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Primary Amenorrhoea

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Abstract
Amenorrhoea is a normal physiological feature in prepubertal girls; however, failure to commence menstruation constitutes primary amenorrhoea. The presence of normal uterovaginal anatomy and a functional hypothalamic-pituitary-ovarian (HPO) axis is essential for menstruation. To ascertain the cause, a detailed and relevant medical/family history is imperative, coupled with a thorough physical examination and appropriate investigations. In the absence of secondary sexual characteristics, hormonal disturbances are the most probable cause, whereas anatomical issues are more likely in the presence of secondary sexual characteristics, alongside physiological delays and endocrinopathies. Primary amenorrhoea warrants investigation in the absence of secondary sexual development by the age of 13 and with secondary sexual development by the age of 15. An early referral is advisable if there is suspicion of chromosomal abnormalities, hyperandrogenaemia, anatomical abnormalities, or if primary amenorrhoea persists for five years after thelarche. A multidisciplinary team approach, offering support to patients and families with evidence-based knowledge for decision-making, is the cornerstone of management. It is crucial to address the patient's and their family's needs on an individual basis for the best outcomes.

Keywords: HPO Axis Problems, Genital Anatomical Problems, Hyperandrogenism, Hyperprolactinaemic

Introduction
The incidence of primary amenorrhoea stands at approximately 0.3% [1, 2]. The framework for its investigation encompasses environmental variables, chronic health issues, dysfunctions within the hypothalamic-pituitary-ovarian (HPO) axis, and anatomical irregularities. These elements may influence the HPO axis either directly or indirectly, leading to amenorrhoea. Understanding the cyclical hormone release throughout the menstrual cycle is essential for identifying the cause of this condition.
The causes of primary amenorrhoea can be broadly categorised into:
- Disorders of the hypothalamus and pituitary gland,
- Primary ovarian insufficiency, often linked to chromosomal anomalies,
- Developmental anomalies of the genital tract,
- Conditions of hyperandrogenaemia.

Normal physiology for menstruation
For menstruation to occur seamlessly, a well-coordinated HPO axis is imperative, involving the hypothalamus, the pituitary gland, and the ovaries, all linked by feedback mechanisms. The secretion of gonadotrophin-releasing hormone (GnRH) pulses from the pituitary predominantly happens nocturnally between the ages of 8 and 13. As puberty approaches, the frequency of daytime GnRH pulses begins to exceed that of the night, aligning with the adult pattern upon reaching puberty [3]. Any disruption in this finely tuned system (Fig 1) can lead to amenorrhoea, manifesting either due to organic or functional discrepancies.

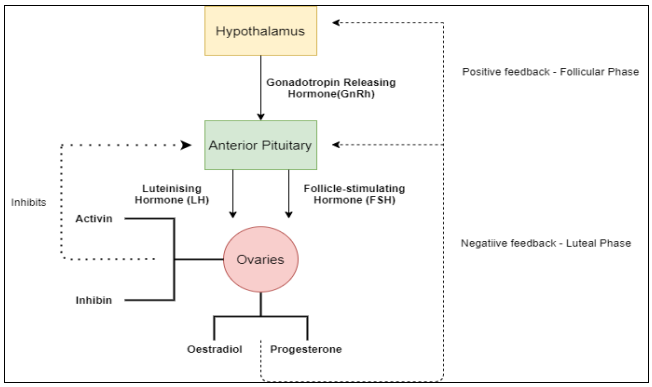

Fig 1: Functional endocrine axis comprising of hypothalamus, pituitary, and ovarian hormonal regulation

This cyclical activity of hormonal release is usually not regular during the first one or two years after puberty and cycles are frequently irregular and anovulatory.

Materials and methods

In this study, we utilised several electronic databases, including MEDLINE, EMBASE, PubMed, the Cochrane Central Register of Controlled Trials, conference abstracts, and the British Library, spanning from January 1995 to June 2023. We limited our search to articles published in the English language. The search terms used were: "primary amenorrhoea," "failure to achieve menstruation," "amenorrhoea with anatomical problems," "amenorrhoea with abnormal chromosomes karyotype," "hyperprolactinaemia," "hyperandrogenaemia," "Androgen Insensitivity Syndrome," "HPO axis," "FHA," "chronic illness," and "constitutional delay." The initial search yielded over 2,000 articles. All articles were screened for relevance based on their titles and keywords, leading to the exclusion of duplications. Subsequently, 301 abstracts were reviewed, and 157 full papers were assessed in detail. Ultimately, 78 articles met our inclusion criteria and were incorporated into this review.
See figure 2:

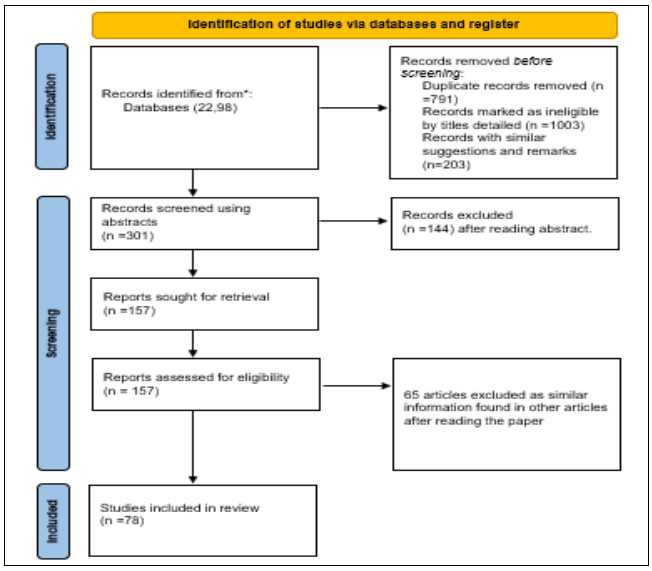

Fig 2

Establishing the underlying cause of amenorrhoea is essential for implementing the most effective, evidence-based management strategies. To this end, a meticulous history, comprehensive clinical examination, and targeted investigations form the cornerstone of our approach.

History taking

A comprehensive collection of the patient's history is crucial in identifying potential contributing factors to amenorrhoea. This includes assessing emotional stress levels, the gynaecological histories of close female relatives, notably the patient's mother and sisters, and any familial incidence of diabetes or genetic disorders. Other critical areas of inquiry encompass delayed puberty, symptoms indicative of thyroid dysfunction or galactorrhoea, notable fluctuations in weight, the presence of hirsutism or anosmia, chronic systemic illnesses, and any prior exposure to radiotherapy or chemotherapy. An in-depth review of the patient's sexual history, contraceptive use, presence of episodic cyclical lower abdominal pain, gynaecological surgeries (including those affecting the vagina or uterus), and current medication regimen is also imperative. Additionally, it is important to address any substance misuse, with a focus on cocaine and opiates, given their potential impact on the menstrual cycle.

