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Review

Review

New and Consolidated Therapeutic Options for Pubertal Induction in Hypogonadism: In-depth Review of the Literature

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Abbreviations: 17β-E_2, 17β-estradiol; BMD, bone mineral density; CDGP, constitutional delay of growth and puberty; CEE, conjugate equine estrogen; CHH, congenital hypogonadotropic hypogonadism; COCP, combined oral contraceptive pill; DP, delayed puberty; EE, ethinylestradiol; ERT, estrogen replacement therapy; FtoM, female to male; hCG, human chorionic gonadotropin; HPG, hypothalamo-pituitary-gonadal; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; rhFSH, recombinant human FSH; TDE, transdermal 17β-estradiol; TRT, testosterone replacement treatment; TS, Turner syndrome; TU, testosterone undecanoate; TV, testis volume; uFSH, urinary FSH

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Abstract

Delayed puberty (DP) defines a retardation of onset/progression of sexual maturation beyond the expected age from either a lack/delay of the hypothalamo-pituitary-gonadal axis activation or a gonadal failure. DP usually gives rise to concern and uncertainty in patients and their families, potentially affecting their immediate psychosocial well-being and also creating longer term psychosexual sequelae. The most frequent form of DP in younger teenagers is self-limiting and may not need any intervention. Conversely, DP from hypogonadism requires prompt and specific treatment that we summarize in this review. Hormone therapy primarily targets genital maturation, development of secondary sexual characteristics, and the achievement of target height in line with genetic potential, but other key standards of care include body composition and bone mass. Finally, pubertal induction should promote psychosexual development and mitigate both short- and long-term impairments comprising low self-esteem, social withdrawal, depression,

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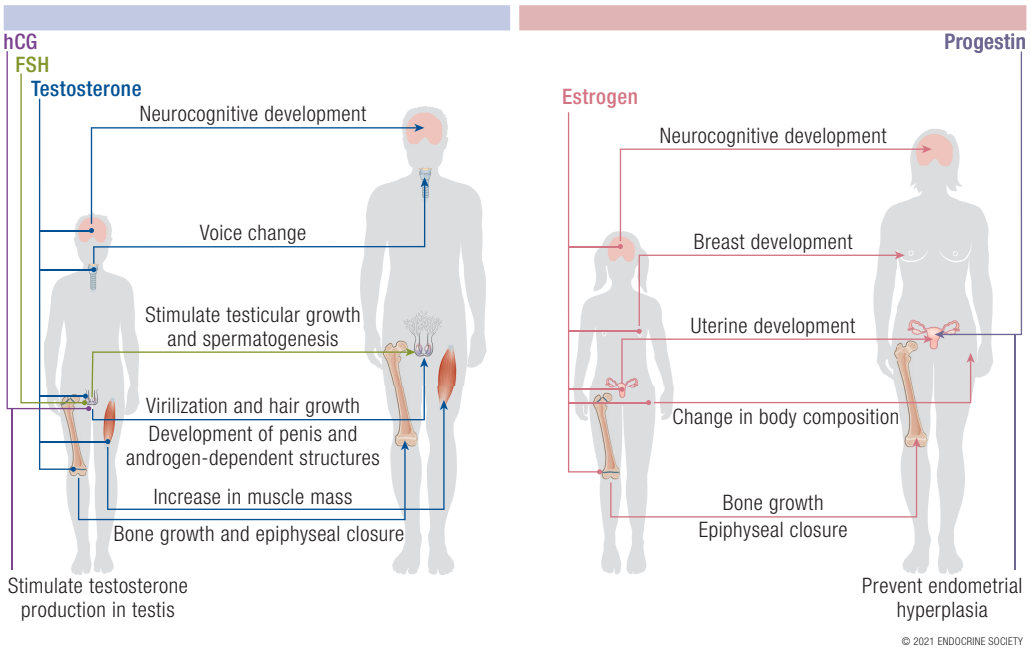
and psychosexual difficulties. Different therapeutic options for pubertal induction have been described for both males and females, but we lack the necessary larger randomized trials to define the best approaches for both sexes. We provide an in-depth and updated literature review regarding therapeutic options for inducing puberty in males and females, particularly focusing on recent therapeutic refinements that better encompass the heterogeneity of this population, and underlining key differences in therapeutic timing and goals. We also highlight persistent shortcomings in clinical practice, wherein strategies directed at "the child with delayed puberty of uncertain etiology" risk being misapplied to older adolescents likely to have permanent hypogonadism.

