 mg tablets are not readily divided and hence not suitable for use in pubertal induction
	Conjugated estrogen Norgestrel medroxyprogesterone acetate	0.625 mg 150 mg 5 mg		Sequential combined therapy: Estrogen is given daily with Norgestrel from days 17–28 Continuous combined therapy: Estrogen and Medroxyprogesterone are given daily throughout	
Transdermal estrogen	25 or 50 mcg patch	Cut 50 and 25 mcg patches into 2–16 or 2–8 pieces to deliver appropriate dose	3.2–6.2 mcg overnight during 1st year; 6.2–12.5 mcg overnight during 2nd year; 16.7–25 ug in two pieces for 12 h overnight during 3rd year, removing one piece in the morning	50-75 (up to 100) mcg patch three times weekly, modifying dose to achieve serum estradiol levels of 150–450 pmol/L	The stability of Evorel, Estraderm and Oesclim patches when cut into 8 pieces and stored at 21 °C or 35 °C has been shown [162]
Oral progestogen	Dydrogesterone Micronised progesterone Medroxyprogesterone acetate	10 mg 100 mg 5–10 mg	Wait until adequate estrogenisation has been achieved before starting progestogen, giving for first 10 days of each calendar month		When breakthrough bleeding occurs, check uterine maturity with ultrasound (see text)

Postpubertal maintenance - what are the options?

The oral contraceptive pill is popular with young women but the conventional 3 weeks on and one week off should be avoided since this involves no estrogen administration for a quarter of the year. Combined sequential or combined continuous replacement regimes are recommended, with 3-monthly cycles using the OCP as a further option. Natural estrogen is preferred where risk for hypertension is increased, as in Turner syndrome [165,166].

What surveillance should girls have before, during and after pubertal induction?

This comprises.

- 4-monthly review of height, height velocity, weight, body mass index, pubertal stage and blood pressure.
- Assessment of compliance, asking the family to gauge how many days per month tablets or patches have been missed.
- Pelvic ultrasound, dual X-ray absorptiometry (DXA) and (in Turner syndrome) echocardiography before and after induction.
- Annual FSH and LH and, in GHD or Turner syndrome, IGF-1 and thyroid function.

Serum 17β-estradiol levels can be used to titrate the estrogen dose. However, a therapeutic range has only been established for transdermal estradiol. This is because values on oral replacement are affected by cross reaction between estradiol and estrone, the latter being produced in significant quantities with oral therapy. In Turner syndrome, annual liver function tests and lipid profile are recommended.

Fertility options and strategies in girls and women

Who is at risk for impaired fertility?

Girls who have undergone gonadectomy will not conceive without help, and conception is not possible at all in conditions in which there is no uterus, for example in 46,XY DSD due to CAIS. Some subjects will have received treatment which is known to cause impaired fertility, e.g. alkylating agents or TBI. Other risk factors include elevation of FSH and low AMH during childhood, primary and secondary amenorrhoea with elevated gonadotrophin levels; and failure to conceive after 1–2 years of unprotected intercourse. Despite all these risk factors, there is considerable variability in fertility and reproductive lifespan outcomes, particularly after chemotherapy and radiotherapy [167]. A controlled study of cancer survivors with regular menstrual cycles showed higher serum FSH levels, lower AMH and lower ovarian volumes in the patient group, indicating the sensitivity of these indices of ovarian function [168].

What options are there and what techniques can be used to achieve assisted conception?

Egg donation from a family member or friend followed by *in vitro* fertilisation is an option for girls with reasonable uterine status, but this technique is expensive, not always successful, and is not available in some countries.

What are the current and future strategies for fertility preservation in girls and women?

Embryo cryopreservation before cancer treatment is started is an option for women but not children and adolescents. Oocyte cryopreservation by vitrification (very rapid freezing), applicable only to postpubertal patients, is effective provided that cancer treatment can be delayed for long enough to carry out ovarian stimulation but experience is limited.

Storage of ovarian tissue is the only strategy available to children. Although previously regarded as experimental, there is now sufficient evidence to justify its implementation in selected cases [169]. Ovarian tissue is removed in the form of multiple biopsy specimens, cut into cortical strips, cryopreserved by slow freezing and re-implanted at a later stage. When there is a risk of malignant cells in the ovarian tissue follicles can be matured and eggs collected *in vitro* for subsequent fertilisation and implantation. The success of this technique has been demonstrated [170] but attention has been drawn to the need for better and more consistent service provision in the UK [171].

In which patients is assisted fertility or fertility preservation problematic?

Successful pregnancies in Turner syndrome have been reported, but the situation is complicated by the risk of abnormal foetal karyotypes; and maternal aortic dissection, particularly with biscuspid aortic valve and aortic coarctation. Implantation of the embryo may be difficult in post TBI and BMT survivors because of altered uterine and endometrial tissue.

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