Examination

The onset of puberty is generally marked by thelarche, the development of breast buds, typically commencing around the age of 11. This is subsequently followed by pubarche, characterised by the emergence of pubic and axillary hair, a phase of accelerated growth, and eventually culminating in menarche. Menarche usually manifests around two to two and a half years post-thelarche.
Importantly, the initiation of pubarche operates independently of GnRH function, with dehydroepiandrosterone playing a key role in this process [1, 3].
The Tanner scale, formulated by paediatrician James Tanner, provides a systematic method for assessing puberty stages in children through an examination of breast size, Diagnostic clarity may be further enhanced through a vaginal examination and an ultrasound scan of the external genitalia. A notable delay in reaching Tanner stage 2 by the age of 13 is indicative of delayed puberty, warranting further investigation [4]. external genitalia, and pubic hair growth [4]. Employing the Tanner staging (Table 1) during physical examination facilitates the identification of underlying conditions [4].

Table 1: Tanner stage for females

Tanner classification	Breast	Pubic hair
Tanner I	The areola follows the skin contours on chest no glandular tissue (prepubertal)	No pubic hair (prepubertal)
Tanner II	Areola starts to widen and appearance of glandular tissue	Pigmentation appears in labia majora small amount of thin pubic hair.
Tanner III	Breast and areola continue to widen and elevate and breast contour remains normal	Pubic hair starts expanding laterally and texture becomes coarse and curly
Tanner IV	Papillary and areola growth continues along with secondary mound development of overall breast tissue	Extension of pubic hair from pubis to medial thighs and texture of hair is almost like adult
Tanner V	Adult size breast with areola recession and papilla extends little above the breast contour	Adult texture hair extends to medial surface of the thighs and inverse triangular appearance superiorly

Investigations

For patients who are sexually active, the foremost step involves ruling out pregnancy. Following this, investigations should adhere to the protocols delineated in Table 2, customised according to the specific characteristics of each case.

Table 2: Investigations with expected results in relation to the underlying condition associated with primary amenorrhoea

Condition	Follicle stimulating hormone (FSH) /AMH	Luteinizing Hormone (LH)	Prolactin	Androgens (testosterone, 17-OHP, Cortisol	Ultrasound scan (Uterus)	Chromosome / Genes	Breast development	Pubic hair
Constitutional delay	↓	↓	↓/ ↔	↓/ ↔	P	46XX	Delayed	Delayed
Turner syndrome	↑	↑	↔	↔	P	45X0/(45X0,46XX	delayed	Delayed
Gonadal dysgenesis Perrault syndrome	↑	↑	↔	↓ / ↔	P	46XX	delayed	delayed
Gonadal dysgenesis Swyer syndrome	↑	↑	↔	↓	P	46XY	delayed	delayed
Mayer-Rokitansky-Kuster Hauser (MKH)	↔	↔	↔	↔	A	46XX	N	N
Complete androgen insensitivity (AIS)	↔	↔/↑	↔	↑	A	46XY	N / As	A
Congenital adrenal hyperplasia (CAH)	↔/ ↓	↔/ ↓	↔/↑	↑/ ↑17OH progesterone	P	46XX	N	N
Cushing's syndrome	↔/ ↓	↔/ ↓	↔/↑	↑ / ↑ Cortisol	P	46XX	N	N
Thyroid dysfunction	↓	↓	↔/↑	↔	P	46XX	N /D	N/ D
Pituitary adenoma (Hyperprolactinemia)	↓	↓	↑	↔	P	46XX	N/ D	N/ D
Congenital Hypothalamic hypogonadism (HH)	↓	↓	↑	↔	P	46XX	D	D
Acquired HH	↓	↓	↑	↔	P	46XX	D	D
Hypopituitarism	↓	↓	↓	↔	P	46XX	D	D
Polycystic ovary syndrome (PCOS)	↔	↔ /↑ slightly in 40% cases	↔ /↑ slightly in 5-30%	↔ /↑ marginally raised	P	46 XX	N	N
Premature ovarian insufficiency (POI)	↑	↑	↔	↔	P	46XX, 46XY 45X0, 47XXX, FMR1 gene	D/N	D/N
Functional Hypothalamic Amenorrhoea (FHA)	↓/↔	↓/↔	↔	↔	P	46XX	D /N	D/N

P- Present, A---Absent, N----Normal, D------delayed, As-----asymmetrical, Antimullerian Hormone (AMH), 17-OH progesterone (17-OHP)

Hormonal Assessments

- **Serum Anti-Müllerian Hormone (AMH)** levels are a reliable marker for evaluating ovarian reserve, offering advantages over serum Follicle-Stimulating Hormone (FSH) due to their independence from the menstrual cycle. Notably low AMH levels in the context of amenorrhoea could signify premature ovarian insufficiency (POI) [5].
- **Follicle-Stimulating Hormone (FSH)**: A level below 5 IU/L is indicative of hypogonadotropic conditions, commonly seen in prepubertal girls or those with hypothalamic and pituitary dysfunctions. In contrast, an FSH level above 20 IU/L suggests a hypergonadotropic state, similar to that observed in menopause. Normal readings for both FSH and AMH, coupled with adequate ovarian androgen levels and chronic anovulation, might point towards conditions such as hyperprolactinaemia and thyroid dysfunction.
- **Prolactin:** Patients are advised to wait for at least 48 hours post-breast examination before proceeding with a serum prolactin test. If serum prolactin concentrations range between 500 mIU/ml and 1000 mIU/ml, a repeat test is warranted [7]. Elevated prolactin levels can arise from various factors, including stress, hypothyroidism, renal dysfunction, and Polycystic Ovary Syndrome (PCOS), with certain medications like risperidone, metoclopramide, and domperidone are known to increase prolactin levels. A reading surpassing 1000 mIU/ml necessitates an endocrinology consultation for a comprehensive evaluation, potentially involving MRI scans for brain to investigate the presence of tumours, bleeding, or vascular irregularities [6, 7]. Cases exhibiting symptoms also require assessments of visual acuity, visual fields, and optic disc examination.
- **Testosterone and Androgen Levels:** Elevated testosterone levels exceeding 5 nmol/L necessitate further examinations to exclude conditions such as Androgen Insensitivity Syndrome (AIS), androgen secreting tumours, Cushing's Syndrome, and late onset Congenital Adrenal Hyperplasia (CAH). Levels between 2 nmol/L and 5 nmol/L might suggest a diagnosis of PCOS [2, 8].