Key Words: idiopathic hypogonadotropic hypogonadism, GnRH deficiency, delayed puberty, testosterone, estradiol, gonadotropin

Graphical Abstract



ESSENTIAL POINTS

1. The optimal age to begin treatment in patients with known hypogonadism has not been universally established yet, but a prompt initiation of treatment after diagnosis of DP is recommended within the physiological time frame whenever possible.
2. Pubertal induction must be tailored according to each patient's clinical history and needs. Treatment should not only lead to genital maturation and development of secondary sexual characteristics, but also promote psychosexual development.
3. The most consolidated therapeutic strategy for pubertal induction in males involves intramuscular testosterone esters (ie, testosterone enanthate); however, in the past few years newer testosterone formulations, such as intramuscular testosterone undecanoate and testosterone gel, have been used with promising results.
4. Gonadotropin treatment for pubertal induction in males with hypogonadotropic hypogonadism represents a good alternative to exogenous androgens, with the advantages of potentially increasing testicular volume, inducing spermatogenesis, and promoting spontaneous descent of testes in patients with cryptorchidism.
5. Pubertal induction in females involves the administration of both estrogens (to promote feminization) and progesterone (to prevent endometrial hyperplasia): the most recent acquisitions in the field seem to suggest that transdermal 17β-estradiol and micronized progesterone represent the most physiological formulations for this purpose.

The conventional normal age ranges for the beginning of puberty are reported to be between 8 and 13 years of age in females, and 9 to 14 years in males, although secular changes are reported. The first clinical signs marking its onset are the enlargement of testis volume (TV) ≥ 4 mL or scrotum enlargement/pigmentation in males (designating progression from Tanner genital stage G1 to G2), and the appearance of breast buds in females (designating progression from Tanner stage B1 to B2) (1). Delayed puberty (DP) is defined as a retardation of pubertal onset beyond the expected age (> 2-2.5 SDs above the mean of the reference population) (2); thus, no breast development by the age of 13 years in girls and no testicular enlargement by the age of 14 years in boys (1). The lack of Tanner stage progression after the beginning of puberty, evaluable on the basis of available nomograms (3, 4), together with a sudden drop in hormonal levels, has to be considered pathologic as well. The most common cause of DP in both sexes is constitutional delay of growth and puberty (CDGP), a self-limited form of delayed puberty resulting from a transient GnRH deficiency often considered an extreme of the normal spectrum of pubertal timing. Pathological causes of DP (Table 1) must always be ruled out with an appropriate diagnostic workup (5-7), particularly in the presence of clinical suspicion.

Differentiating between CDGP and functional and congenital hypogonadotropic hypogonadism (CHH) during early adolescence is particularly challenging because of the many overlapping clinical, biochemical, and radiological features (5, 6, 8, 9), but the distinction is a necessary one as these conditions then diverge hugely in respect of their long-term outcomes. Permanent forms of hypogonadism causing DP require specific treatment to induce or complete pubertal development, and reaching a correct and timely diagnosis thereof is crucial to promote patients' somatic and sexual maturation and psychosocial well-being. It is also vital not to misdiagnose as CDGP those patients with retardation of growth and puberty resulting from parasellar lesion or systemic illness such as bowel disease or an eating disorder.

We present here an in-depth review of the literature regarding therapeutic options available for pubertal induction in both males and females with hypogonadism, beginning with the more consolidated approaches and then proceeding to more novel advances in the field, considering the heterogeneity of this population and underlining differences in therapeutic timing and goals. We emphasize that CDGP should be a diagnosis of exclusion and not of default: notably, among older adolescents approaching the end of their teenage years, CDGP is no longer the majority and should not be seriously considered beyond 18 to 20 years of age. Based on Bayesian principles, the adverse consequences of not intervening to pharmacologically induce puberty in a minority of minors with hypogonadism outweigh those of intervening—perhaps unnecessarily—in the majority with CDGP.

Goals and Timing

Therapy should lead to the maturation of genitalia and of secondary sexual characteristics of patients with DP, whether young adolescents or older individuals who "slipped through the net," and linear growth, body composition, muscle mass, and normal bone density should also be achieved (2, 10, 11).

During the first phase of pubertal development, sex hormones play a crucial role in inducing the pubertal growth spurt and permit the achievement of body proportions and adult height in line with genetic potential. For instance, in CHH, a delayed growth spurt in those with a background of otherwise preserved linear growth can lead to both taller stature (12) and segmental disproportion (13) compared with genetic potential, whereas failure to recognize and treat a systemic disease or parasellar lesions causing DP and growth retardation leads to a shorter adult height (14-16). Moreover, an excessive delay in puberty and/or in pubertal induction may adversely affect bone health, although the data are not entirely consistent. According to several studies (17-20), adult men with history of DP present significantly decreased bone mineral density (BMD) levels at all sites, hence being at greater risk of osteoporotic fractures, and pubertal delay can lead to a lower peak bone mass in girls as well (21). On the other hand, Bertelloni (22) and Yap (23) conclude that male patients with CDGP tend to have normal volumetric BMD, with conventional areal BMD appearing low because of altered skeletal phenotype. In particular regarding CHH, De Rosa and colleagues (24) found an inverse correlation between spinal BMD and the age at pubertal induction, supporting the importance of a timely diagnosis and intervention. Notably, histories of deficient treatment (inadequate dosage and/or long pauses) after diagnosis are also associated with impaired bone mass (25). Finally, pubertal induction must promote psychosexual development (26-28) and prevent psychosocial damage, including low self-esteem, body image concerns, social withdrawal, and sexual inactivity in later life (29-31). In males with CHH, combined gonadotropin treatment can also promote the normal maturation (or indeed descent) of the testes (see the following section).

