DOI: 10.4274/jcrpe.galenos.2024.2024-2-7
J Clin Res Pediatr Endocrinol 2024;16(4):379-400

A Current Perspective on Delayed Puberty and Its Management

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Abstract

Delayed puberty is defined as the lack of development of secondary sex characteristics in childhood. Based on a review of the literature, delayed puberty can be divided into three main categories: (i) hypergonadotropic hypogonadism (congenital and acquired); (ii) permanent hypogonadotropic hypogonadism (congenital and acquired); and (iii) transient hypogonadotropic hypogonadism [constitutional delay of growth and puberty (CDGP) and functional hypogonadotropic hypogonadism]. CDGP is the most common cause of hypogonadism in both males and females, accounting for 60% and 30% respectively. Testosterone is the primary treatment for male hypogonadism, while estrogen and progesterone are used for female hypogonadism. However, in recent years, physiological induction therapy protocols such as human chorionic gonadotropin (hCG) monotherapy, hCG + follicle-stimulating hormone combined therapy, and gonadotropin-releasing hormone infusion have been recommended for the treatment of hypogonadotropic hypogonadism to increase long-term fertility success. There is no clear consensus on treatment protocols for physiological induction treatment and its effect on fertility. This review will discuss the clinical approach to hypogonadism, as well as traditional and physiological induction protocols.

Keywords: Hypogonadism, classification, treatment

Introduction

Puberty is a period of transition and developments resulting from a series of events starting *in utero* that are coordinated by the complex and timely interactions between the hypothalamus, pituitary gland, and gonads (1,2,3). In the normal tempo of maturation, in healthy infants, gonadotropin-releasing hormone (GnRH) secreting neurons in the hypothalamus, originating from the olfactory placode and the neural crest, start secreting GnRHs during the first six months of life in boys and two years in girls (4,5). During this period, also known as “mini-puberty”, the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) reach pubertal ranges between 1-3 months of life and decrease to prepubertal levels around six months in boys, while they can remain elevated for up to 3-4 years in girls (6).

In boys, LH induces the maturation of Leydig cells, which secrete testosterone and insulin-like peptide 3 (INSL3), both of which are responsible for inguinoscrotal testicular descent and penile growth (7). Increased FSH levels stimulate Sertoli and germ cell proliferation, which constitute 90% of testicular volume (7). Androgens are of major importance in the onset of spermatogenesis. However, spermatogenesis is not observed during the mini-puberty period due to very low androgen receptor (AR) expression in Sertoli cells during the first year of life (as experimentally confirmed in mice) (7,8). In girls, LH induces ovarian follicular theca cells to secrete androgens, and in granulosa cells under FSH stimulation, they are aromatized to estrogens (3).

The development of pubic hair (pubarche) is not usually considered a sign of pubertal onset because pubarche can result from the maturation of the adrenal glands (adrenarche), and the appearance of pubic hair can be independent of activation of the hypothalamic-pituitary-gonadal (HPG) axis. Adrenarche is the maturation of the zona reticularis of the adrenal gland, resulting in increased production of adrenal androgens. These androgens are associated with secondary

Cite this article as: Abacı A, Besci Ö. A Current Perspective on Delayed Puberty and Its Management. J Clin Res Pediatr Endocrinol. 2024;16(4):379-400

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Conflict of interest: None declared
Received: 07.02.2024
Accepted: 31.03.2024
Epub: 05.04.2024
Publication date: 04.12.2024

sexual characteristics, such as the development of pubic and axillary hair, body odor, and acne (9).

After this transitory period, GnRHs are inactivated until they are reset again at the onset of puberty, which is influenced by several factors, including genetic differences, exercise, nutrition, endocrine-disrupting chemicals, psychosocial factors, and body mass (9,10). Physiologically, starting between the ages of 8 and 13 in girls and 9 and 14 in boys, GnRH neurons start pulsatile GnRH secretion, activating FSH and LH, which in turn further stimulate the production of sex steroids (10,11). Pubertal sex steroids induce the development of secondary sexual characteristics and fertility, starting with breast development and uterine growth in girls and testicular and penile growth in boys (12). Sex steroids in puberty also influence the accrual of bone mineral density, changes in body composition and height, metabolic responses, and general well-being in both sexes (3,13). Any damage to this complex network can cause hypogonadism in both males and females (2,12).

Definition of Delayed Puberty

Delayed puberty is defined as lack of the initial signs of sexual maturation by an age that is more than 2-2.5 standard deviation above the mean for the population (1,12,14). Delayed pubertal onset is considered in the absence of testicular enlargement (testicular volumes <4 mL) by the age of 14 years in boys and breast development (absence of glandular breast tissue) by the age of 13 years in girls. Even if the onset is within the normal ranges, delayed pubertal progression or pubertal arrest is considered when the period between the onset and completion of puberty is longer than five years in boys, or there is a lack of menarche by 15 years of age, or within three years of thelarche in girls (3,15).

Classification of Delayed Puberty

There is no clear consensus in the literature on the classification of delayed puberty. Based on a review of the literature and text books, delayed puberty can be divided into three main categories (Table 1) (9,15,16,17,18): (i) hypergonadotropic hypogonadism (congenital and acquired); (ii) permanent hypogonadotropic hypogonadism (congenital and acquired); and (iii) Transient hypogonadotropic hypogonadism [constitutional delay of growth and puberty (CDGP) and functional hypogonadotropic hypogonadism (FHH)] (Table 1) (1,15,17,19). The etiological distribution of delayed puberty in the study by Sedlmeyer and Palmert (20) (n=232, 158 males) is summarized in Figure 1.

Hyper- and Hypogonadotropic Hypogonadism

Hyper- and hypogonadotropic hypogonadism can be congenital (permanent or transient) or acquired (3).

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism, characterized by low gonadotropins, can be caused by either a permanent (isolated or in combination with other pituitary hormone deficiencies) or a transient deficiency caused by either a primary delay in HPG axis maturation (known as CDGP) or a secondary delay in HPG maturation (known as FHH) (15,19).

Congenital Hypogonadotropic Hypogonadism

There has been no definitive epidemiological investigation of the prevalence of congenital hypogonadotropic hypogonadism (CHH). There is a scarcity of estimates for the incidence of CHH and Kallmann syndrome (KS). Studies based on French and Sardinian military screening suggest varied incidences, with CHH occurring in 1 in 10,000 males and KS occurring in 1 in 84,000 males. In the Finnish population, the incidence of KS is estimated to be 1 in 30,000 for males and 1 in 125,000 for females (21,22,23). Male patients experience it two to five times more frequently than female patients. CHH may be sporadic or familial (9). An increasing number of genetic loci involved in either the development and migration of GnRH neurons or the secretion and action of GnRH have been implicated in CHH. More than 30 genes have been identified for isolated or multiple anterior pituitary hormone deficiency associated with CHH (24). There are several known mechanisms of transmission, including autosomal dominant transmission, X-linked recessive transmission, autosomal recessive transmission, and transmission connected to an imprinting locus (3).

It is also worth noting that isolated CHH is a complex entity. Approximately half of patients with CHH exhibit a condition called KS, which is characterized by an impaired sense of smell. The other half have been reported to have normosmic (normal sense of smell) HH (15). There is a wide spectrum of phenotypes due to multiple causes and incomplete penetrance. KS has been associated with mutations in genes, including *ANOS1, SEMA3A,* and *TUBB3*, and normosmic types have been reported to be caused by mutations in *GNRH1, KISS1, TAC3,* and *NR0B1* (25). Some of these mutations (*FGF1, PROK2, PROKR2, GNRHR*) can also result in partial loss-of-function, leading to partial hypogonadism, which is characterized by arrest of pubertal development, and even reversible HH with relatively low gonadotropin levels (3,9,26).

KS can be sporadic or familial (autosomal dominant, recessive, X-linked, digenic, and oligogenic inheritance patterns) and present with diverse phenotypical features (21). During the neonatal period, the identification of micropenis and undescended testes (5-40%) in males are important physical examination findings in CHH. Conversely, there is no particular finding that relates to females. Male patients with partial hypogonadism may have no clinical findings in the neonatal period (8,9). The presence of severe hypospadias excludes the diagnosis of CHH (2,12,27). Absent virilization or low libido in males and the absence of breast development or amenorrhea in females are among the presenting signs during adolescence (27). Most CHH patients have eunuchoid proportions, which are characterized by arm spans that are 5 cm longer than their height. This is related to the delayed closure of the long bone epiphysis in the absence of gonadal hormones. On average, CHH adolescents attain their mid-parental height (12).

CHH can be associated with nonreproductive phenotypes, and presenting symptoms including anosmia/hyposmia (55-100%), hearing loss (5-15%), mirror movement (19-31%), dental agenesis (NA), and renal agenesis (8-15%), eye movement disorders 3-27%), cleft lip/palate (4-7%), scoliosis (13%) and syndactly, polydactyly, and camptodactyly (5%), all of which can be useful diagnostic clues in the differential diagnosis (2,11,12).

Combined forms may be present with other hormone deficiencies (idiopathic or associated with mutations in *PROP-1, HESX1,* and *LHX5*) and/or could be a component of a genetic syndrome (e.g., Prader-Willi syndrome, Noonan syndrome, CHARGE syndrome, Bardet-Biedel syndrome, Waardenburg syndrome, and Hartfield syndrome) (3,15,19).

Congenital Hypogonadotropic Hypogonadism and Spontaneous Remission

It is believed that lifelong hormone therapy is required to maintain sexual function and secondary sexual characteristics in men with IHH (28). Even though the precise pathophysiological mechanisms are unknown, it should be noted that the onset of puberty may occur spontaneously in 10 to 20% of cases later in life, more frequently in males (12). However, there are currently no definitive clinical parameters for predicting the reversibility of CHH. Hence, the cases should be evaluated every two years for the reversibility of the HPG axis (12,29). It should be emphasized that the restoration of reproductive axis function may be temporary, as some people may relapse into GnRH deficiency. Thus, long-term monitoring of reproductive function is necessary (12). Pathogenic variations that induce CHH and reversibility in clinical follow-up can be identified as occurring in *FGFR1* (13%), *GnRHR* (8%), *TACR3* (8%), *PROKR2* (5%), *TAC3* (3%), and *HS6ST1* (3%) (26,29).