Imaging Techniques

While the utility of transabdominal ultrasonography is generally limited, transvaginal sonography provides invaluable insights into ovarian morphology and can reveal anomalies in the upper genital tract, such as the absence of the uterus. Magnetic Resonance Imaging (MRI) is the recommended modality for a comprehensive visualization of the genital tract [8].

Genetic and Chromosomal Testing

Chromosome karyotyping plays a pivotal role in confirming diagnoses, especially in cases suggesting congenital origins based on physical, radiological, and biochemical findings. In instances of suspected POI, sequencing of the fragile X mental retardation gene (FMR1) should be performed alongside chromosomal karyotyping [8]. Routine screening for autoantibodies associated with ovarian pathology is generally not recommended [1, 8].

Causes

The causes of Primary amenorrhoea can be categorised based on the presence or absence of secondary sexual characteristics development.

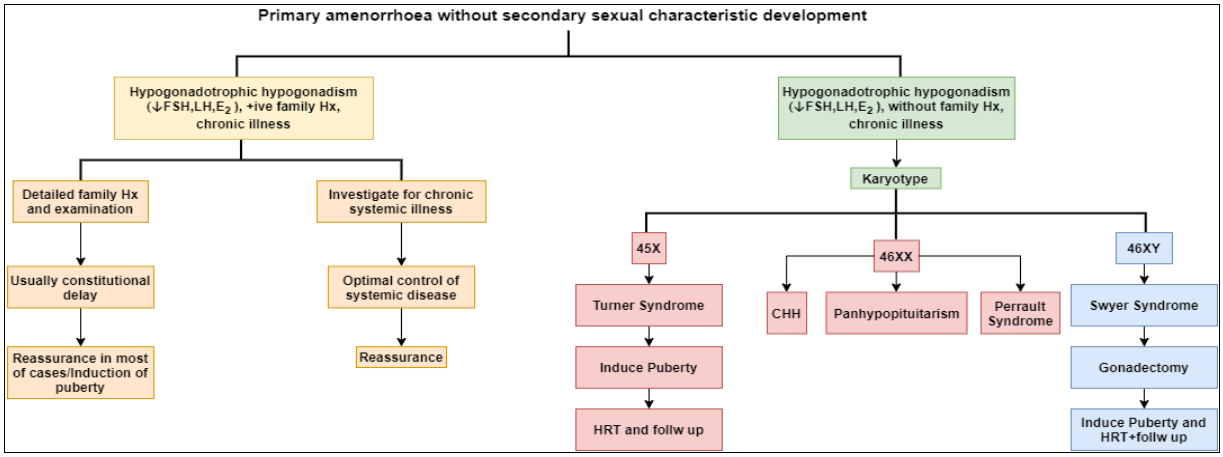

Fig 3: Flow chart for Primary amenorrhoea causes with absent or impaired secondary sexual characteristics

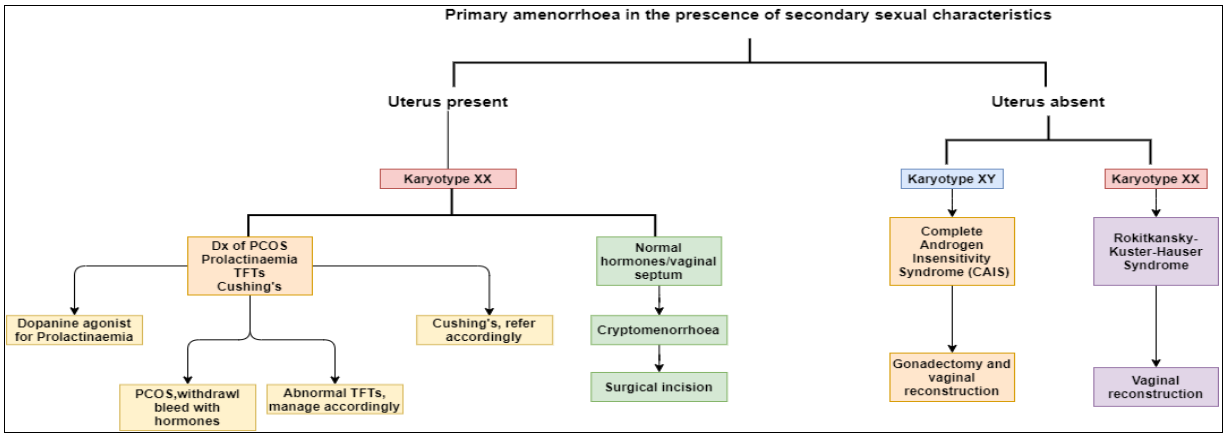

Fig 4: Flow chart for Primary amenorrhoea causes with normal or impaired development of secondary sexual characteristics

Hypogonadotrophic hypogonadism

Constitutional delay

Constitutional delay refers to the delayed maturation of the hypothalamic-pituitary-ovarian (HPO) axis, often manifesting with similar patterns within familial lines, such as in mothers or elder siblings. Clinically, it is characterised by a delayed progression in both skeletal growth and sexual maturity when compared to age-matched peers, contributing to between 30% and 56% instances of primary amenorrhoea [9]. Typically, a supportive approach emphasizing reassurance and expectant management, alongside recommendations for a healthy lifestyle, suffices as effective management.

Hypothalamic and pituitary disorder

Functional Hypothalamic Amenorrhoea (FHA)

FHA is recognised as a diagnosis of exclusion, adhering to the Endocrine Society's criteria. These include extended menstrual cycles beyond 45 days or the presence of amenorrhoea for more than three months, coupled with signs of weight loss, excessive exercise, stress, hypogonadotropic hypogonadism, a negative progesterone challenge test, and normal MRI findings of the brain [10]. The onset of FHA is attributed to a combination of physical, emotional, and nutritional stressors that interrupt the regular pulsatile release of gonadotropins, leading to anovulation [10, 11]. Significant life event responses, diminished body fat, and rapid weight loss are identified triggers [11].

Eating disorders play a significant role in disrupting hypothalamic functions related to appetite, hydration, thermoregulation, sleep, and endocrine balance, with leptin being a key regulator [11]. In amenorrhoeic, lean women, leptin levels are notably lower than in their menstruating counterparts [12], contributing to nutritional deficiencies, oestrogen deficits, decreased bone mineral density (BMD), and heightened fracture risks [12].

Additionally, a spectrum of chronic conditions including physical and sexual abuse, emotional neglect, thyroid dysfunction, type I diabetes, chronic renal failure, and various congenital disorders can impede HPO axis functionality [11]. These stressors can provoke FHA and hypogonadotropic hypogonadism, affecting metabolic equilibrium and the timing of puberty [11, 12]. Epilepsy and certain antiepileptic drugs may also induce hypothalamic

disruptions or hyperprolactinemia [13].