Thus, it is essential to promptly define the underlying pathogenesis to identify a tailored program of care. In contrast to CDGP, in which a "wait-and-see" strategy would be an appropriate management because puberty should start spontaneously, pathological forms of DP need a specific therapeutic approach. A possible exception is represented by DP associated with functional central hypogonadism, in

Table 1. Main etiologies of pubertal delay

Classification	Nonpathologic form	Pathologic forms		
	CDGP	Central hypogonadism		Primary hypogonadism
		Organic	FHH	
Common causes	Unknown (genetic background?)	• CHH (normosmic CHH; Kallmann syndrome) • CHARGE syndrome • CHH with CAH • MPHD • Hypothalamic-Pituitary region lesions (eg, craniopharyngiomas) • Metabolic diseases (eg, hemochromatosis) • Hypophysitis • Infiltrative diseases • Thalassemia • Infection • Inflammatory (eg, Langerhans cell histiocytosis • Granulomatous disease (eg, sarcoidosis) • Iatrogenic causes (eg, radiotherapy) • Other genetic syndromes (eg, Prader-Willi, Laurence Moon-Biedl)	• Chronic illness • Malnutrition • Excessive exercise • Stress • Medications • Other endocrine disorders (eg, hyperprolactinemia, hypothyroidism)	• Klinefelter syndrome • Tuner syndrome • Anorchia • Enzymatic defects • DSD (eg, gonadal dysgenesis) • LH/FSH resistance • Acquired forms (eg, chemo- and/or radio-therapies; autoimmune diseases, trauma, gonadal torsion)
Frequency (%)				
Male	60-65	10	20	5-10
Female	35	20	20	25

Adapted from: Bollino A, Cangiano B, Goggi G, et al. Pubertal delay: the challenge of a timely differential diagnosis between congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty. *Minerva Pediatr*. 2020;72(4):278-287.

Abbreviations: CAH, congenital adrenal hypoplasia; CDGP, constitutional delay of growth and puberty; CHARGE, Coloboma, Heart defect, Atresia choanae, Retarded growth and development, Genital abnormalities, Ear abnormalities; CHH, congenital hypogonadotropic hypogonadism; DSD, disorders of sex development; FHH, functional hypogonadotropic hypogonadism; MPHD, multiple pituitary hormone deficiencies.

which the first line of action would be to remove or treat the underlying cause of hypothalamo-pituitary-gonadal (HPG) axis disorder, aiming to restore its correct functionality.

The optimal age to begin treatment in patients with known hypogonadism has not yet been universally established. Whenever genetics, clinical history, or physical examination allow a timely diagnosis of either congenital or acquired forms of hypogonadism, a treatment within the physiological time frame of puberty is possible and therefore recommended (8). In particular, clinical syndromes associated with hypergonadotropic hypogonadism can be diagnosed before birth or in early infancy, and gonadal insufficiency is confirmed by rising gonadotropins at the anticipated time of puberty. More rarely, patients with reproductive or nonreproductive clinical features typically associated with CHH (the so-called "red flags") might also receive a timely diagnosis (Table 2) (6). Therefore, to recapitulate normal physiology and mitigate adverse outcomes, in such patients, pubertal induction should be initiated no later than 14 years in males and 13 years in females. However, this injunction to clinicians necessarily assumes an unrealistically precocious diagnosis of hypogonadism that is rarely achieved in practice, especially in those cases with a challenging differential diagnosis with CDGP. For the vast majority of hypogonadal patients, the diagnosis cannot even begin to be considered until after a period of pubertal delay. This means that patients necessarily will receive their final diagnosis outside their physiological time frame, making it even more important to promptly complete the diagnostic workup and begin appropriate treatment. In practice, the diagnosis and treatment of CHH patients is typically delayed until very late adolescence even in developed countries, which is unacceptable. These delays reflect both the inertia of clinical referral pathways and the frequent misapplication by clinicians of management principles aligned with CDGP to adolescents with likely organic hypogonadism (32).

Whenever it is not possible to differentiate between CDGP and CHH, a short treatment with low doses of sex steroid hormones should be considered (ie, testosterone gel 10 mg every second day or IM testosterone enanthate 25-50 mg monthly): in fact, besides helping these patients psychologically, it can also help the clinician differentiate the self-limiting delayed puberties from persistent conditions because CDGP patients will then generally initiate endogenous pubertal development (6, 33).