Acquired Hypogonadotropic Hypogonadism

The acquired causes of hypogonadotropic hypogonadism may be related to infections (e.g., tuberculosis, meningitis), tumors (e.g., craniopharyngiomas, germinomas), infiltrative diseases (e.g., sarcoidosis, hemochromatosis), autoimmune diseases, radiotherapy, surgery, trauma, drugs, and be functional or idiopathic (Table 1) (30).

Constitutional Delay of Growth and Puberty

CDGP is the most common cause of delayed puberty in both sexes. It accounts for 65-73% of boys and 30-43% of girls (17). It is self-limited and has classically been described as representing late variants of the normal spectrum of pubertal timing (15,19). CDGP also has a substantial genetic component, with 50% to 80% of cases reporting a family history of delayed puberty, frequently in an autosomal dominant fashion (8,9,15,17,19). Although the exact etiological cause of CDGP is not known, increased total energy expenditure and insulin sensitivity are among the possible causes. As linear development slows in comparison to peers entering puberty, the growth chart may indicate a steady downward crossing of centiles. The development of pubic hair (adrenarche) may also be delayed in CDGP, in contrast to CHH, when adrenarche occurs at the normal population age (15,19). Puberty begins at a later age than usual but continues spontaneously (3). The tempo of pubertal development does not follow the chronological age but is concurrent with the bone age, which is delayed compared to chronological age (3,17). It is a self-limited normal variant and is considered an exclusion diagnosis (12,31,32). It is often clinically difficult to distinguish adolescents with CDGP from those with a form of permanent HH. Differentiating between these conditions is particularly difficult in the initial evaluation because adolescents with both conditions are often prepubertal on examination and have low levels of gonadotropins (LH and FSH).

The diagnosis of CHH is the most difficult clinical situation, especially when the clinical presentation overlaps with CDGP and gives no diagnostic signs. The “gold standard” for distinguishing between these two conditions is clinical monitoring until the age of 18 years for signs of endogenous activation of the HPG axis (progressive testicular enlargement or breast development). The presence of endogenous, progressive pubertal development by the age of 18 years is the “gold standard” for differentiating CDGP from IHH (33).

Functional Hypogonadotropic Hypogonadism

FHH can be due to an underlying systemic illness (e.g., celiac disease, asthma, cystic fibrosis), endocrinopathy (e.g., growth hormone deficiency, hypothyroidism, hyperprolactinemia), medications (e.g., antipsychotics, some antidepressants, opioids), intense exercise, or excessive weight loss (12,20,34). While the underlying etiology can be identified in only 20% of all cases, it is seen more frequently in girls and typically displays reversibility once the underlying pathology is treated or restored (2). The treatment approaches for selective cases are discussed later in this review.

Transient FHH accounts for 10-20% of cases diagnosed with delayed puberty and is likely to affect more girls than boys (Table 1). Nutrition has been suggested to play an important role in the control of GnRH secretion by a mechanism that has not yet been identified. Suboptimal nutritional status results in a hypogonadotropic state and the arrest of pubertal maturation. In malnutrition and chronic diseases, weight loss below the level of 80% of the ideal body weight can cause delayed or arrested pubertal development (9). This pathophysiological condition is usually observed in female patients with anorexia nervosa or excessive physical activity. Chronic disorders, such as sickle cell anemia, thalassemia, cystic fibrosis, inflammatory bowel disease, celiac disease, and chronic renal disease may also be associated with delayed puberty (Table 1). Malnutrition also contributes to short stature, decreased bone mineral density (osteopenia and osteoporosis), and a low mood, which are often observed in these patients (9).

A history of abdominal pain, constipation, or diarrhea, indicating a gastrointestinal disorder; weight gain/loss or temperature intolerance, indicating a thyroid disorder; disordered body image or eating, indicating a restrictive eating disorder; significantly slowed growth, indicating a growth hormone deficiency; or participation in high-demand athletics (gymnastics, ballet, or long-distance running), could be suggestive of an underlying etiology for FHH. On physical examination, children with FHH may be underweight for their height or have physical signs consistent with a specific illness (for example, goiter or abdominal distention) (15,33).

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism, characterized by high levels of gonadotropins, is typically caused by primary gonadal insufficiency. It may develop due to congenital and acquired causes (9,15,33). Conditions associated with primary gonadal failure are listed in Table 1.

Congenital Hypergonadotropic Hypogonadism

Permanent and transient forms of CHH are recognized. CHH may result as a consequence of several etiologies, such as genetic or chromosomal abnormality syndromes (e.g., Turner syndrome in girls or Klinefelter syndrome in boys, Noonan syndrome, Fragile X syndrome, trisomy 13), metabolic disorders (e.g., galactosemia), steroidogenesis defects (e.g., 5-alpha reductase type 2 deficiency, 17-hydroxylase deficiency, aromatase deficiency), FSH and LH β subunit mutations, FSH and LH receptor mutations, androgen defects (e.g., complete androgen insensitivity syndrome), vanishing testes syndrome (in boys), and autoimmune oophoritis (in girls) (Table 1) (3,9,35).

Klinefelter syndrome (47, XXY or 48, XXXY) seen with a prevalence of 1 in 660 males, is the most common chromosomal aneuploidy and primary hypogonadism etiology in boys. Most affected people naturally reach puberty at a normal age, but in the years that follow, testosterone levels progressively drop as pubertal arrest occurs and seminiferous tubule degeneration is accompanied by Leydig cell degeneration in Tanner stages 4-5. Individuals with Klinefelter syndrome usually present with tall stature, gynecomastia, behavioral and neurocognitive problems, and their testicular volumes are usually less than 5-6 mL.

Turner syndrome is the most frequent type of hypergonadotropic hypogonadism in females, occurring in 1 in 2,000 to 2,500 live births. Individuals present with specific phenotypical and clinical characteristics (e.g., facial appearance, neck webbing, short stature, cardiovascular, skeletal, and renal anomalies) and are diagnosed in the presence of one intact X chromosome with complete or partial absence of the other chromosome. The 45, X karyotype is seen in almost half of all girls with Turner syndrome. Puberty is frequently missing or delayed in Turner syndrome, and it is followed by progressive ovarian failure. Importantly, up to 30% of females will experience spontaneous pubertal growth, and 2% to 5% will experience spontaneous menstruation (9).

Acquired Hypergonadotropic Hypogonadism

Acquired defects may be due to radiation, chemotherapy, autoimmunity (e.g., autoimmune polyglandular syndrome), gonadal infections (e.g., mumps), or idiopathic (Table 1).

Diagnostic Evaluation of a Patıent with Delayed Puberty

The diagnostic evaluation should consider the medical history, including height and weight charts, developmental milestones, nutritional status, medications (chemotherapy, radiation, steroid), history and/or symptoms of chronic disease, and psychosocial functioning, trauma, or infection, family history, and physical examination (current height, weight, pubertal staging, and syndromic features) (15,36). Symptoms suggestive of thyroid dysfunction, androgen excess, hyperinsulinism, or an underlying chronic disease should be evaluated carefully to exclude the diagnosis

Table 1. Etiology of delayed puberty

	Hypergonadotropic hypogonadism		Permanent hypogonadotropic hypogonadism		Transient hypogonadotropic hypogonadism	
					Functional hypogonadotropic hypogonadism	**CDGP**
Frequency **Girls** **Boys**	 **15-25%** **5%**		 **10-20%** **10%**		 **20-30%** **10-20%**	 **30-55%** **60-80%**
	Congenital	**Acquired**	**Congenital (> 30 gene implicated)**	**Acquired**	• Systemic illness or infections ✓ AIDS	
	• Genetic syndromes and related disorders ✓ Noonan syndrome ✓ Klinefelter syndrome ✓ Down syndrome ✓ Fragile X syndrome (FMR1)	• Trauma • Testicular torsion	• Isole or multiple PHD ✓ Anosmic (Kallmann syndrome) ✓ Normosmic (Isolated) ✓ HPG axis developmental disorders (Rathke's pouch cyst) • Monogenic obesity (LEP, LEPR, and PCSK1)	• CNS ✓ Tumors/infiltrative diseases ✓ Astrocytoma ✓ Germinoma ✓ Glioma ✓ Craniopharyngioma ✓ Prolactinoma ✓ Langerhans cell histiocytosis ✓ Sarcoidosis	• Rheumatic disease ✓ Juvenile rheumatoid arthritis • Respiratuvar disease ✓ Asthma • Renal disease ✓ Chronic renal disease • Hematologic and oncologic disease ✓ Sickle cell disease ✓ Hemosiderosis ✓ Thalassemia ✓ Langerhans cell histiocytosis ✓ Leukemia and lymphoma	
	✓ Gonadal dysgenesis ✓ Turner syndrome (45, X, or mosaic) ✓ 46, XX pure gonadal dysgenesis	• Chemotherapy	• Syndromic obesity ✓ Prader-Willi syndrome ✓ Bardet-Biedl syndrome CHARGE syndrome • Midline defects ✓ Sept-optic dysplasia ✓ Congenital hypopituitarism	• Prior CNS infection Meningitis Encephalitis	• Endocrinopathy ✓ Diabetes mellitus ✓ Hypothyroidism ✓ Hyperandrogenism ✓ Hyperprolactinemia ✓ Growth hormone deficiency ✓ Hypercotisolism	
	• Testicular regression syndrome (Anorchia)	• Radiation therapy		• Radiation therapy	• Gastrointestinal disease ✓ Cystic fibrosis ✓ Celiac disease ✓ Inflammatory bowel disease ✓ Hepatic disease	
	• Defects in steroidogenesis ✓ 5-alpha reductase deficiency ✓ 17, 20 lyase deficiency Congenital lipoid adrenal hyperplasia (StAR) ✓ 17-hydroxysteroid dehydrogenase deficiency • Resistance to androgen receptor • Sertoli cell only syndrome • Gonadotropin resistance	• Gonadal infection ✓ Mumps, ✓ Coxsackie		• Chemotherapy	• Excessive exercise	
	• Metabolic disease ✓ Galactosemia	• Autoimmune orchitis • Autoimmune oophoritis		• Trauma	• Malnutrition • Anorexia nervosa/bulimia	
		• Gonadectomy		• Cranial surgery	• Drug (eg.glucocorticoid)	

CNS: central nervous system, CDGP: constitutional delay of growth and puberty, AIDS: acquired immune deficiency syndrome, HPG: hypothalamic-pituitary gonadal