Intervention aimed at the root causes is pivotal for reinstating normal HPO axis operations and resuming menstrual cycles. Behavioural adjustments, such as moderated exercise and improved dietary habits, generally restore metabolic and endocrine balance, although some individuals may persistently experience amenorrhoea [14]. In severe scenarios, hospitalization might be necessary, especially for life-threatening energy and electrolyte imbalances. The National Institute for Health and Care Excellence (NICE) also recommends bone density tests for malnourished individuals [14]. Non-pharmacological treatments and short-term transdermal 17β oestradiol therapy are advocated, while the Endocrine Society advises against the use of combined oral contraceptives (COCs), bisphosphonates, testosterone, and leptins solely for enhancing BMD in adolescents with FHA. However, COCs can be considered for birth control purposes [10, 14].

Congenital Hypothalamic Hypogonadism (CHH)

CHH, affecting 10-20% of adolescents and showing a higher prevalence in males (5:1 ratio), is marked by a Gonadotropin-Releasing Hormone (GnRH) deficit that results in incomplete or absent pubertal milestones [15]. Kallmann syndrome, a variant of CHH, stems from embryonic development issues, presenting as anosmia or a diminished sense of smell and delayed puberty. The differentiation between constitutional delay and Kallmann syndrome is often made based on the presence of pubic hair and olfactory function [16].

CHH might also be associated with syndromes like Prader-Willi, Laurence-Moon, and Gordon Holmes, exhibiting similar symptoms to constitutional delay but with distinct genetic markers or clinical symptoms [17]. Diagnosis entails genetic testing, MRI brain scans, ultrasound of genitalia, bone density evaluations, and olfactory testing, noting that anosmia or hyposmia is reported in up to 50% of CHH cases [18, 19]. Treatment is personalised, focusing on puberty induction tailored to individual needs [19].

Acquired Hypogonadotrophic hypogonadism (AHH)

AHH during adolescence is relatively rare but can be attributed to pituitary adenomas, which are generally benign. These adenomas, along with craniopharyngiomas, meningiomas, gliomas, chordomas, epidermoid cysts, Rathke's cleft cysts, and metastatic tumours, become more prevalent etiological factors in adults [20, 21]. Additional influences include medical interventions and disorders impacting the pituitary fossa, such as surgical operations, radiation therapy, ischemic events, infarctions, and infiltrative diseases including lymphocytic hypophysitis, sarcoidosis, tuberculomas, syphilis, and hemochromatosis [20, 21]. Among these, prolactinomas are the most common, present in 46%-66% of cases, followed by corticotropinomas (30%), somatotropinomas (5-15%), and other adenoma types [22].

Impairments in GnRH or gonadotropin production often result in hyperprolactinemia, disrupting the inhibitory effects of dopamine on the pituitary gland [22]. Adenomas are differentiated by size into microadenomas (≤10mm) and macroadenomas (>10mm), with diagnosis confirmed through MRI scans employing gadolinium contrast [23]. Symptoms typical of macroadenomas include bitemporal hemianopia and generalised headaches. Treatment strategies vary; symptomatic macroadenomas usually necessitate trans-sphenoidal surgery and pharmacological intervention, whereas microadenomas are often managed effectively with medications such as cabergoline or bromocriptine [22, 24]. Achieving normal prolactin levels is crucial for the restoration of menstrual cycles, ovulation, and fertility [23, 24].

Non-functional adenomas are resistant to pharmacological management. Persistent, mildly elevated prolactin levels in the absence of hyperthyroidism, acromegaly, or Cushing's syndrome suggest the presence of a non-functioning adenoma [25]. Conditions such as primary hypothyroidism may also lead to hyperprolactinemia due to increased proliferation of thyrotrophs and lactotrophs within the pituitary gland [25, 26]. Chronic kidney disease contributes to elevated serum prolactin through decreased renal clearance and increased production, a consequence of suppressed dopamine activity [26].

Certain medications, including neuroleptics like risperidone and SSRIs such as fluoxetine, elevate prolactin levels by inhibiting dopamine receptors [25, 26]. Likewise, drugs such as metoclopramide, domperidone, and specific antihypertensives like verapamil and methyldopa modestly increase prolactin secretion by diminishing dopamine secretion [26, 27]. Furthermore, opioid use can lead to decreased LH production, culminating in clinical hypogonadism [27].

Hypopituitarism

Hypopituitarism manifests as diminished hormone production and secretion from the anterior or posterior lobes of the pituitary gland. It can arise from various insults to the hypothalamic-pituitary (HP) axis, including infection, inflammation, ischaemia, haemorrhage, or traumatic brain injury [28]. Hypophysitis, an inflammatory condition of the pituitary, though infrequent, has been reported in specific instances during pregnancy and among early adolescents presenting with primary amenorrhea [28].

Idiopathic hypopituitarism cases prompt exploration into infiltrative diseases like haemochromatosis, lymphocytic hypophysitis, sarcoidosis, and tuberculosis, potentially leading to either reversible or irreversible hypogonadotropic hypogonadism. Addressing these underlying conditions may restore glandular function [28, 29].

Sheehan's syndrome, characterised by ischemic pituitary damage following significant post-partum haemorrhage induced by hypovolaemic hypotension, is more commonly associated with secondary amenorrhea. The severity and range of symptoms depend on the extent of pituitary damage incurred.

The pituitary gland, situated within the Sella Turcica at the brain's base, may also be involved in Empty Sella Syndrome (ESS). Contrary to what the term might suggest, ESS involves a congenital defect of the Sellar diaphragm, allowing cerebrospinal fluid from the subarachnoid space to enter the Sella, leading to the condition's manifestation [30]. Secondary ESS, often a consequence of pituitary adenoma treatment through surgery or radiation, tends to occur without symptoms, though it can present with hypopituitarism signs. Notably, ESS appears more frequently in women who are obese or hypertensive and exhibit amenorrhea [30].

Comprehensive assessment of the HP axis structure and functionality is essential, entailing extensive blood tests for serum prolactin, cortisol, insulin-like growth factor 1 (IGF-

1), thyroid-stimulating hormone (TSH), free thyroxine (FT4), luteinising hormone (LH), follicle-stimulating hormone (FSH), testosterone, and oestradiol. These samples are best collected early in the morning for optimal accuracy [31]. MRI scans of the pituitary gland are recommended to identify any anatomical irregularities.

In managing hypopituitarism, hydrocortisone serves as the primary therapy in 98% of cases, with subsequent hormone replacements including thyroxine, sex steroids, growth hormone (GH), and desmopressin as needed [31]. Continuous monitoring of the patient's health, including bone mineral density evaluations and addressing the root cause, remains a crucial component of the treatment regimen [28, 31].