Induction of Puberty in Males

Testosterone is the most frequently adopted pharmacological treatment in hypogonadal boys with DP because of its efficacy at inducing secondary sexual characteristics,

Table 2. Red flag features of CHH

Indicators of absent minipuberty			Nonreproductive phenotypes		
	% among CHH patients	% among general population		% among CHH patients	% among general population
Microphallus	10	0.15 at birth	Anosmia	45	2, but not exclusively congenital
Cryptorchidism	40	1.5 at 3 mo	Deafness	6	0.12 at birth (otoacoustic emission test)
Bilateral cryptorchidism	20	0.4 by 3-12 mo	Cleft lip or palate	5	0.15 at birth
Absent erections on morning diaper change	-	-	Digital abnormalities	5	0.1 at birth
			Family history of CHH		

Abbreviations: CHH, congenital hypogonadotropic hypogonadism.
Composite data: Bonomi, EJE 2018 (181); Quinton, Clin Endo 2001 (182); Stamou, EJE 2017 (183); Pitteloud, JCEM 2002 (184); Swee, Front Endocrinol 2019 (185); Quinton personal data.

growth spurt, bone maturation, and psychosexual development and its limited side effects and costs. However, testosterone alone cannot stimulate testicular growth or induce spermatogenesis, nor induce testicular descent in those with cryptorchidism (1, 2, 8, 29), whereas these goals can all be achieved in most CHH patients with combined gonadotropin treatment (2, 34-36), which may also give these patients significant psychological encouragement and enhance their self-confidence (2). Early induction of spermatogenesis may reduce the time required to reinduce it in adult life (29, 35, 37). CHH patients treated with gonadotropins achieve an androgen profile (both testosterone precursors and metabolites) that is closer to normal biochemistry than what is achievable with testosterone treatment (38), although the clinical relevance of this finding remains uncertain.

There are no longer concerns as to whether prior testosterone replacement treatment (TRT) negatively affects the subsequent spermatogenesis or TV response to gonadotropins in CHH patients: although earlier studies suggested that prior TRT was a negative prognostic factor (39), especially in patients with severe phenotype of CHH (TV < 3 mL) (40), a metanalysis found no differences in the success rate of sperm appearance or in the mean sperm concentration between patients who previously received testosterone and those who did not (41). Moreover, a recent study by Rohayem et al. (34) showed that gonadotropin treatment in young CHH males successfully induces testicular growth and spermatogenesis, irrespective of previous testosterone treatment; however, although being not a statistically significant difference, it must be taken into account that both the rate of TRT-naïve patients who reached a normal sperm concentration and also their mean sperm concentration itself were around double those of patients who previously completed pubertal induction with TRT (34).

Finally, another strategy to induce puberty in CHH involves pulsatile subcutaneous infusion of GnRH through a wearable minipump (42), but data on its comparative efficacy to gonadotropins are conflicting (43-45). Overall, considering its high cost, limited availability, and the associated discomfort (44, 46), the GnRH pump is a less feasible strategy in clinical practice to induce puberty in males, compared with exogenous gonadotropins.

Testosterone: types and routes of administration

There remains a dearth of studies directly comparing different testosterone protocols to induce pubertal development in males and thus no truly evidence-based guidelines regarding the optimal formulation and regimen for this purpose have ever been drawn up (47).

IM testosterone esters are the most frequently prescribed formulation for pubertal induction (48-51), in consideration of their relatively low cost (10, 52) and wide experience of efficacy at inducing an adequate virilization (52). Nevertheless, intermediate-acting testosterone esters come with some disadvantages: younger adolescents may find it hard to tolerate such frequent and relatively painful injections (50), which could contribute to poor adherence. Moreover, they exhibit suboptimal pharmacokinetics with wide serum testosterone level fluctuations between injections, from supraphysiological levels for the first few days, to subtherapeutic levels leading up to the next injection, sometimes associated with undesirable swings in energy, mood, and libido (53-56), and greater risk of erythrocytosis (57).

Another consolidated formulation of testosterone is oral testosterone undecanoate (TU): being a tablet its administration is better tolerated than injections; moreover, because it is absorbed through the enteric lymphatic system, it bypasses hepatic first effect, therefore relatively lower doses can be administered compared with older oral formulations. However, its absorption is highly sensitive to the lipid content of meals, which often makes it unreliable; besides, its short half-life makes it necessary to administer multiple daily doses, with fluctuations in testosterone levels through the day (and possible compliance issues); finally, it is also not widely available (47, 58). Most data concerning the safety and efficacy of oral TU in young boys derive from studies performed on patients with CDGP rather than hypogonadism and, despite differences in doses and duration of therapy, they all found that treatment with oral TU was able to induce the maturation of secondary sexual characteristics (4, 59, 60), increase TV (59, 60), and stimulate growth without an inappropriate advancement of bone age (4, 59-62). However, the effects on predicted adult height were inconsistent, being either increased (4), reduced (62), or unchanged (60). Overall, these studies provide significant evidence that the administration of oral TU in boys with CDGP is both safe and effective at promoting pubertal development and growth without excessively advancing bone age. Similarly, long-term treatment with oral TU was also proved to be both safe and effective at inducing pubertal development in boys with anorchia (63, 64).