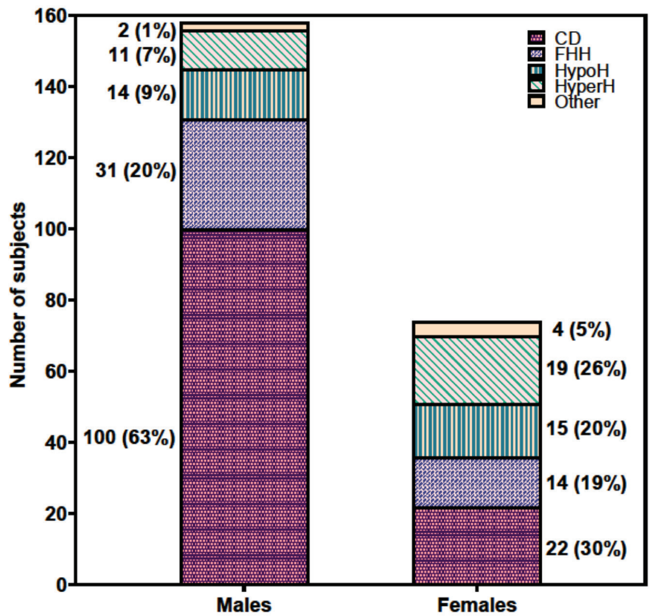

Figure 1. Distribution of diagnostic categories among males and females (20)

CD: constitutional delay of growth and puberty, FHH: familial hypogonadotropic hypogonadism, HypoH: hypogonadotropic hypogonadism, HyperH: hypergonadotropic hypogonadism, Other: etiology not clearly classified

of FHH. While hypogonadism is typically diagnosed during adolescence, patients with features suggestive of hypogonadism, such as micropenis and bilateral undescended testis in boys, or those with hypogonadism in the family history, can also be evaluated during mini-puberty (2). A thorough history should include evidence of anorexia and the intensity of exercise. A comprehensive family history is essential, including childhood growth trends, age at pubertal onset of both parents and siblings, and any history of infertility, anosmia, and midline abnormalities of parents and siblings (9).

Physical signs, such as cleft lip or palate, bimanual synkinesia, congenital ptosis and abnormal visual spatial attention, abnormal eye movements, sensorineural hearing loss, unilateral renal genesis, agenesis of one or more teeth (hypodontia), obesity, features suggestive of CHARGE syndrome, and digital and other skeletal abnormalities, will raise suspicion of the diagnosis of CHH. A genetic condition may be involved if there is delayed cognitive development accompanied by obesity or dysmorphic traits (9).

A bone age assessment, early morning basal testosterone, LH, and FSH levels (to detect hypergonadotropic hypogonadism), and a biochemical analysis that includes a full blood count should all be part of the initial screening process for delayed puberty. It is advised to do tests for sedimentation rate (or C-reactive protein), renal and liver function, thyroid function, electrolytes, celiac screen (anti-transglutaminase IgA), insulin-like growth factor-1 (IGF-1), and prolactin to rule out any additional pituitary hormone insufficiency or underlying chronic illness (15). Serum testosterone levels show a diurnal rhythm, with a decrease in the afternoon and evening. As a result, blood samples should be taken at the same time every day (ideally in the morning) (37).

Other tests, such as pelvic ultrasound for gonad and uterine evaluation and renal ultrasound in X-linked CHH, may be necessary due to probable anosmin (*ANOS*) mutations associated with renal malformation or unilateral agenesis (9).

Olfactory function is a hallmark of the clinical assessment of CHH, as ~50% of patients have a defect in the sense of smell or KS, also known as "olfactogenital dysplasia" (12). Both objective (Pennsylvania Odor Test) and subjective (detailed interview) olfactory tests should be performed (9). Self-reporting anosmia is sensitive and specific, whereas self-reporting normal olfaction is unreliable. Therefore, formal olfactory testing should be performed in all patients with CHH (12).

Magnetic resonance imaging (MRI) plays a significant role in diagnosing hypogonadism. Brain MRI can be used to rule out an acquired form of hypogonadism, such as a central nervous system tumor, and to identify features of CHH, such as defects in the olfactory bulbs, corpus callosum, semicircular canals, and cerebellum. In association with anosmia or hyposmia, patients with KS typically present with unilateral or bilateral olfactory bulb agenesis, olfactory tract agenesis, and/or gyrus malformation. An MRI should be obtained for any patient with suggestive clinical features of intracranial pathology (12,15).

In the presence of hypergonadotropic hypogonadism, a karyotype analysis, or comparative genomic hybridization (to identify lesser levels of mosaicism) can be used to diagnose Turner or Klinefelter syndrome (15).

A spermiogram is the quantitative and qualitative analysis of semen for the assessment of an adult man's fertility potential (12). However, it is not recommended in routine practice to perform spermiograms at certain intervals (every 3-6 months) during the physiological puberty induction protocol in adolescents. In the second or third year of physiological induction therapy, a spermiogram may be carried out for those who are curious about the fertility status or for cryopreservation (banking) of sperm (38). According to a meta-analysis, gonadotropin therapy resulted in a mean sperm concentration of 5.2 million/mL [95% confidence interval (CI), 4.7-7.1]. The median time to achieve sperm in the ejaculate was 7.1 months (95% CI, 6.3-10.1), and the median time to conception was 28.2 months (21.6-38.5) (39). The latest World Health Organization criteria for semen analysis interpretation were published in 2010. They were based on semen samples from >4500 men in 14 countries and defined lower reference limits for the following parameters: 1.5 mL for semen volume, 15 million/mL for sperm count, 40% for total motility, and 4% for normal morphology (40).

Elevated basal plasma FSH and LH levels in the early morning (FSH level >25 IU/L to >40 IU/L) indicate hypergonadotropic hypogonadism, while undetectable, low, at the lower limit of normal levels should suggest hypogonadotropic hypogonadism (3,41,42). The differential diagnosis of CDGP and CHH does not benefit from baseline gonadotropin values, but basal gonadotropin levels are frequently elevated in primary hypogonadism because of conditions like Turner or Klinefelter syndrome (9).

Over the past 30 years, various basal and stimulation tests have been proposed to differentiate between adolescents with CDGP, FHH, and persistent HH. Basal gonadotropins, GnRH, and human chorionic gonadotropin (hCG) stimulation tests all have limitations in diagnostic specificity and sensitivity to differentiate between the groups (2,42). Basal FSH and LH levels can be identified at prepubertal levels (3). In addition, low total testosterone levels in boys (free testosterone should be calculated if sex hormone-binding globulin is below the reference range) or low estradiol levels in girls may suggest the presence of hypogonadism (1,43). Since sleeping patterns, food consumption, acute illness, and immunoassay type may profoundly affect the values measured, a single cut-off for assessment cannot be given; however, levels of total testosterone >20 ng/dL in boys and >12 pg/mL in girls indicate the pubertal onset, while >12 nmol/L (>346 ng/dL) for testosterone and >50 pg/mL for estradiol have been suggested to rule out the diagnosis of hypogonadism (3,44). An 8 a.m. testosterone level >20 ng/dL predicts the onset of puberty within 12 to 15 months. At testosterone levels >100 ng/dL, structural growth is accelerated (42).

Levels of FSH, LH and total testosterone stimulated by dynamic testing using GnRH, GnRH analog (buserelin, leuprolide, nafarelin, triptorelin), and hCG can be useful (3). However, the diagnostic utility of these tests in distinguishing between youth with CHH and CDGP is limited due to significant overlap in diagnostic thresholds (15). However, in the absence of a standardized protocol, threshold values (peak LH, FSH, and total testosterone) vary widely and reliability is low (33). A predominant LH response in comparison to FSH or peak LH levels >5 IU/L in the GnRH test can indicate pubertal onset or FHH/CDGP (45). However, prepubertal GnRH test response should not rule out the diagnosis of CDGP and FHH (33). Furthermore, it should be noted that while a stimulated peak LH level above 5 IU/L (as measured by immunochemiluminometric assays) may suggest a diagnosis of CDGP, a normal response may still be observed in cases of partial HH.

Minipuberty provides a window of opportunity for evaluation of the functionality of the HPG axis before puberty for infants with CHH (7). As serum placental estrogen levels decline during the first postnatal week, increasing pulsatile GnRH production leads to increased gonadotropin and sex steroid levels in both sexes (12,42). Gonadotropin levels in healthy infants start to increase during the first week of life and then decrease toward the age of six months, except for FSH levels in girls that remain elevated until 3-4 years of age (9). In the neonatal period, low (1.2 IU/L) or undetectable levels of FSH are suspicious findings and may indicate CHH (2,46). During childhood, the diagnosis is difficult due to the physiological hypogonadism normally present during this period. The gonadotropic axis is resting, and LH is only detectable by ultrasensitive assays, whereas FSH plasma concentrations are variable (9). In adolescents, total plasma testosterone is low for the age, and baseline FSH and LH are also low or low normal. The response to the GnRH test is variable and depends on the severity of the gonadotropin deficiency, e.g. it may show no response in profound HH but be normal in partial HH (42).

Incorporating the markers of gonadal function, such as Inhibin B concentrations (marker of Sertoli cell), INSL3 concentrations (marker of Leydig cells), and anti-Müllerian hormone (AMH) levels (marker of granulosa cells and Sertoli cell) can assist in confirming the diagnosis (2,47,48). While the relationship between testosterone level and AMH is positively correlated due to low AR expression (2-15%) in Sertoli cells in the first four years of life, this relationship reverses with the increase in AR expression in the pubertal period. After the age of eight years, AR expression in Sertoli cells reaches 90% and spermatogenesis can be induced by the effect of increasing intratesticular testosterone concentration in the pubertal period (8). In the pubertal period, total testosterone level is positively correlated with inhibin B and negatively correlated with AMH levels. Normogram values of AMH and inhibin levels were determined according to age and sex (8,12,42). Low AMH levels might be indicative of ovarian failure (49).