Genital tract problems

Genital tract complications predominantly stem from congenital anomalies, though post-surgical issues can also arise. The formation of the female genital tract involves a complex process where the Müllerian (paramesonephric) ducts migrate medially and fuse along the midline, giving rise to the uterus, fallopian tubes, cervix, and the upper portion of the vagina. The remaining lower section of the vagina develops from the urogenital sinus invagination, merging vertically with the Müllerian structure to complete the reproductive tract. The hymen is formed through the posterior vaginal wall's invagination from the urogenital sinus. Müllerian anomalies can range widely, including vaginal, cervical, and uterine agenesis, as well as conditions like transverse vaginal septum and imperforate hymen.

An imperforate hymen is often identified by primary amenorrhoea alongside cyclical lower abdominal pain or urinary retention symptoms. A vaginal examination may reveal a protruding membrane at the introitus's lower limit, which is more evident during the Valsalva manoeuvre, aiding in the diagnosis. Surgical incision and drainage are the standard treatments, typically resulting in favourable long-term outcomes [32].

A transverse vaginal septum occurs when the vaginal plate, originating from the sinovaginal bulb, fails to dissolve during embryogenesis. In young patients, this may manifest as a shortened vagina with an unseen cervix and common occurrences of haematocolpos (blood accumulation behind the septum). MRI scanning is excellent for evaluating the septum's thickness and its proximity to the introitus. Notably, in about 75% of instances, the septum is located less than 3cm from the introitus, and instances where it is over 6cm away are quite rare, with nearly half of these septa exceeding 1cm in thickness [33]. Management of such cases is best conducted in specialised facilities. The administration of continuous combined oral contraceptive pills (COCP) or GnRH analogues can mitigate pain. Endometriosis, frequently caused by retrograde menstruation, is a common complication in these scenarios [33, 34].

Cervical agenesis, though exceedingly rare, leads to haematometra (uterine blood accumulation) and retrograde menstruation, potentially resulting in endometriosis and pelvic adhesions [35]. Complete outflow obstruction due to cervical stenosis represents an unusual complication following cervical surgical interventions.

Asherman's syndrome is suspected in patients exhibiting a history of uterine surgery succeeded by secondary amenorrhoea. Characteristic signs include transitioning from hypomenorrhoea to amenorrhoea and a diminished or non-existent reaction to sequential treatments with exogenous oestrogen (1.25 mg daily) for 21 days and progestin (medroxyprogesterone acetate (MPA) 10mg daily for 5-7 days), suggesting endometrial dysfunction and aiding diagnosis [36].

Mayer-Rokitansky-Kuster Hauser (MKH) Syndrome

Mayer-Rokitansky-Kuster-Hauser (MRKH) Syndrome is characterised by the incomplete development of the Müllerian ducts, leading to the absence or underdevelopment of the uterus and cervix, and often a shortened vagina, varying with the defect's extent. In cases of MRKH syndrome, individuals typically lack a functional uterus and the upper two-thirds of the vagina, while retaining normal secondary sexual characteristics and a 46XX karyotype. The syndrome affects approximately 1 in 5,000 individuals [37]. The presentation often includes primary amenorrhoea, with some cases reporting haematometra [38]. Ovaries remain unaffected, though positioned higher in the pelvis. MRKH syndrome is frequently accompanied by renal anomalies, such as unilateral renal agenesis or a horseshoe kidney, and can also involve vertebral, cardiac, and auditory irregularities [38, 39].

Therapeutic approaches aim to establish a functional vagina, employing either vaginal dilators or surgical techniques to create a neovagina, enhancing the individual's quality of life and enabling sexual intercourse, if desired [39]. The functionality of the ovaries allows for the assessment of ovarian reserve via serum Anti-Müllerian Hormone (AMH) levels. A notable minority of women with MRKH and their mothers possess genes linked to classical galactosaemia [40], potentially heightening the risk for Premature Ovarian Insufficiency (POI). Fertility options may include surrogacy or uterine transplantation [39].

It's imperative for clinicians to approach discussions around MRKH syndrome with empathy and sensitivity, offering support to patients and their families. Directing to support networks and resources, such as https://www.mrkh.org.uk, play a vital role in providing holistic care.

Androgen insensitivity syndrome (AIS)

Complete Androgen Insensitivity Syndrome (CAIS), historically referred to as testicular feminisation or male pseudohermaphroditism, is observed in approximately 4 in 100,000 individuals [41]. This condition is characterised by a 46XY karyotype, with individuals possessing male gonads that produce testosterone and Anti-Müllerian Hormone (AMH) [42]. CAIS is notably the third leading cause of primary amenorrhoea, after gonadal dysgenesis and Müllerian anomalies [41, 42]. It originates from mutations in the androgen receptor (AR) gene situated on the X chromosome's long arm, resulting in a lack of response to androgens at the target organs [41, 42]. Typically, individuals with CAIS exhibit elevated serum testosterone levels, with normal to high levels of serum LH and a standard conversion rate of testosterone to dihydrotestosterone.

Approximately half of CAIS cases are inherited via an X-linked recessive pattern, while the remainder arise from new mutations on the maternal X chromosome [42]. Clinically, CAIS manifests as a phenotypical female experiencing primary amenorrhoea, with breast development but lacking pubic hair during adolescence. The presence of an inguinal hernia significantly indicates AIS, with clitoromegaly varying according to the degree of AR sensitivity.

The management of CAIS is twofold: ensuring the possibility of sexual relationships through a functional vagina and mitigating the malignancy risk of undescended testes. Progressive vaginal dilatation is the preferred initial method for creating a neovagina, reliant on patient dedication and consistent dilator use. Surgical options like vaginoplasty are considered after thorough discussions within a multidisciplinary team, based on patient preference and anatomical considerations [42, 43]. Notably, in CAIS, there is an absence of the uterus, fallopian tubes, ovaries, cervix, and the upper portion of the vagina. Delaying gonadectomy until after puberty permits natural hormonal development, with tumour risk in prepubertal CAIS being extremely low [43, 44]. Post-puberty, the removal of gonads is advised due to the minimal but existent malignancy risk, supplemented by regular monitoring throughout puberty [43, 44]. Elevated AMH levels in CAIS, indicative of testicular Sertoli cell activity, are a diagnostic marker [44].

Diagnosing an individual with a 46XY karyotype and a female phenotype during adolescence presents significant challenges, potentially evoking psychological distress and identity queries for both the patient and their family. It's crucial that discussions are conducted factually, empathetically, and sensitively. Psychological support, alongside referrals to support networks and educational resources like the Androgen Insensitivity Syndrome Support Groups and www.dsdfamilies.org, is highly recommended for comprehensive care.