Finally, among the oldest testosterone formulations are testosterone pellets, implanted subcutaneously using a trocar into the lower abdominal wall. However, although relatively stable levels of testosterone are achievable, the overall pharmacokinetic profile is inferior to IM TU (see the following section). They also require a minor surgical procedure and are associated with risk of extrusion or infection (53). The literature data regarding the use of testosterone pellets for pubertal induction in young hypogonadal boys are very scarce: the only evidence available on this issue showed that 18 months of treatment with testosterone pellets in boys affected with different forms hypogonadism

led to an appropriate growth, pubertal progression, and psychosocial development, proving to be safe, effective, and well tolerated by all patients (65)

Over the past few years, interest has grown in newer testosterone formulations that have begun to be used more widely in clinical practice. However, no formulations were designed for inducing puberty in patients with hypogonadism, but rather as hormonal replacement therapy (HRT) for adult hypogonadal males; therefore, no standard of care exists for their use in pubertal induction, and their pharmacokinetics and doses may not always be suitable for treating young prepubertal boys (see the following section). However, over the last decade a few studies have provided some evidence (see the following section).

Testosterone gels have the advantages of guaranteeing more stable serum testosterone levels over 24 hours and of providing good flexibility in dose adjustments (in particular, multidose dispenser can deliver as low doses as 10 mg per pump) (57, 66). Unintentional skin-to-skin testosterone transfer is one of the most important adverse events of such formulation because it can lead to inappropriate virilization of passively exposed women and children. Moreover, high costs might be a deterrent. To date, few studies have investigated its efficacy in young boys with delayed puberty, but data seem promising regarding both efficacy and safety, although concerns remain as to degree of adherence by the average teenager. Rogol et al. reported a significant rise to normal age-matched serum testosterone levels in young patients affected with primary hypogonadism treated with increasing doses of testosterone gel 1% for 6 months, despite no clinical meaningful changes being observed in terms of physical examination (more than one-half of patients were not naïve to testosterone, an occurrence that may have limited such changes) (56). Chioma et al. reported that low daily doses of testosterone gel 2% administered for 3 months in boys with CDGP were able to significantly increase growth rate in such patients, with no significant differences compared with IM testosterone (67). Finally, Contreras et al. showed the effects of 3 different testosterone gel regimens (with different formulations and dosages) in 3 young hypogonadal patients with hepatic dysfunction, reporting the advancement of secondary sexual characteristics, an increase in height and in testosterone levels, and a decrease in liver enzymes in each of them (68).

Another recent introduction in the field is long-acting intramuscular TU, although data are still scarce among patients with DP. The 3 studies (55, 69, 70) that investigated its efficacy at promoting pubertal development showed good results without significant adverse events, but they only recruited older adolescents (≥17 years) or prepubertal hypogonadal adults. TU has a much lower frequency of injections compared with testosterone enanthate, which handily corresponds to the typical interval between clinic visits, potentially facilitating greater compliance; moreover, testosterone levels remain relatively stable between injections and, therefore, unpleasant swings in mood, energy, or libido are generally not experienced by patients. On the other hand, its fixed dose and long half-life make it harder to progressively modulate serum testosterone levels in children undergoing pubertal induction; hence, there is a theoretical risk in younger boys of advancing bone age too rapidly and thereby inducing precocious maturation and fusion of epiphyses, impairing final adult height (71).

The US Food and Drug Administration recently approved a new oral TU formulation characterized by a self-emulsifying drug delivery system: TU is dissolved within a mixture of lipids and a hydrophilic surfactant, and this composition allows the solubilization of testosterone so that it can be absorbed through the intestinal lymphatic system irrespective of the lipid content of the previous meal (in contrast with the older oral TU that needs a fat-rich meal to be efficiently absorbed). To date, only a few phase 3 clinical trials have evaluated the effects of this new formulation in the treatment of hypogonadism in adult males; data look promising in terms of both efficacy and safety (although the administration of new oral TU was shown to be associated with milder gastrointestinal adverse effects and a 3-5 mmHg increase in systolic blood pressure) (72, 73). Unfortunately, no data exist yet on their use for pubertal induction in prepubertal hypogonadal boys; hopefully, in the future, trials will be performed on these patients as well.

Finally, the US Food and Drug Administration also recently approved the administration of subcutaneous testosterone enanthate via an autoinjector as a new formulation of TRT for hypogonadal patients. Different studies show that a weekly administration of subcutaneous testosterone enanthate is safe, well tolerated, and effective at allowing patients to achieve testosterone levels within the reference range (74-77). Besides its efficacy, subcutaneous testosterone enanthate via an autoinjector has also several advantages: first of all, testosterone can be easily self-administered through the autoinjector, a device that also reduces the sensation of needle entry and pain; second, because of a reduced variability of testosterone levels, swings of mood, energy, and libido are less likely to happen compared with IM injections; and finally, the ease of administration and its weekly regimen may lead to higher compliance compared with other formulations (74). To date, however, no data are available on the use of subcutaneous testosterone for pubertal induction in hypogonadal patients. Despite this, some evidence can be extrapolated from induction protocols used in transgender female-to-male (FtoM) patients. Several different testosterone esters