Recent studies suggest that inhibin B may be an informative and simple first-line test. Inhibin B levels, controlled through FSH, reflect Sertoli cell number and activity. Serum inhibin

B levels correlate well with testicular size, and low inhibin B levels are a negative predictor of fertility (12). Despite considerable diversity in the threshold level of inhibin B to differentiate between CHH and CDGP, preliminary investigations evaluating the significance of baseline inhibin B concentrations were encouraging but have not been verified. Undetectable inhibin B (<10 pg/mL) is considered diagnostic of anorchia, but low, close to undetectable levels are also seen in severe forms of CHH. Its levels correlate with testicular volume and are therefore a good marker of the spermatogenesis and severity of HH. Inhibin B transiently peaks around 2-4 months after birth, decreases during childhood, and increases again during puberty (50). Rohayem et al. (50) observed that a threshold value of ≥28.5 pg/mL for inhibin B was sufficient to distinguish CDGP from HH in male patients, with a sensitivity of 95%, and specificity of 75%. Coutant et al. (48) showed in genital stage 1 that a single inhibin B level of 35 pg/mL or less had a sensitivity and specificity of 100% and a positive predictive value (PPV) of 93% for distinguishing persistent HH patients from CDGP patients. In another study, the PPV was 73% when the inhibin B threshold was set at 100 pg/mL. The predictive value increased to 100% when only patients with persistent HH with a testicular volume of less than 3 mL were considered. However, the sensitivity and specificity of inhibin B were reported to be lower when comparing patients with HH diagnosed as part of multiple pituitary hormone deficiency with the CDGP group. Combined markers have also been used to differentiate CDGP and persistent isolated HH. Combined markers have also been used to differentiate CDGP and persistent isolated HH. Binder et al. (51) reported that a combination of basal LH and inhibin B provided 100% sensitivity and 98% specificity for discrimination of the two conditions when basal LH <0.3 U/L and inhibin B <111 pg/mL were used as combined decision limits. Although the exact role of inhibin B during female puberty is not known, its increasing serum concentration at early puberty reliably reflects the secretory maturation of the ovarian follicles, which is driven by gonadotropins.

Inhibin B is secreted by granulosa cells in women and is a marker of the number of antral follicles. Very few studies have investigated the levels of circulating inhibin B levels in females with CHH (12). There have been a limited number of studies in female patients, and the threshold value for inhibin B in terms of discriminating CDGP and HH was determined to be <20 pg/mL in the study by Binder et al. (51).

In pubertal patients with central hypogonadism, AMH is low for the Tanner stage - reflecting lack of FSH stimulation - but high for age - reflecting lack of testosterone inhibition (52). When compared to prepubertal levels, the AMH decrease during Tanner stages 2 and 3 coincides with the increase in intratesticular testosterone and the meiotic onset of germ cells in the seminiferous tubules during puberty. Although AMH serum levels have been reported to be a useful marker in discriminating CDGP and HH in male patients, they are not as discriminative as inhibin B (8,50). Serum prolactin, free T4, thyroid-stimulating hormone, cortisol, IGF-1, and IGF binding protein-3 may be determined to characterize combined pituitary hormone deficiencies (2).

Treatment of Hypogonadism

Hormonal therapy is used to induce puberty in adolescent males based on published consensus and expert opinion. However, there are currently no evidence-based guidelines regarding the optimal timing and regimen for inducing puberty in either males or females (53,54,55,56).

The choice of preparation and administration route for estrogen or testosterone is based on the advantages and disadvantages of the available regimens (12). Among the different forms of testosterones, oral forms have the disadvantage of shorter half-lives, transdermal forms may cause skin reactions, and subcutaneous implants require a surgical intervention (57,58).

Treatment Approach in Transient Hypogonadism

Delayed puberty can cause psychological distress and low self-esteem in adolescent males. It also negatively affects metabolic profile, fat distribution, muscle mass, bone mass, and growth. It is important to address this issue promptly to prevent further complications (53). Therefore, for individuals with a possible diagnosis of FHH or CDPG, it is recommended that puberty be induced in the short term by low-dose administration of sex steroids. In addition, induction of delayed puberty can help to trigger a pubertal "jump-start" or confirm the diagnosis of a permanent or transient etiology (59).

Management of Constitutional Delay of Growth and Puberty

Although the "watchful waiting" strategy is one of the main approaches in CDGP, puberty can be induced with low doses of testosterone and estrogens when chronological age reaches 14 years and bone age reaches 12 years in boys and chronological age reaches 13 years and bone age reaches 11 years in girls (3,32,60). Inductions are administrated for a cycle of 3-6 months, followed by a 3-6-month window period of clinical follow-up to allow pubertal "jump start" (60). If progression fails, a second trial with higher dosages can be administered, before commencing lifelong hormone replacement treatment (14). Parenteral testosterone is commonly used to induce puberty in boys with hypogonadism and CDGP due to its flexible dosing administration (53). In girls, 17-β-estradiol (oral or transdermal), ethinylestradiol (oral), or conjugate equine estrogens (oral) are available to induce puberty (32,60). The dosing equivalents of various estrogen preparation vary significantly: 0.1 mg transdermal 17-β-estradiol equates to 2 mg oral 17-β estradiol, 20 mg oral ethinyl estradiol or 1.25 mg oral conjugated estrogen (61). The typical starting dose of estrogen is 0.25 to 0.5 mg oral 17-β-estradiol (or 5 micrograms/kg) daily. Alternatively, if the transdermal route is preferred, 3.1 to 6.2 mg (1/8 to 1/4 of a 25 mg/24 h 17-β-estradiol patch) can be used (15). The recommendation for pubertal induction protocols in cases with suspected CDGP is summarized in Figure 2 for male subjects and Table 2 for female subjects (60).

The use of aromatase inhibitors (anastrozole or letrozole) for a six-month duration has been shown to induce puberty and accelerate growth in boys (32,62). Mauras et al. (63) demonstrated that oral letrozole (2.5 mg/day) or anastrozole (1.0 mg/day) may be a viable alternative treatment option to intramuscular testosterone therapy for pubertal induction in male patients with CDGP after six months of use (11,62).

Management of Functional Hypogonadotropic Hypogonadism

Although there is no clear consensus on the general approach to FHH, the underlying etiological cause should be treated primarily (17). In these patients, the process normalizes spontaneously when the energy deficit is corrected or the underlying disease is treated (17). Delayed puberty is also frequently observed in chronic renal failure. However, spontaneous recovery of gonadotropin secretion has been observed in these patients after successful renal transplantation (9). In FHH, if attempts to modify nutritional, psychological, and exercise-related variables are unsuccessful in establishing menses, clinicians may consider estrogen replacement. Even after as little as 6-12 months of amenorrhea, bone outcomes may be compromised. Therefore, clinicians may consider short-term transdermal E2 with cyclic oral progestin therapy after 6 to 12 months of nutritional, psychological, and exercise-related interventions in those with low bone density and/or evidence of skeletal fragility. It should be noted that E2 replacement therapy may not protect bone health if there are ongoing nutritional factors or energy deficits (36).

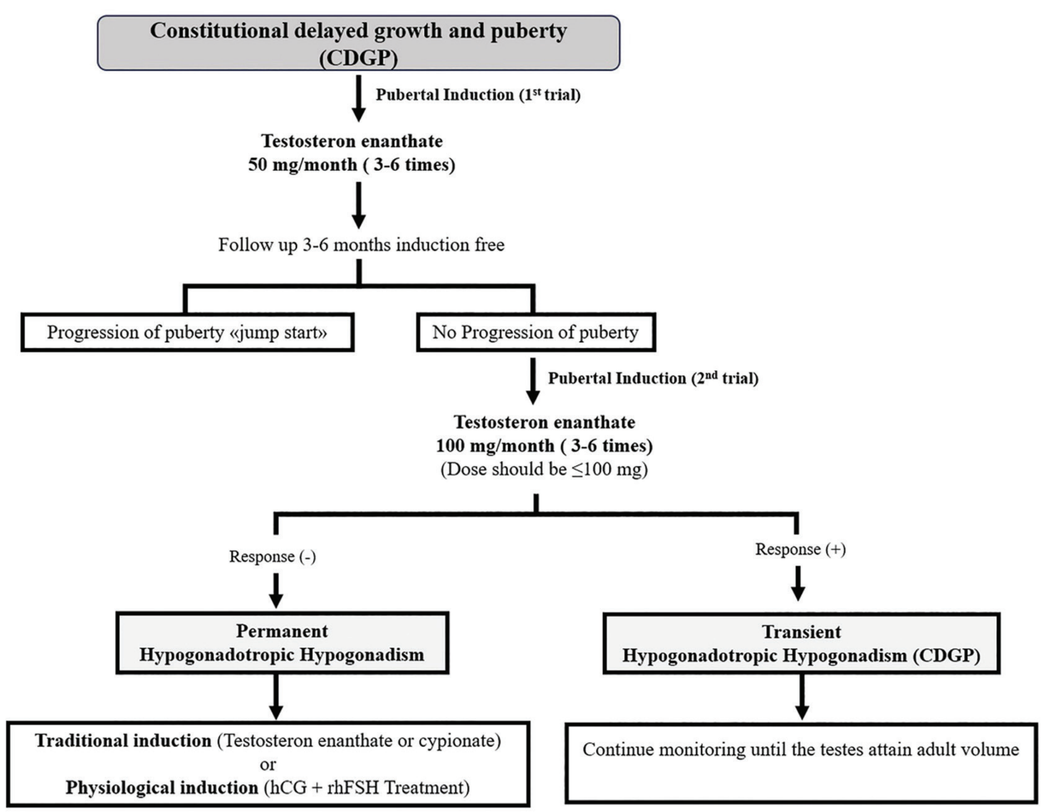

Figure 2. Pubertal induction protocol in boys with CDGP

CDGP: constitutional delay of growth and puberty, hCG: human chorionic gonadotropin hormone, rhFSH: recombinant human follicular stimulating hormone

 J Clin Res Pediatr Endocrinol
2024;16(4):379-400

Table 2. Pubertal induction protocol in girls with CDGP

	Route	Dose	Duration	Start
Ethinyl estradiol	PO	0.05-0.1 μg/kg/day (2.5 μg/day for 6-12 months. Increase after 6 months to 5 ug/day if necessary	Until breast development reaches B3	≥11-12 years BA ≥13 years CA
Conjugated estrogens	PO	0.3 mg on alternate days for 6-12 months. Increase after 6 months to 0.3 mg/day if necessary		≥11-12 years BA ≥13 years CA
17-β-estradiol	PO	5 ug/kg/day Increase after 6 months to 10 ug/day if necessary		≥11-12 years BA ≥13 years CA
	T.D	As shown in Figure 3		≥11-12 years BA ≥13 years CA

IM: Intramuscular, SC: subcutaneous, BA: bone age, CA: chronological age, PO: per oral, TD: trans-dermal, CDGP: constitutional delay of growth and puberty

Treatment of Permanent Hypogonadism

Approach to Hypogonadism in Male Patients

There are two possible approaches to pubertal induction in boys. The first is parenteral or transdermal testosterone esters, which are used in the treatment of both hypergonadotropic and hypogonadotropic hypogonadism (traditional pubertal induction). The second is GnRH and gonadotropin therapy (physiological pubertal induction), which is recommended in the treatment of permanent HH (38).