Chromosomal and Ovarian disorders (Hypergonadotropic Hypogonadism)

Gonadal dysgenesis represents a range of conditions marked by the defective or incomplete development of the gonads, predominantly due to chromosomal anomalies, which can be structural and/or numerical. A notable fraction of women with this condition have abnormalities linked to the X chromosome, and around 25% exhibit a conventional 46XX karyotype but with minor X chromosome irregularities [45]. Among these, Perrault syndrome is distinguished by its combination of gonadal dysgenesis within a 46XX karyotype and sensorineural hearing loss. On the other hand, a 46XY karyotype exhibiting gonadal dysgenesis is indicative of Swyer syndrome [45].

The most commonly encountered chromosomal disorder within this context is Turner's syndrome, generally associated with a 45X0 karyotype. These conditions highlights the complexity and variety of chromosomal influences on gonadal development and underscores the importance of a comprehensive genetic evaluation in the diagnostic process of hypergonadotropic hypogonadism.

Turner's syndrome

Turner's syndrome is distinguished by its unique phenotypic features, including a webbed neck, short stature, lack of sexual development, low-set ears, and widely spaced nipples. In instances where this classic phenotype is not observed, affected individuals might experience delayed growth, primary amenorrhoea, and the absence of secondary sexual characteristics, indicative of hypergonadotropic hypogonadism. Karyotyping typically uncovers a 45X0 chromosome composition in 40%-60% of cases, while others exhibit mosaicism (e.g., 45X0/46XY, 45X0/46XX) [46]. Occurring in around 5 in 10,000 individuals, Turner syndrome is the most common cause of Premature Ovarian Insufficiency (POI) during adolescence [46].

Women with Turner syndrome face an elevated risk of various medical issues. Notably, about one-third of these individuals have cardiovascular abnormalities—including problems with the aortic and mitral valves, coarctation of the aorta, aortic aneurysm, and the risk of spontaneous aortic dissection—which contribute to a threefold increase in mortality, predominantly from cardiac complications. The risk of morbidity and mortality significantly escalates during pregnancy [47]. Renal anomalies, such as horseshoe kidney, unilateral renal agenesis, and duplicated renal collecting systems, are also common. Additionally, autoimmune disorders like thyroiditis, hepatitis, type 1 diabetes, thrombocytopenia, and coeliac disease occur more frequently in those with Turner syndrome, necessitating thorough and ongoing surveillance. This includes initial and follow-up echocardiograms, renal ultrasounds, and regular screening for thyroid, liver, and renal function, as well as coeliac disease and lipid levels [48]. Audiometry is advised at diagnosis and then every decade afterwards. Furthermore, Attention Deficit Hyperactivity Disorder (ADHD) has a higher prevalence in individuals with this syndrome [48].

Mangement

Prompt diagnosis followed by the initiation of growth hormone (GH) treatment can significantly improve height outcomes, potentially achieving heights up to 150cm. Puberty induction is usually the next step. The ultimate height reached largely depends on the GH dosage and the length of treatment before introducing oestrogen, which might be delayed until the age of 14 to maximise growth potential [47, 48]. Late diagnosis may limit the height gains from GH therapy. Oxandrolone, a synthetic anabolic steroid, has been effective in enhancing height outcomes [48]. Although spontaneous pregnancies have been reported in women with Turner syndrome, these cases are often complicated by miscarriages and an elevated risk of sex chromosome aneuploidy. Egg donation emerges as the most feasible path to successful pregnancy for these women [47, 48].

Swyer syndrome

Swyer syndrome is marked by an outwardly female phenotype, despite the presence of 46 XY chromosomes and anatomically male gonads, which sometimes manifest as inguinal hernias. These gonads are non-functional and appear as streak gonads, incapable of producing Anti-Müllerian Hormone (AMH) or androgens. This leads to what is known as complete gonadal dysgenesis. Consequently, the internal reproductive anatomy, including the vagina, cervix, uterus, and fallopian tubes, develops typically, while external genitalia remain unmasculinised [49]. The incidence of 46XY gonadal dysgenesis is estimated at around 1.5 in 100,000 [49]. Individuals with Swyer syndrome often experience delayed development of secondary sexual characteristics and present with primary amenorrhoea. Early gonadectomy is advised following diagnosis to decrease the risk of gonadal malignancy, given the elevated malignancy potential of cryptorchid testes [43, 49].

Ovarian dysgenesis with 46 XX karyotype

Individuals diagnosed with this condition typically manifest a normal female phenotype and do not display prominent somatic anomalies, often coming to medical attention due to delayed puberty [50]. The underlying cause includes

mutations in the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) receptor genes, which result in gonadotropin resistance and impede oestrogen production by the ovaries. In some cases, enzymatic deficiencies and certain autosomal genes can also disrupt steroidogenesis within both the ovaries and adrenals, further complicating the clinical picture.

Perrault syndrome

Perrault syndrome is a rare genetic disorder that results in sensorineural hearing loss coupled with ovarian dysgenesis in individuals possessing a 46XX karyotype. The spectrum of clinical presentations can range from gonadal dysgenesis and primary amenorrhoea to Premature Ovarian Insufficiency (POI) characterised by progressive follicular dysgenesis, all of which are linked to specific gene mutations [51]. Additional manifestations might include learning difficulties, developmental delays, cerebellar ataxia, as well as sensory and motor neuropathies, highlighting the syndrome's systemic impact [63].

Management strategies for Perrault syndrome are multifaceted, encompassing auditory interventions such as the use of hearing aids or cochlear implants, alongside hormonal treatments for inducing puberty and preserving bone mineral density. For fertility considerations, egg donation and the preservation of eggs represent viable options for those facing the risk of POI [52]. This comprehensive approach underscores the importance of individualised care and the need for multidisciplinary involvement to address the wide array of challenges associated with this condition.

Premature ovarian insufficiency (POI)

Premature Ovarian Insufficiency (POI) is a form of hypergonadotropic hypogonadism that occurs before the age of 40, displaying a wide array of causes and phenotypes. It primarily manifests as secondary amenorrhoea, though it can occasionally present before menarche. The risk of POI is notably higher among those with primary amenorrhoea, estimated at 10-28%, and slightly lower, at 4-18%, for those with secondary amenorrhoea [53]. In POI, ovarian characteristics resemble those seen in the menopausal stages. Its prevalence differs across ethnic groups, with higher rates observed in Caucasian and African American populations compared to Chinese and Japanese women [53, 54]. Factors contributing to POI encompass chromosomal anomalies, fragile X syndrome, autoimmune diseases, exposure to chemotherapy and radiation, infiltrative and infectious diseases, and pelvic surgery [54, 55].

Galactosaemia, an autosomal recessive condition, leads to POI through accelerated follicular atresia, a consequence of the damaging effects of galactose metabolites on germ cells [68]. All women diagnosed with POI are advised to undergo screening for the Fragile X mental retardation (FMR1) gene, facilitating discussions around the potential risks of transmission to future generations [8, 56].