and different regimens have been used so far for such a purpose (78-81): for example, Olson et al. (79) administered progressively increasing doses of subcutaneous testosterone cypionate for 6 months (first a biweekly dose of 25 mg for the first 8 weeks, then an increase to 25 mg weekly for 4 weeks and, if tolerated, increased to 50 mg weekly, with most patients ending up on a final dose of 25-75 mg per week) to a small cohort of young (13-24 years old) FtoM patients. They reported that such treatment was overall effective at achieving testosterone levels within the normal male range and at inducing amenorrhea, with little adverse impact on physiologic parameters (body mass index, systolic blood pressure, and alanine aminotransferase increased to statistically but nonclinically significant levels) and a few, mild side effects. Similarly, Spratt et al. (78) showed that progressively increasing doses of subcutaneous testosterone cypionate in FtoM patients (starting dose of 50 mg weekly, then sequentially increased to achieve testosterone levels within the adult normal range) were able to achieve testosterone levels within or above the normal male range, induce amenorrhea, suppress estradiol levels, and induce male secondary sexual characteristics, proving to be an effective, safe, and well-accepted option. Thus, subcutaneous testosterone esters, also with the possibility of administering lower doses at shorter time intervals might be a promising alternative for pubertal induction of hypogonadic cisgender boys.

The main characteristics of the formulations illustrated, with their advantages and disadvantages, are summarized in Table 3 (47, 53, 54, 57, 58, 66, 72-74).

Testosterone: proposed therapeutic schemes

In younger adolescents, a regimen of gradual testosterone titration is essential to recapitulate the progressive increase of serum testosterone during the physiology of normal puberty; in this way, both psychosexual and secondary sexual characteristics develop gradually, whereas optimization of growth and adult height is also ensured (29). Although concrete data are conspicuously lacking, there are reasonable concerns that too-rapid escalation of testosterone dose might lead to abrupt virilization (10) and accelerated psychosexual development, potentially increasing the risk of precocious sexual activity (8) and relational problems (10), and could also compromise adult height by inducing premature epiphyseal fusion (10, 29, 46). However, these concerns are largely theoretical and not relevant to older adolescents or adult males with CHH.

The best established regimen for pubertal induction involves using intermediate-acting intramuscular testosterone esters (in particular, testosterone enanthate); ideally, it should be started around the age of 12 years (1, 46, 82) with low doses of testosterone esters (eg, 50 mg of testosterone enanthate) every 4 weeks (1, 2, 8, 29), then the dose being increased gradually (eg, escalations of 50 mg every 6-12 months (1, 2)), over a course of 24 to 36 months (2, 8, 29) until reaching full adult dose (eg, 200-250 mg every 2-4 weeks) (1, 2). In hypogonadal patients that come to medical attention only at late adolescence or in adulthood, testosterone dose escalation should be faster because adult height is no longer of concern (29), segmental disproportion may already be established, and patients are by and large desperate to complete puberty as rapidly as possible (8): a higher initial dose (eg, 100-200 mg of IM testosterone enanthate monthly) (2, 8) can be quickly increased to 250 mg monthly (2).

Regarding the use of intramuscular TU for pubertal induction, a few, small observational studies have recently been developed (55, 69, 70). To ensure a more gradual rise in testosterone levels, the investigators either omitted the usual loading dose at 6 weeks (69, 70) or started with oral TU for a few months before switching to intramuscular TU (55). As already stated, because of a lack of data and the theoretical risks of advancing bone age too rapidly, intramuscular TU is not recommended to induce puberty in young boys with hypogonadism and is therefore reserved for older patients (≥17 years) with hypogonadism who have achieved the majority of their linear growth potential (71), which in practice comprises around 50% of CHH patients, partly because of referral delays and medical procrastination (31).

Because of its favorable pharmacokinetics, lack of injections, and flexibility for dose adjustment, testosterone gels administered through a multidose dispenser offer significant advantages for pubertal induction in young adolescents, although the actual evidence remains scarce (56, 67). An adequate starting dose for a 12 to 14 year old boy would be 10 mg every second day (8), progressively increased to full adult dosing over the course of 24 to 36 months, depending on clinical response. Further studies are necessary to establish a standard of care.

Once again, despite the paucity of studies on the use of oral TU for pubertal induction in hypogonadal boys making it difficult to design solid standards of care, it is reasonable to start with low doses and then progressively increase them as pubertal development proceeds; therefore, it has been suggested to start with 40 mg every other day for 3 months, then 40 mg daily for 6 to 12 months, and then progressively increase the dose up to 80 to 120 mg daily within 18 to 24 months from the beginning of treatment, when a switch to a parenteral formulation is usually made. The rate of increasing doses needs to be decided for each patient according to the progress of his linear growth, virilization, and bone age advance (71).