Parenteral Testosterone Replacement Treatment

Adolescents with delayed puberty should start puberty induction therapy around the average age of normal male puberty (12 years). In cases where the distinction between permanent HH and CDGP can not be made, it is recommended to wait until the chronological age is 14 and the bone age is 12 before starting testosterone replacement treatment (TRT) (53,60,64). In addition, in prepubertal children who are short for their age, postponing treatment may be an option to increase their final height (53,65). The most commonly used method of exogenous TRT is known to induce virilization, enhance sexual function, increase bone density, and promote lean body mass. Gonadal development cannot be stimulated via TRT, since it suppresses serum LH secretion, decreasing intratesticular testosterone levels by 98%. Some authors argue against its use as a therapeutic option for hypogonadal males, and favor gonadotropins, since it may cause atrophy of the germinal epithelium and decrease spermatogenesis (7,66,67,68). Thus, in adolescent males with permanent HH, hCG, with or without FSH, appears to be more physiological and potentially safer than testosterone in initiating spermatogenesis and testicular growth (53). A meta-analysis by Rastrelli et al. (69) found no significant difference in sperm count in patients receiving TRT before gonadotropin treatment (5.84 million/mL vs. 4.88 million/mL, $p = 0.684$). This lack of association implies that previous testosterone exposure may not exert an adverse effect on fertility rates. However, the authors also underlined the impact of the potential interferences in the interpretation of data, such as the ecological fallacy, or the availability of small numbers of cases. Furthermore, the potential reversibility of the cases included in the analysis was also highlighted, since 20% of the >300 patients with hypogonadism have shown reversibility (70).

In adolescent males with CDGP or permanent hypogonadism, TRT is the most commonly used therapy to induce puberty. Compared with other treatments, testosterone is an effective, convenient, safe, well-tolerated, and cost-effective option (53). Currently, the only formulations approved by the US Food and Drug Administration for delayed puberty are intramuscular testosterone esters, particularly testosterone enanthate, cypionate, undeconate, and subcutaneous testosterone pellets (71,72). In addition, several new formulations, including transdermal, nasal, subcutaneous, and oral formulations, have recently been developed to improve the pharmacokinetic profile and ease the administration route, thereby increasing patient compliance in adult males with hypogonadism (Table 3) (53,71). However, during the early pubertal period, parenteral testosterone is preferred due to the difficulty of dose titration with other forms of testosterone. All these formulations are not approved for the pediatric age group, although some of them are used as "off-label" regimens (71).

The most commonly used form of TRT is intramuscular injection of testosterone esters. Unmodified testosterone has a half-life of only 10 minutes and would have to be injected very frequently. Esterification of the testosterone molecule at position 17, for example with propionic or enanthic acid, prolongs the activity of testosterone in proportion to the length of the side chain when administered intramuscularly (37,73). Intramuscular injections of these testosterone esters (testosterone propionate and testosterone enanthate) result in supraphysiological testosterone levels early after administration and subphysiological levels near the end of the dosing interval. Attempts have been made to overcome

Table 3. Available preparations and dosing strategies for patients with permanent hypogonadism

Preparation and route of administration	Initial dosage (pubertal induction dose)	Dose increment, interval	Adult dose
Induction of puberty in boys			
Testosterone enanthate, cypionate, or a mixture of testosterone esters (IM)	25-50 mg/ every 4 weeks or 1 mg/kg/ every 4 weeks	Increase of 50 mg every 3-12 months (ideally 6 months) until the dose of 150-200 mg every 4 weeks	150-200 mg/every 2 weeks
Testosterone undecanoate (IM)	No data available	No data available	750-1000 mg/every 10-14 week
Testosterone undecanoate (oral)	20-40 mg/day	Every 6 months	40-80 mg/day 2 twice daily
Testosterone gels, 1% or 2% (transdermal)	1% gel 0.5 g up to 5 g daily 2% gel 10 mg daily for 3 months	No data available	1% gel: 50-100 mg daily 2% gel: 40-70 mg daily
Testosterone patch (transdermal)	Age 12.5-15 years 2.5-5.0 mg for over 8-12 h/daily for 8 weeks	5 mg for 8-12 h/ daily application for 6 months	Adult dose: 5-10 mg over 24 h daily
Subcutaneous testosterone pellets	No data available	No data available	Adult dose: 8-10 mg/kg every 6 months three doses (or 150-450 mg every 3-6 months
Induction of puberty in girls			
Ethinyl estradiol, oral	0.05-0.1 µg/kg/day (2.5 µg/day)	Every 6-12 months	10-20 µg/day
17-β-estradiol, oral	5 µg/kg/day (0.25 mg/day)	5 µg/kg, every 6 -12 months	1-2 mg/day (max 4 mg)
17-β-estradiol, transdermal*	0.08-0.12 µg/kg/day for 10 hours	Detailed in Figure 5	50-100 µg/day twice a week

*The transdermal treatment protocol is detailed in Figure 5.
IM: intramuscular, max: maximum

this effect by combining short- and long-acting esters (e.g. Sustanon, Testoviron Depot). However, it has been observed that these products result in even higher initial serum levels of testosterone, without any corresponding increase in the duration of their effects. Testosterone propionate must be administered every 2-3 days, whereas testosterone enanthate and testosterone cypionate only need to be administered every 2-3 weeks (72). Although long-acting testosterone undeconate has been reported as safe for continuing pubertal induction after the age of 18 years, there is no data on its use in children (74,75).

The lower limit of 'normal' serum testosterone concentration is controversial, and the generally used or suggested lower serum testosterone concentration for starting therapy varies in the four European countries (Germany, France, the UK, and Spain) surveyed by the authors. These lower thresholds range from 216-346 ng/dL. According to research, serum total testosterone concentration of 300 ng/dL (10.4 nmol/L) may be clinically relevant for starting TRT in patients with symptoms of permanent hypogonadism (37).

During puberty, TRT should be increased gradually to mimic normal pubertal physiology and can be stopped when the HPG axis is significantly activated, as indicated by an increase in the testicular volume of 6 to 8 mL (53,71). In adolescents with permanent hyper- or hypogonadism, it is recommended to initiate treatment with a low dose of intramuscular testosterone enanthate or cypionate (25-50 mg every four weeks or 1 mg/kg per month) and gradually increase the dose by 50 mg every 6-12 months over a period of 2-3 years. After reaching a monthly dose of 150-200 mg, the dosing interval is increased to every 2 weeks. The recommended adult dose is 150-200 mg every two weeks. Table 3 summarizes the doses of pubertal induction with different testosterone products (11,12,59,62,71,76). The Endocrine Society Clinical Practice Guidelines recommend testosterone enanthate or cypionate 75-100 mg/week or 150-200 mg/two weeks for young adults and adult with hypogonadism (73,77). To prevent accelerated bone age and short final adult height, it is recommended to avoid high-dose testosterone therapy at the start of pubertal induction (76). Continuous monitoring of endogenous puberty is recommended. Testicular volume can be assessed every six months, and testosterone and LH levels can be measured one month following the most recent injection. If endogenous puberty does not occur by the age of 18 years, the diagnosis of permanent HH is established (15).

Sustanon (250 mg of Sustanon corresponds to 176 mg actual testosterone) is the most commonly used, commercially available form of testosterone ester mixture, consisting of testosterone propionate (30 mg), testosterone phenylpropionate (60 mg), testosterone isocaproate (60 mg), and testosterone decanoate (100 mg). Various testosterone esters exhibit distinct elimination half-lives throughout the body (44,64). A single dose of Sustanon 250 mg leads to an increase of total plasma testosterone with peak levels of approximately 70 nmol/L (2019 ng/dL) (C_{max}), which is reached approximately 24-48 h (t_{max}) after administration. Plasma testosterone levels return to the lower limit of the normal range in males in approximately 21 days (https://www.medicines.org.uk/emc/product/5373/smpc#gref) (78). The actual testosterone content in 100 mg of testosterone enanthate and cypionate is 70 and 73 mg respectively, while 100 mg of Sustanon contains a similar amount (70.4) of actual testosterone. Table 4 summarizes the peak effects, half-lives, and actual testosterone amounts within 100 mg of parenteral testosterone products (73,74,75,79,80,81).

Infancy

To date, hormone therapy during the neonatal period has only been used in male patients with micropenis/cryptorchidism and HH in the neonatal period (12). In infancy, cryptorchidism in males should be corrected by orchiopexy at 6-12 months of age to preserve future fertility potential (2,82). There are currently no additional data on the use of hCG or GnRH supplements during the minipubertal period for future fertility. Some publications suggest that high-dose hCG may have negative effects on germ cells, including increased apoptosis, intratesticular hemorrhage, inflammation, and potential harm to future fertility (12,83,84). On the other hand, it has been reported that hCG and GnRH treatments have a beneficial effect on increasing penis size, increasing testicular volume, and facilitating descent of undescended testicles in the minipubertal period, although the negative effect on the testes remains controversial (12,85,86,87). Smaller doses of FSH (2.5 IU/kg twice a week) and hCG (20 IU/kg twice a week) have been recommended during infancy; however, larger prospective randomized controlled trials are needed (85,88). In addition to this treatment, parenteral TRT can increase the size of the penis in boys with central hypogonadism and primary hypogonadism-associated micropenis (2,76,89). Administration of testosterone cypionate or enanthate in oil (25 mg) every 3-4 weeks for 3-4 months or topical 5α-dihydrotestosterone gel (5%) are two possible approaches (89,90). The 5% testosterone gel can either be applied 3 times a day for 5 weeks or 0.2-0.3 mg/kg once daily for 3 months (76,90,91).