There is a significant correlation between autoimmune adrenal and ovarian insufficiency, highlighting the importance of anti-adrenal antibody testing in women with POI [57]. Ovarian biopsy is generally not recommended for diagnosing POI [55, 57]. Women testing positive for 21-hydroxylase require further adrenal function assessments, with ongoing surveillance recommended to pre-emptively identify and manage adrenal complications [57]. Autoimmune thyroiditis is also notably more common in women with POI [57].

The detrimental effects of radiotherapy on oocytes are contingent upon the radiation dose, exposure duration, and age at the time of exposure. Abdominal radiation can induce structural changes in the myometrium, cervix, and endometrium, while cranial radiation can disrupt the hypothalamic-pituitary-ovarian (HPO) axis, potentially causing amenorrhoea and anovulation [58]. Conversely, extra-pelvic radiation poses a minimal risk to ovarian function with proper pelvic shielding. Strategies such as ovarian transposition and urgent gamete or tissue preservation should be considered prior to radiotherapy [58, 59]. Interestingly, no evidence indicates an increased risk of birth defects in children born to mothers who underwent radiation or chemotherapy. The impact of chemotherapeutic agents on fertility is dose-dependent, with the age and regimen complexity being crucial factors [59]. Utilizing long-acting GnRH analogues pre-treatment to induce a hypo-gonadal state may reduce the incidence of irreversible POI(<10%) compared to those not receiving such therapy (40-70%) [59].

Approximately 5-10% of women with POI may achieve spontaneous pregnancy [61]. It's imperative to provide comprehensive counselling on contraception for those not seeking pregnancy. The diagnosis of POI can be profoundly distressing, underscoring the need for robust support, including information on fertility preservation options like donor oocytes, hormone replacement therapy, bone density monitoring, and psychological support. For prepubertal cases, the approach to puberty induction mirrors that for hypogonadotropic hypogonadism.

Eugonadotropic hyperandrogenism

Eugonadotropic hyperandrogenism, defined by increased androgen levels amidst normal gonadotropin readings, is a potential cause of primary amenorrhoea. Signs suggestive of an early onset of adrenarche might include pronounced acne, hirsutism, alopecia akin to male-pattern hair loss, clitoromegaly, and voice deepening. To pinpoint the source of androgen excess, clinicians typically assess total and free serum testosterone, Dehydroepiandrosterone Sulphate (DHEAS), Androstenedione, and 17-Hydroxyprogesterone levels. Total free serum testosterone values falling within the 2-5 nmol/L range are often indicative of Polycystic Ovary Syndrome (PCOS), while readings surpassing 5 nmol/L prompt further investigations to locate the androgen origin [2, 62]. An increased 17-Hydroxyprogesterone level calls for an ACTH stimulation test to rule out Congenital Adrenal Hyperplasia (CAH), ensuring a comprehensive diagnostic approach to eugonadotropic hyperandrogenism and its associated conditions.

Polycystic ovary syndrome (PCOS)

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder primarily diagnosed through exclusion and is notably the most prevalent condition associated with mild hyperandrogenism. The Rotterdam criteria, universally recognised for diagnosing PCOS in adults, include:

1. Ultrasound findings revealing over 10-12 follicles, each under 10mm in diameter, arrayed around the ovary's periphery, or an ovarian volume surpassing 10cm³.
2. Biochemical markers such as raised serum free testosterone, an elevated LH/FSH ratio, and diminished or normal levels of Sex Hormone Binding Globulin

(SHBG).
3. Clinical signs including oligomenorrhoea, hirsutism, acne, and male pattern baldness.

The diagnosis of PCOS is confirmed with the presence of at least two of these three criteria [62].

PCOS is seen in 6%-18% of adolescent girls, typically manifesting as oligomenorrhoea or secondary amenorrhoea, and less commonly, primary amenorrhoea. Adolescents with PCOS who present with primary amenorrhoea tend to experience more pronounced metabolic complications such as increased testosterone levels, dyslipidaemia, and acanthosis nigricans, alongside a heightened risk of depression and anxiety, compared to their counterparts with secondary amenorrhoea [63, 64].

Applying the Rotterdam criteria to adolescents may coincide with standard pubertal development, potentially complicating diagnosis [66, 67]. According to the World Health Organisation, adolescence spans from 10 to 19 years, a phase characterised by significant growth and developmental changes. Globally agreed upon guidelines for PCOS assessment and management in adolescents seek to mitigate the risks of misdiagnosis [67]. Diagnostic considerations include evaluating oligomenorrhoea relative to the years since menarche, owing to the typically erratic menstrual cycles within the initial three years, alongside identifying hyperandrogenism through clinical examination or biochemical testing [67].

In adolescents, neither pelvic ultrasound nor Anti-Müllerian Hormone (AMH) levels are deemed diagnostic for PCOS. Cases suggestive of PCOS, yet not conclusively diagnosed, warrant regular monitoring and symptomatic care, with an ultrasound review recommended after 8 years post-menarche should symptoms continue [68].

Management strategies emphasise healthy lifestyle modifications, including effective weight management, and symptomatic relief. The use of combined oral contraceptive (COC) pills and metformin, despite metformin not being officially approved in the UK for treating PCOS, offers benefits in regulating hormonal imbalances, menstrual cycles, and in alleviating acne and hirsutism for some individuals [68, 69]. For lean adolescents, a daily metformin dose of 750mg often suffices, whereas overweight individuals may require dosages ranging from 1.5gm to 2.5gm [69, 70]. COC pills help lower circulating free androgens by reducing ovarian androgen production and enhancing sex hormone-binding globulin levels. Short-term use of antiandrogens like cyproterone acetate and flutamide, which block testosterone's conversion to the more potent dihydrotestosterone, is effective for managing severe acne and hirsutism [69,70].

The high serum DHEA-S or androstenedione, suspect androgen producing adrenal tumours. Rarely these are ovarian in origin [85]. The ACTH and CRH release from neuroendocrine tumours can result in over production of cortisol from the adrenals, results in hyperandrogenism. Glucocorticoids used in high doses can also cause Cushing's syndrome [86]. A 24 hours urinary free cortisol, midnight salivary cortisol or 1mg dexamethasone suppression test (Oral administration of 1 mg Dexamethasone at midnight and check serum cortisol at 0800 hrs in the morning) to rule out Cushing's syndrome after this screening do diagnostic tests to find the cause [86]. The Dexamethasone-CRH test is performed by giving 0.5mg of dexamethasone every 6 hours for 48 hours total of eight doses and check serum cortisol after 15 minutes of CRH 1µg/kg body weight, which is given two hours after the last dexamethasone dose. The serum cortisol less than 1.4 µg/dL rule out Cushing's syndrome.