Table 3. Testosterone: types and routes of administration and characteristics

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
Intermediate-acting testosterone esters for IM injection: enanthate or mixture of different esters	100-, 200-, 250-, 400-, 1000-, 2000-mg vials	Viscous, oily liquid The esterification of its 17β-hydroxyl group makes its absorption much slower Plasma half-life is about 4.5 d Steady state is achieved in 1-2 injections Standard doses for adult hypogonadal males (TRT): 100 mg IM every 7-10 d, or 250 mg every 2-3 wk Initial dose for pubertal induction in younger adolescents with DP might be 50 mg monthly	Wide experience and known efficacy in adolescents with delayed puberty Low cost Self-administration is possible Widely available	Wide fluctuations of testosterone levels may deeply affect mood, energy, and libido Frequent, painful injections (compliance at risk) Higher risk of erythrocytosis and other side effects
Long-acting TU for IM injection	1000 mg/4-mL vials	Its viscous, oily solution and its long aliphatic hydrophobic side chain make its absorption slower and its half-life considerably longer (about 34 d) Testosterone serum levels reach their maximal levels about 10-12 d after each injection Recommended injection interval in adult hypogonadal males (TRT) is 10-14 weeks. To achieve steady state more rapidly in such patients, a shorter interval (6 weeks) between first and second doses is recommended It can be used for pubertal induction only in older adolescents and adult males with delayed puberty, although with a few precautions (see text); it is not recommended in younger adolescents	Much less frequent injections than with intermediate-acting testosterone esters (possibly better compliance) More stable testosterone concentrations than with intermediate-acting testosterone esters; therefore, no swings in mood, energy, or libido	Relatively painful injections Slow washout after withdrawal High cost Self-administration is impractical Poor flexibility in dose modifications Limited clinical experience in adolescents with delayed puberty
Transdermal testosterone gels	Single-use sachets or tubes and multidose dispensers Testosterone concentration: 1%, 1.62%, and 2%	It must be applied every morning on dry, intact skin Once applied, it is rapidly absorbed through skin and is stored within the stratum corneum of epidermis, from which it is slowly and progressively released into the bloodstream After the first administration, testosterone levels increase up to 4- to 5-fold within 24 h, reaching the steady state after 2-3 d Testosterone levels remain stable as long as gel is regularly administered: once withdrawn, testosterone serum levels fall within 96 hours	Self-administration Flexible dose modification using the multidose dispenser Stable testosterone concentrations; therefore, no swings in mood, energy, or libido Short half-life; therefore rapid washout after drug withdrawal (eg, after adverse events)	Daily administration (compliance issues) Allergic reactions Skin irritation Risk of skin-to-skin transmission Limited clinical experience in adolescents with delayed puberty High cost
Oral TU	Tablets 40 mg	It has a long, aliphatic, lipophilic chain that allows it to be absorbed through the intestinal lymphatic system, bypassing hepatic first-pass effect and avoiding inactivation It takes 2-6 h after ingestion to reach its maximum serum levels It has a very short half-life and a 7% bioavailability	Easy and discreet self-administration Short half-life; therefore rapid washout after drug withdrawal (eg, after adverse events) No liver toxicity (as opposed to methyltestosterone and 17-α derivatives)	Its absorption is unreliable and dependent on dietary fat intake; without a fat-rich meal, it is minimally absorbed Short duration of action Because of its short half-life, multiple daily administrations may be required, which could lead to compliance issues Even with multiple daily administrations, there are daily swings in testosterone levels Not widely available

Table 3. Continued

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
Subdermal testosterone pellets	Subdermal implants 100 mg, 200 mg	They are implanted subcutaneously within the lower abdominal wall Testosterone is progressively and constantly absorbed into the systemic circle through a uniform erosion of the pellet's surface, through which it is released Because in this way testosterone does not undergoes hepatic first-pass effect, its absorption and biodisponibility are virtually complete A single 200-mg pellet releases about 1.3 mg/d of testosterone In adult hypogonadal men, the implantation of 3-6 200-mg pellets provides physiological daily dosage of testosterone for 4-6 months	Flexible dosage is feasible by combining different pellets of 100 or 200 mg Stable testosterone concentrations with no swings in their levels except for a rapid increase in the first days after implantation from an accelerated release of testosterone No need for injections or frequent administration; therefore, better compliance (useful for noncompliant patients)	Minor surgery is required for pellet implantation and regular substitution Risk of implant extrusion, bleeding, or infection High cost
Oral TU (SEDDS formulation)	Oral capsules	Self-emulsifying drug delivery system that combines a mixture of hydrophilic and lipophilic excipients that enable the solubilization of TU in the gut, so that after oral administration it can be absorbed through the intestinal lymphatic system irrespective of the lipid content of the previous meal It bypasses first hepatic effect and therefore inactivation Once absorbed into the systemic circulation, its undecanoic acid molecules are cleaved by nonspecific esterases and subsequently metabolized; TU is therefore turned into testosterone. After administration, peak testosterone levels are achieved after about 2-4 h and then progressively decrease down to the lower end of normal range after about 12 h from administration	Easy and discreet self-administration The lipid content of meals does not interfere with TU absorption No liver toxicity	Being a SEDDS formulation, capsules cannot be cut High degree of enzymatic cleavage of testosterone from TU can occur during blood sample handling, possibly leading to artefactually higher levels of testosterone when dosed Mild gastrointestinal adverse effects Increase in mean systolic blood pressure of 3-5 mmHg No studies have evaluated its efficacy for pubertal induction
Subcutaneous enanthate testosterone	Autoinjector	Testosterone enanthate is administered in an oil solution, via an autoinjector devised to inject highly viscous solutions through a 5/8-inch, 27-gauge needle subcutaneously Weekly injections of 50 mg of subcutaneous testosterone led to a rapid, yet temporary, increase of testosterone levels, which progressively fall to baseline right before the following administration; Conversely, weekly injections of 100 mg subcutaneous testosterone led to progressively higher levels of testosterone during the first 3 weeks, when a steady state is reached: after 4 wk, testosterone exposure is greater than that provided during the first week of treatment	Reduced sensation of pain and fine-needle entry Autoinjector is easy to use; therefore, self-administration is easily feasible No risk for skin-to-skin transmission Reduced variability of testosterone levels through time; therefore, less risk for fluctuations of mood, energy, or libido	Higher frequency of injections compared with other injectable formulations No studies have evaluated its efficacy for pubertal induction