Table 4. Actual testosterone content of testosterone-containing products

Testosterone product	Peak after injection (hours)	Terminal ($t^{1/2}$) Median-residence time (days)	Actual testosterone (per 100 mg)	Actual testosterone content of commercially available products (implementation periods)
Testosterone undecanoate (IM) 750 mg 1000 mg	 240 168	20.9-34.9	61	*Marketed under the brand names Nebido (1000 mg/4 mL) vial (Bayer), IM (10-14-week interval) Total actual testosterone 610 mg
Testosterone decanoate	24-48	12-14	62	*Marketed under the brand names Sustanon (250 mg/1 mL vial) (Organon), IM (2-4 weeks interval) Total actual testosterone 176 mg
Testosterone Isocaproate		7-9	72	
Testosterone phenylpropionate		3-4	66	
Testosterone propionate		0.8-1.5	83	
Testosterone cypionate 200 mg	 48-120	6-7	70	1-4 weeks interval, IM or SC
Testosterone enanthate 100 mg/week 200 mg/2 week 300 mg/3 week 400 mg/4 week	 96-120 48 36-48 36-48	4.5-8.5	 73	1-4 weeks interval, IM or SC

*The Sustanon ampoule (a mixture of testosterone esters) is a parenteral product. The contents of the ampoule are indicated by the grey-shaded boxes.
IM: intramuscular, SC: subcutaneous

Monitoring of Parenteral Testosterone Replacement Treatment

It is recommended to monitor testosterone levels 3 to 6 months after starting TRT. Although there are different recommendations regarding the measurement of testosterone levels (before injection, one week after injection, etc.) due to the different half-lives of testosterone products, the general recommendation is the midpoint of the two injection times (89). For patients receiving long-acting parenteral testosterone therapy, such as testosterone cypionate and enanthate, which have a short half-life of seven days, it is recommended that testosterone levels be measured four weeks after the start of treatment and one week after injection. For testosterone undecanoate, the levels should be measured before each subsequent injection (77).

When the adult dose is attained in the second or third year of treatment, the total testosterone level should be kept within the mid-normal reference range (350-700 ng/dL) (77,92). If testosterone is >700 ng/dL or <350 ng/dL, the dose or frequency should be adjusted. The major disadvantage of parenteral testosterone treatment is wide fluctuation of plasma testosterone levels, which are not in the physiological range for at least 50% of the time. After a single intramuscular injection, serum testosterone levels rise above physiological ranges, then decline gradually into the hypogonadal range by the end of the dosing interval (77). Preparations are generally well tolerated, but they may cause side effects, such as local reactions, gynecomastia, priapism, increased hematocrit (polycythemia), deranged liver function, and inappropriate behavioral changes (38,76). Polycythemia, which is defined as a hematocrit level greater than 52%, is a known side effect of TRT. It is recommended to determine hematocrit levels at baseline, at 3 to 6 months, and then annually. If the hematocrit level exceeds 54%, therapy should be discontinued until the hematocrit level decreases to a safe level (77). Patients should be monitored for the development of this condition and therapeutic phlebotomy may be required if it becomes severe (79). Testosterone esters should be used with caution in cases of renal impairment and avoided in cases of hepatic impairment or hypercalcemia (76).

Physiological Pubertal Induction in the Management of Hypogonadotropic Hypogonadism

TRT aims to induce virilization but does not stimulate spermatogenesis. On the other hand, pulsatile GnRH and gonadotropin treatments for 6-24 months result in testicular growth in almost all individuals and stimulate spermatogenesis in 80-95% of patients without undescended testes (38,87,92). Thus, mimicking the HPG axis during the mini-pubertal period to treat infants with congenital etiologies or to induce puberty at appropriate pubertal ages can be used as an alternative to parenteral TRT (92).

Various physiological pubertal induction protocols, including the use of hCG alone or in combination with recombinant FSH (rFSH), have been proposed in guidelines and studies for adolescent boys with permanent HH (Figure 3) (38,50,53,66,93). Various hCG products, which are derived from pregnant women's urine (Pregnyle, N.V. Organon, The Netherlands, or Choriomon, Institut Biochimique SA, Switzerland) or from recombinant DNA technology (Ovitrelle, Merck Serono) are commercially available, and no difference in efficacy has been reported between the two forms (94,95). One study showed that after administration of urinary hCG (5000 IU) and recombinant hCG (6500 IU), there was no significant difference in peak testosterone and estradiol levels (96). Urinary hCG preparations are currently marketed in lyophilized vials containing 1500 or 10,000 IU for intramuscular use. In contrast, recombinant hCG is available in prefilled syringes or pen devices containing 250 mg of pure hCG equivalent to approximately 6500 IU of urinary hCG. Although Ovitrellin single injection pens (0.5 mL, 250 μg = 6500 IU hCG) are not suitable for physiological pubertal induction, there are ready-to-use pens with adjustable doses that are more practical to use. However, these pens are not widely available in many countries (39). Recombinant hCG is purer than hCG derived from urine and

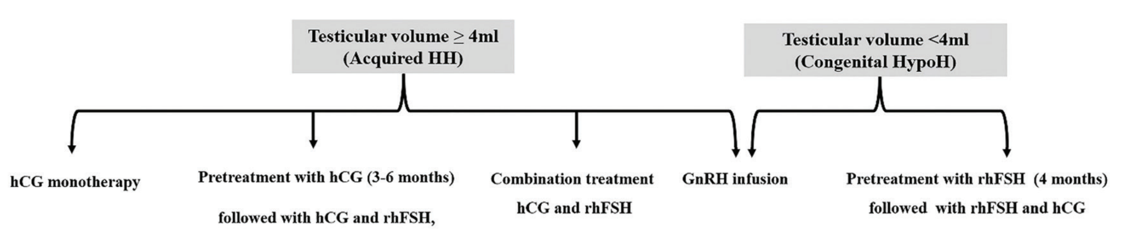

Figure 3. Physiological pubertal induction protocols in hypogonadotropic hypogonadism with gonadotropins

hCG: human chorionic gonadotropin hormone, rhFSH: recombinant human follicular stimulating hormone, HypoH: hypogonadotropic hypogonadism, GnRH: gonadotropin-releasing hormone, HH: hypogonadotropic hypogonadism

J Clin Res Pediatr Endocrinol
2024;16(4):379-400

has a better quality and safety profile than its counterparts derived from urine (94,95). Patients using urinary hCG for induction of HH may develop antibodies to hCG, which can lead to testosterone unresponsiveness (28,97,98).

rFSH has been reported to have a better safety and quality profile than its urinary counterparts. In general, rFSH preparations are purer than urine-derived FSH, and the inclusion of mass and vial filling has virtually eliminated batch-to-batch variation and enabled accurate dosing. The most common FSH preparations are recombinant, administered subcutaneously two or three times a week for 3-6 months at doses ranging from 75 to 300 IU (95). Long-acting FSH preparations have also been developed in recent years. Corifollitrophin alpha, a long-acting FSH analogue, needs to be administered every two weeks. Although it has been proven effective, FSH analogues are not commonly used (39).

There is consistent evidence that recombinant rFSH/hCG combination therapy is significantly more effective than hCG alone in both inducing spermatogenesis and increasing testicular volume (69). There is also some evidence that pre-treatment with rFSH followed by combination with hCG or GnRH is even more effective in optimizing Sertoli cell maturation and inducing spermatogenesis in extremely small testes (<4 mL) (28,38,66). This treatment led to a significant increase in TV (bi-testicular volume: from 5±5 to 34±3 mL) and to the induction of spermatogenesis in 91% of the patients (93). Although hCG alone can increase testicular volume, combined treatment with hCG and FSH has been shown to result in a better response in terms of final testicular size (53). In a meta-analysis study conducted by Alexander et al. (99) in 2023, which included 103 studies with a mean age of less than 25 years, gonadotropin therapy was found to increase testicular volume, penile size, testosterone levels, and spermatogenesis success. The success rate was 86% (82-91%) in patients who received hCG+FSH therapy and 50% (25-56%) in patients who received hCG monotherapy. However, it was emphasized that the treatment options, doses, durations, and results were heterogeneous, and therefore new randomized control studies are needed. Rastrelli et al. (69) conducted a meta-analysis and found that patients who received hCG monotherapy had a significantly lower sperm count compared to those who received hCG+FSH treatment (0.47 million/mL vs 11.57 million/mL, respectively, $p<0.001$). Various factors affecting the fertility success of physiological induction are summarized in Table 5 (12,87,99).

It is important to note that the physiological pubertal induction protocol has several significant disadvantages, including the requirement for five injections each week, consisting of two hCG injections and three FSH injections. Moreover, acquiring the essential medications may pose challenges contingent upon the economic circumstances prevailing in the country (especially hCG), and the cost is higher compared to traditional parenteral TRT. Moreover, it remains unclear whether a physiological protocol for inducing puberty should be applied to individuals in the pubertal age group. The use of physiological pubertal induction therapy is limited due to its high cost and impractical lifelong use. Although there is no strong evidence to support switching to parenteral TRT after completing physiological pubertal induction therapy, it is reported that TRT can be used once physiological induction therapy is completed (6-24 months) until fertility is desired. However, it is recommended to perform a spermiogram before switching to TRT treatment. If there is enough sperm in the ejaculate, it is advisable to consider sperm cryopreservation (banking), especially in cases of severe oligospermia, to improve future fertility. In cases of azoospermia, individuals may be classified as ‘poor responders’ to gonadotropin stimulation. In such cases, a testicular sperm extraction procedure may be considered. It is unclear whether spermatogenesis will begin more quickly with repeated physiological induction therapy after TRT treatment. Therefore, a spermiogram should be performed before the transition (38).

Table 5. Factors affecting fertility success during physiological pubertal induction therapy

	Factors affecting success
- History of bilateral undescended testis?	Yes
- Bilateral undescended testis operation time?	>12 month
- Dysgenetic condition of the testes?	Yes
- Etiology of HypoH? Is it congenital or acquired?	Congenital?
- Prior exposure to androgens?	Controversial
- Is the minipubertal period physiologically mimicked in permanent hypogonadotropic hypogonadism?	No long-term data
- Testicular volumes before treatment?	<4 mL
- Basal inhibin B levels?	<10 pg/mL

HypoH: hypogonadotropic hypogonadism

For cases with acquired permanent HH where pubertal arrest (TV >4 mL) has occurred or minipuberty has been experienced, fertility probabilities are greatly increased with the application of a physiological induction protocol in advanced ages, even if the physiological induction protocol was not applied during the pubertal period (53).