Adult-onset congenital adrenal hyperplasia (CAH)

Adult-onset Congenital Adrenal Hyperplasia (CAH) predominantly results from a deficiency in 21-hydroxylase and can become apparent in adolescence or adulthood. Common manifestations include primary amenorrhoea or oligomenorrhoea, early onset of pubarche, pronounced acne, hirsutism, or clitoromegaly [71]. This autosomal recessive disorder is estimated to occur in 1 in 1,000 individuals, with higher prevalence rates noted among certain ethnic groups, including Ashkenazi Jews [72]. An early morning follicular phase measurement of 17-hydroxyprogesterone (17-OHP) exceeding 6 nmol/L indicates CAH. An ACTH stimulation test provides a conclusive diagnosis for those with 17-OHP levels in the 2 nmol/L to 6 nmol/L range. For primary amenorrhoea linked to CAH, hydrocortisone therapy has been shown to effectively reduce androgen excess.

Elevated androgen levels might also signal hyperandrogenic disorders such as Cushing's syndrome, glucocorticoid resistance, or androgen-secreting adrenal and ovarian tumours [73]. High serum concentrations of DHEA-S or androstenedione can suggest adrenal tumours as the source of androgens, though ovarian origins are comparatively rare [73]. Neuroendocrine tumours that release ACTH and CRH can provoke excess cortisol production by the adrenal glands, leading to hyperandrogenism. Excessive glucocorticoid therapy, similarly, can trigger Cushing's Syndrome [73]. Initial screening for Cushing's syndrome involves tests such as a 24-hour urinary free cortisol, midnight salivary cortisol, or a 1 mg dexamethasone suppression test (1 mg of Dexamethasone taken at midnight with serum cortisol measured at 0800 hrs the next morning). Follow-up diagnostic procedures aim to pinpoint the specific cause [73]. The Dexamethasone-CRH test, which entails administering 0.5 mg of dexamethasone every 6 hours for 48 hours (a total of eight doses), followed by a serum cortisol check 15 minutes post-CRH injection (1µg/kg body weight) two hours after the last dexamethasone dose, is used to exclude Cushing's syndrome if serum cortisol is below 1.4 µg/dL.

Ambiguous genitalia

In cases of ambiguous genitalia, chromosomal karyotyping plays a crucial role and is usually carried out early in infancy. A 46XY karyotype with 5α-reductase deficiency is a condition where testosterone cannot be converted into dihydrotestosterone (DHT), a hormone vital for the masculinisation of external genitalia. Consequently, the external genitalia may strongly resemble that of female genitalia.

Incomplete Androgen Insensitivity Syndrome (IAIS) represents a range of androgen signalling responses, leading to ambiguous genitalia in affected newborns. As these individuals reach puberty, common signs may include sparse pubic hair, breast development, and clitoromegaly [74]. An early gonadectomy is often recommended for IAIS to avert unintended virilisation during puberty [43, 74]. The phenotypical presentation in IAIS cases usually leans towards female, although the appearance of the external genitalia varies, dependent on the degree of androgen

exposure. This variation significantly influences whether individuals are raised as male or female, based on their responsiveness to androgens [42,74].

For those with a 46XX karyotype, Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency is responsible for 95% of cases. Here, an overproduction of androgens results in the masculinisation of the external genitalia [75]. Although rare, placental aromatase deficiency can also expose the foetus to significant levels of androgens, leading to ambiguous genitalia, and may even cause maternal symptoms like severe acne and hirsutism during pregnancy [76].

The management of ambiguous genitalia demands a comprehensive, multidisciplinary approach, placing a significant emphasis on involving parents and family members in decision-making processes and gender assignment. This period can be particularly strenuous for parents, who may require extensive counselling and support, both emotional and psychological. It's critical that each case is considered individually, ensuring parental involvement in all decisions from diagnosis to management [90]. Consistent follow-ups and appropriate hormonal support are crucial for the child's overall wellbeing. Moreover, any surgical procedures, whether they involve gonadectomy or genital reconstruction, should be undertaken by surgeons with specific expertise in this area.

Induction and maintenance of puberty

The induction of puberty plays a crucial role in stimulating the development of secondary sexual characteristics such as breast growth and uterine development, alongside promoting a growth spurt in height and ensuring age-appropriate accumulation of bone mass. Under normal circumstances, ovarian function begins around age 11. Consequently, a low dose of oestrogen is introduced at ages 11-12 for females with both hypo- and hypergonadotropic hypogonadism. The commencement of treatment may be delayed for those undergoing treatment for underlying conditions, such as cancer, or in instances where maximising height potential with growth hormone (GH) therapy is a priority. The overarching aim is to emulate the natural progression of puberty as closely as possible.

Oestrogen therapy typically starts with a low dose of 0.25-0.5 mg, preferably administered via the transdermal route, utilising natural 17 β-oestradiol, which is favoured over synthetic variants [77]. The oestrogen dose should be tailored based on body weight or increased at fixed intervals every 3-6 months, with periodic assessments of Tanner staging to monitor treatment efficacy [77, 78]. These adjustments are pivotal in ensuring optimal growth in height.

Progestin introduction follows approximately two to two and a half years post the initiation of oestrogen therapy, coinciding with the occurrence of breakthrough bleeding or when the endometrium is deemed ready on ultrasound, generally when its thickness exceeds 7-8 mm. Progestin administration for 10 days every 1-3 months is crucial for safeguarding against endometrial hyperplasia and regulating menstrual bleeding. For individuals with a definitive diagnosis, continuation of therapy is advised until the average onset of menopause, around 51-52 years, though this may be adjusted based on a personalised assessment of risks and hormone replacement therapy needs [78].

Summary

The presentation of primary amenorrhoea in adolescence is associated with emotional, psychological and physical problems. An early diagnosis, hormone replacement if required and psychological support is mandatory as part of the management plan. The approach is via a multidisciplinary team in the presence of adolescent gynaecologist and paediatric endocrinologist. The treatment of underlying cause and oestrogen replacement for promoting normal sexual development is essential. Each case should be assessed individually and genetic testing helps in cases with CHH, CAIS and POI, but routine autosomal genetic testing is not required. Decisions for gonadectomy and surgery needs thorough counselling of patients and parents to help them make an informed decision. In central HP axis problems reproductive outcome is usually reassuring. In POI case, timely diagnosis can facilitate fertility preservation in some situations.

Author contributions

All authors contributing for the preparation of this review. The first literature search and draft were prepared by A.Khan with the help of K.Younas. All figure and tables by A.Khan. K.younas cross checked literature search and helped with draft preparation. E. Kevelihan did the final proof reading and helped with final submission.

Conflicts of interest

All authors have no conflict of interest to declare.

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