Should Physiological Induction Be Performed During the Minipuberty or Prepubertal Period?

FSH is necessary for the development of the Sertoli cell population. Following birth, Sertoli cells proliferate under the control of FSH during the first few months of life and in early puberty. The number of Sertoli cells is directly related to sperm production capacity. Each of these somatic cells can only support a limited number of developing spermatogenic cells. In line with these findings, it has been suggested that male patients who have not experienced mini-pubertal periods as a result of CHH have a poor response to pulsatile GnRH in terms of testicular growth and spermatogenesis (28,32).

Infants with micropenis associated with HH require TRT to increase the length of the penis. However, gonadotropin therapy with recombinant human FSH (rhFSH) and rhLH can also be used to enhance testicular enlargement before orchiopexy and correct micropenis (28,85,86,87,100). In recent years, it has been proposed to use physiological mimicry of the mini-puberty process with rhFSH and rhLH to increase the fertility potential of patients with CHH in adulthood. It is important to note that this approach is still being researched and is not yet widely used. In small groups of patients, different physiological induction protocols (continuous pump infusion or intermittent subcutaneous injection) have been used during the postnatal period of 0.7-6 months (mini-puberty), resulting in increases in testicular volume, penile length, testosterone, and inhibin B levels (85,86,87,101). However, these trials, which physiologically mimicked mini-puberty, did not provide information on adult fertility outcomes (85,86,87,101).

In an experimental study conducted in 2005, it was shown that administering FSH for four months before induction of puberty increased testicular volume and inhibin B levels, and also increased the number of Sertoli cells and type A spermatogonia (102). Following this experimental study, in 2007, Raivio et al. (103) proposed a new treatment for prepubertal boys with congenital and acquired HH, using rhFSH to increase sperm production by inducing the proliferation of immature Sertoli cells before hCG treatment. This study included 14 prepubertal male patients with different diagnoses: two patients with idiopathic HH, two with KS, four with idiopathic panhypopituitarism, and six with organic panhypopituitarism. The patients were aged between 9.9 and 17 years and had a testicular volume of less than 3 mL. The patients underwent rhFSH priming (1.5 IU/kg/dose, 3 times a week) for two months-2.8 years. The study found that there was a significant increase in testicular volume and inhibin B levels (0.9 ± 0.6 mL to 1.8 ± 1.1 mL, $p < 0.005$ and 27 ± 14 to 80 ± 57 pg/mL, $p < 0.01$, respectively). Spermatogenesis was successful in 6 out of 7 boys (86%) who provided semen samples, with a maximum sperm count ranging from 2.9 to 92 million/mL (median 8.5 million/mL). It was emphasized that the proliferation of the germ cell pool is important for fertility success and that FSH priming is necessary before hCG treatment (103). They emphasized that poor inhibin B responses in three patients who did not have postnatal hypothalamic-pituitary axis activation (indicating that they did not experience mini-puberty) would negatively affect future fertility success. One of the major limitations of this study is that patients were not classified by etiology, presence or absence of cryptorchidism, and the duration of FSH was not standardized. To achieve better outcomes in future studies, it is recommended to classify patients according to etiology and standardize FSH duration. The high fertility success observed in this study may be related to the inclusion of patients diagnosed with acquired HH with pubertal arrest.

Following the demonstration of the effect of FSH priming on fertility success in the experimental study by Pitteloud and Dwyer et al. (66) in 2005 (102), an open randomized controlled trial was conducted by the same authors in 2013 in 13 male patients with CHH and no history of undescended testicles. Seven patients received rhFSH (75-150 IU SC QD) for four months, followed by treatment with GnRH and the other group ($n = 6$) received only GnRH from the start of treatment. At the 24th month of treatment, testicular volume, sperm count, and fertility success were significantly higher in the FSH-primed group compared with the non-FSH-primed group (testicular volumes; 9.3 ± 1.7 mL and 6.6 ± 1.3 mL; sperm counts; 5.8 ± 2.3 and $2.6 \pm 1.5/10^6$ mL, fertility success, 100% and 66% respectively). In this study, although the mini-puberty period was not experienced in the FSH priming group, all cases were considered to be fertile (66). The results of this study suggest that, contrary to the study by Raivio et al. (103), the physiological mimicry of the minipubertal period is not mandatory (66). Randomized studies with large patient populations are needed to reach firmer conclusions in this area.

Although these two studies (66,103) make a valuable contribution to the literature, the most important drawbacks are the small number of cases included in the groups studied. In addition, the heterogeneity of the diagnoses in the study group of Raivio et al. (103) does not provide strong evidence for the mimicry of the mini-pubertal period. The physiological induction protocols that have been used in the mini-pubertal period have been used as non-standardized protocols in isolated cases or small groups of patients, and there is a lack of data on the fertility outcomes. However, published studies show that combined gonadotropin therapy has a more beneficial effect than parenteral TRT on testicular (Sertoli cell proliferation and seminiferous tubule growth) and genital development (increase in TV and penile length) in male patients with CHH during the mini-pubertal period (85,86,87,101). Despite the beneficial effects of gonadotropin therapy, there is still a need for strong evidence to support the physiological mimicry of the mini-pubertal period. Existing studies suggest the need for robust randomized and controlled trials with large patient populations in which: (i) the groups are diagnostically homogeneous; (ii) the mini-pubertal period is mimicked or not with physiologically standardized protocols; and (iii) the cases with or without undescended testes are grouped. Although there are different physiological induction protocols for gonadotropins in the mini-pubertal and pubertal periods in boys, there are no data on physiological induction in the mini-pubertal period in girls with CHH. In contrast, there are gonadotropin protocols for ovulation induction in adult female patients with CHH (12).

Physiological Pubertal Induction in Male Patients

In male patients, the physiological pubertal induction protocol can be started from the age of 12 years in cases with confirmed congenital HH. In cases of unconfirmed diagnosis, it should be started after the necessary differential diagnoses have been made (11,38).

Normal levels of both gonadotropins are necessary for appropriate spermatogenesis induction during puberty (53). The optimal treatment regimen should be used when a patient has inadequate pubertal development and a testicular size of less than 4 mL. Treatment of prepubertal patients (testicular volume <4 mL) initially with rhFSH for four months, to maximize the Sertoli cell pool, followed by combination treatment with rhFSH and hCG has been suggested as the most favorable strategy for future fertility (93,104). FSH maximizes Sertoli and germ cell counts, and increases seminiferous tubule growth (7). Recombinant hCG, which shares a receptor with LH, increases serum testosterone levels, both of which lead to normal spermatogenesis (66). For patients experiencing spontaneous onset of puberty or pubertal arrest (due to any etiological reason), with a testicular volume of 4 mL or more, hCG monotherapy or hCG combined therapy with FSH can be initiated as the primary treatment option (53,93).

Rohayem et al. (93) studied a relatively large group (n = 34) of adolescents with delayed puberty, with the majority of them having no signs of puberty. The adolescents were treated with low doses of hCG (250-500 IU twice a week) with gradual increases of 250-500 IU every six months, and when the target pubertal level of serum testosterone (5.2 nmol/L = 150 ng/dL) was reached, rFSH was introduced (93). Typically, it is advised to provide hCG at a dosage of 500-2500 IU per dose, 2-3 times per week, towards the conclusion of the treatment (12). The literature states that the recommended dosage ranges from 3000 to 10000 IU each dose, administered 2 to 3 times per week (105). The initial dosage of FSH is typically 75-150 IU (or 1 IU/kg/dose) administered every other day (or 3 times per week) (106). To attain a serum FSH concentration within the physiological range of 1-7 IU/L, the dosage should be increased if deemed required (93). The physiological induction protocol in male patients was revised according to the recommendations of Rohayem et al. (93) (German Adolescent Hypogonadotropic Hypogonadism Study Group). Figures 3 and 4 summarize the general recommendation for physiological pubertal induction in male patients (53,93).

In addition to the assessment of the development of the secondary sex characteristics, serum levels of FSH, inhibin B, total testosterone, and hemoglobin should be monitored at 3-month intervals to assess safety and efficacy (12). The dose of hCG should be adjusted according to testosterone levels, while the dose of FSH is typically modified based on the clinical signs and FSH levels (Figure 4) (53,66,93). The half-life of hCG is approximately 36 hours. Therefore, total testosterone and estradiol concentrations obtained before the subsequent injection are the most informative indicators for ensuring that the target testosterone concentration is being maintained. Normal serum testosterone concentrations may not be achieved due to poor adherence or, rarely, the development of antibodies (106).

For patients with CHH who have GnRH deficiency but normal pituitary function, pulsatile GnRH treatment may be a viable option for both sexes (12). The most physiological approach is to use GnRH infused in a pulsatile fashion, with pulse intervals of 90-120 minutes (53). Pulsatile GnRH treatment stimulating the release of endogenous FSH and LH is effective in normalizing the gonadal axis of the majority of the patients with HH (except GnRH receptor defect) (73). Even in patients with combined pituitary hormone deficiencies, in the presence of a pituitary reserve, the pituitary-testis axis function is restored in 60% of all cases, while displaying no association with the pituitary

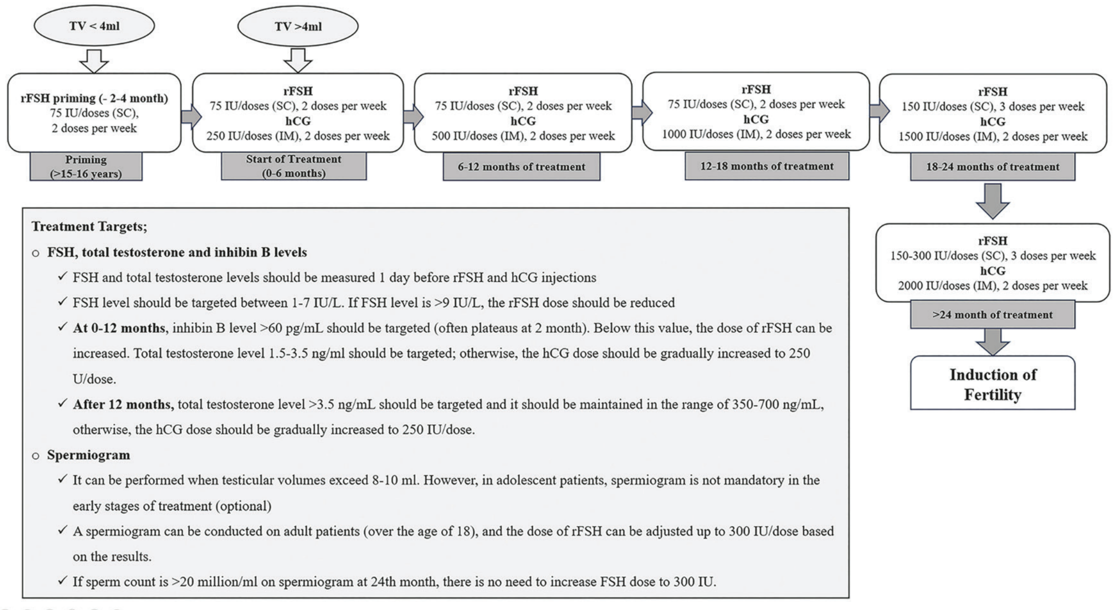

Figure 4. Graphic timeline of recombinant FSH and hCG hormone treatment plan, assessments, and treatment goals

FSH: follicular stimulating hormone, hCG: human chorionic gonadotropin hormone, rFSH: recombinant FSH, IM: intramuscular

height or integrity. Liu et al. (107) found that pulsatile GnRH therapy for two years in adolescents with the complete form of CHH does not significantly enhance testicular growth, accelerate the onset of sperm production, or increase sperm output compared to hCG/human menopausal gonadotropin therapy. While there are several different regimens, it is recommended to start GnRH treatment with 5-25 ng/kg per pulse administrated at 90-120-minute intervals with an increase of 2 ng every month, targeting testosterone levels in the mid-normal adult ranges (13,108).

A review by Young et al. (12), evaluating the efficacy of GnRH (n = 11 trials) and combined gonadotropin therapy (n = 33 trials) among 1118 patients, demonstrated that the median testicular volume increased from 3.4 mL to 9.8 mL and median sperm count increased from 7.59 million/mL to 15.3 million/mL. Persistent azoospermia was found in 17% (38/219) of patients treated with GnRH infusion, compared to 21% (190/899) of patients treated with combined gonadotropins (FSH + hCG).

Physiological Protocols for Inducing Puberty in Female Subjects

The overall goal of sex hormone replacement therapy in girls with hypogonadism is to establish an age-appropriate endocrine milieu resulting in normal growth, bone mass accrual, uterine growth and maturation, and development of secondary sexual characteristics and cognitive functions, at a tempo consistent with their peer group. The hormone replacement therapy process in adolescent girls consists of three main stages. It has been suggested that low levels of estrogen in healthy pre-pubertal girls may promote the maturation of the bones and growth. Therefore, it is recommended to start with very low doses of estrogen therapy in the early stages, which will not cause breast development but will contribute to growth and bone maturation. In the second stage, low doses of estrogen therapy should be initiated to ensure the physiologic pubertal developmental stages and the development of secondary sex characteristics, and the dose should be increased in certain intervals. In the last stage (2-3 years of treatment), when the final estrogen dose is reached, progesterone should be added to the treatment to ensure menarche in patients who have completed pubertal development. This treatment should be continued until the age of menopause (56).

Determining the optimal route, drug, dose, and timing of estrogen replacement treatment for girls with hypogonadism is an active area of research. There is currently no agreement in the literature regarding the most suitable approach. Treatment should be individualized (56). The most common and preferred form of estrogen replacement is 17-β-estradiol. Oral 17-β-estradiol products (ethinyl estradiol, conjugated equine estrogen) can be initiated as biphasic or triphasic sequential hormone replacement regimens combined with progestins. The triphasic regimens are useful in providing lower estrogen doses during the treatment-free week of the biphasic regimens, hence effectively controlling vasomotor symptoms, which can be particularly valuable in those with established diagnosis and

J Clin Res Pediatr Endocrinol
2024;16(4):379-400

older ages (109). However, oral forms have been associated with a higher risk of thromboembolism than transdermal products. Therefore, transdermal estrogen therapy should be the first choice for pubertal induction because it bypasses hepatic metabolism and has been shown to result in more stable serum estradiol concentrations with no reduction in IGF-1 concentrations compared to oral forms (15,110,111). However, no significant differences in body composition, height, or bone mineralization have been found in studies directly comparing oral and transdermal estrogen (15). Table 2 shows the dosage and route of administration of different estrogen preparations for pubertal induction. Although different protocols are available, the transdermal estrogen protocol used in this review is based on the protocol published by Ankarberg-Lindgren et al. (12,54,55,56) in 2001 and 2014. The transdermal estrogen protocol is summarized in Figure 5.

Progestins are initiated for withdrawal bleeding after 2-3 years of estrogen treatment or when a significant breakthrough bleeding occurs under estrogen treatment (111). Micronized crystalline progesterone (100-200 mg/day) or medroxyprogesterone acetate (5-10 mg/day) are preferred and are administrated for 5-10 days each month to prevent endometrial hypertrophy (Figure 6) (56,110). Gonadotropin or GnRH infusion therapy protocols are not commonly used for pubertal induction in adolescent girls. In contrast, gonadotropin treatment protocols are used for the induction of ovulation in female patients expected to be fertile in adulthood (12).

Conclusion

Hormone treatment is essential in hypogonadism as sex hormone deficiency can lead to several complications, including osteoporosis, changes in body composition,

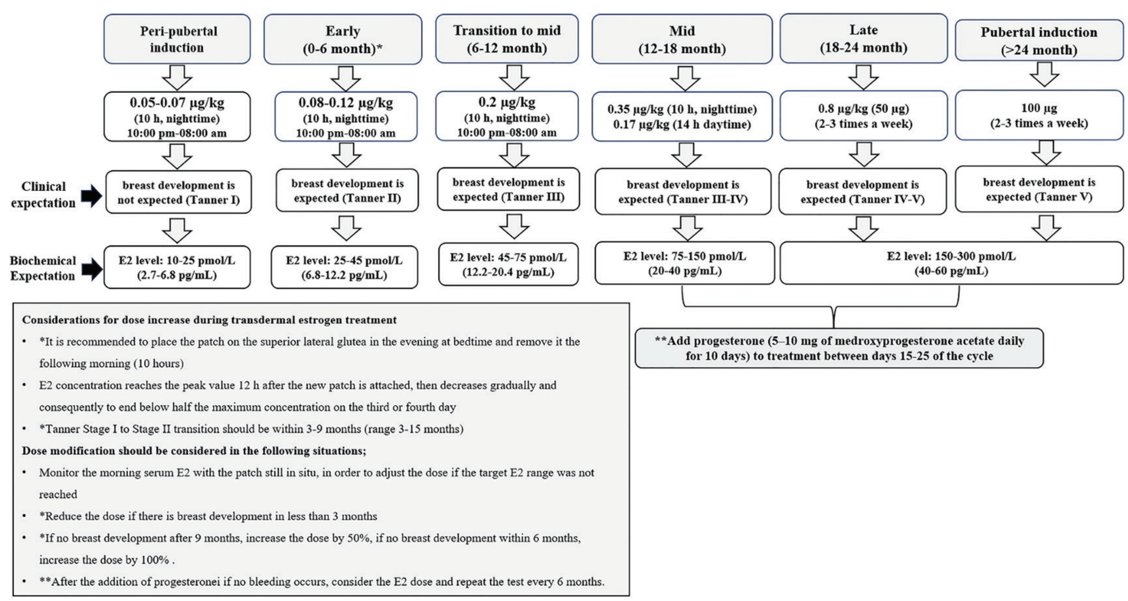

Figure 5. Pubertal induction protocol with transdermal estrogen therapy in girls

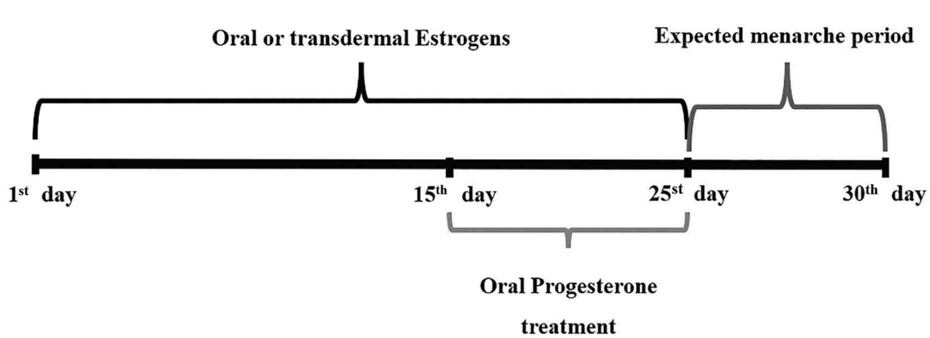

Figure 6. Monthly administration of estrogen and progesterone after the induction of puberty in an adolescent girl

metabolic abnormalities, cardiovascular risks, and mood disorders. The aim is to help achieve physiological and psychological adolescent maturation for the normal development of secondary sexual characteristics, uterine/testicular growth, bone mass, and growth spurt. Treatment strategies should be individualized with meticulous dose titrations to balance the expectations on pubertal progression and expected adult height.

In recent years, physiological pubertal induction protocols (recombinant gonadotropins or GnRH analog) have been recommended to increase fertility success in cases of persistent HH. The protocol allows induction of spermatogenesis in the majority (80-90%) of cases after two years. The treatment is generally well accepted and tolerated by patients.

However, due to the inconvenience of application, difficulties in obtaining drugs, and the lack of strong evidence that TRT decreases fertility success, the use of physiological induction protocols from mini-puberty and adolescence is still controversial. Randomized, case-controlled studies are needed to support the use of physiological induction protocols in mini-pubertal and adolescent populations.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayhan Abacı, Concept: Ayhan Abacı, Design: Ayhan Abacı, Data Collection or Processing: Ayhan Abacı, Özge Besci, Analysis or Interpretation: Ayhan Abacı, Literature Search: Ayhan Abacı, Özge Besci, Writing: Ayhan Abacı, Özge Besci.

Financial Disclosure: The authors declared that this study received no financial support.

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