Abbreviations: CHH, congenital hypogonadotropic hypogonadism; DP, delayed puberty; SEDDS: self-emulsifying drug delivery system; TRT, testosterone replacement therapy; TU, testosterone undecanoate.

The main studies that evaluated the effects of different testosterone formulations for pubertal induction are listed in Supplementary Table 1 (83) (https://zenodo.org/record/5572710#.YWm24RpBxPZ).

Gonadotropins: types and routes of administration

Pituitary gonadotropins are administered by subcutaneous self-injections and their use has gained ground in clinical practice in recent years, with several novel themes having emerged.

FSH is required to induce proliferation of immature Sertoli cells and spermatogonia, and to sustain TV and spermatogenesis (2, 84, 85). Three different preparations are available: human urinary FSH (uFSH), whether highly purified or not, and recombinant human FSH (rhFSH) (41), with regular uFSH being far cheaper. A small retrospective study found that, compared with highly purified uFSH, rhFSH achieved higher spermatozoa forward motility, a trend toward shorter time to achieve pregnancy, and significantly faster appearance of spermatogenesis, despite achieving similar final sperm concentrations (86). However, a metanalysis (41) reported no significant difference in the achievement of spermatogenesis or sperm concentrations among CHH adult patients treated with any form of FSH, suggesting no evident advantages of the newer preparations.

Because of its structural and functional homology with human LH and far longer half-life, human chorionic gonadotropin (hCG) is used to stimulate the production of endogenous testosterone from testicular Leydig cells, thereby inducing virilization, growth spurt, bone maturity, and psychological development in the same manner as exogenous testosterone. Moreover, hCG also achieves physiologically high intratesticular testosterone concentrations that—via binding to androgen receptors on Sertoli cells—act in concert with FSH to induce germ and Sertoli cell proliferation and maturation and thereby achieve spermatogenesis (85). Both urinary and recombinant forms are now available, with a single subcutaneous injection of 2500 U of recombinant hCG—after a delay of about 12 hours—achieving a major increase in testosterone levels, peaking 72 to 96 hours later (mean peak level, 34 nmol/L) and returning to baseline after 8 days (87). Finally, by inducing aromatase, hCG can also lead to gynecomastia from increased conversion of androgens into estrogens (88). Although hCG can be used alone, in CHH, it should generally be used in support of FSH therapy (see the following section). Despite its possible advantages on testicular growth and potentially in future fertility, gonadotropin treatment is considered off-label for pubertal induction.

Gonadotropins: proposed therapeutic schemes and applications

Among studies that have investigated gonadotropin use for pubertal induction, few were randomized controlled trials and, overall, were heterogeneous in terms of patient numbers, ages (ranging from adolescence to adulthood), length of observation, and therapeutic protocol adopted, whether with hCG alone or in combination with FSH. The main studies are listed in Supplementary Table 2 (83) (https://zenodo.org/record/5572710#.YWm24RpBxPZ).

hCG monotherapy induces testicular growth (35, 89-91) and spermatogenesis (35, 89, 90, 92), especially in patients with a postpubertal onset of CHH (basal TV > 4 mL) (89, 90), but combination therapy with FSH achieves notably better results in males starting off with prepubertal TV, with significantly more patients showing evidence of spermatogenesis and at greater sperm densities (35, 91, 93-96) and higher final TV (34, 35, 93, 95, 96). However, larger randomized controlled trials comparing the effects of hCG-alone vs hCG + FSH are required to better define these advantages.

Animal studies (97, 98) have suggested a limited time window in prepubertal subjects wherein FSH can optimally induce Sertoli and germ cell proliferation in immature testes, but that then begins to close with the onset of testosterone secretion by Leydig cells (41, 85). Therefore, several investigators trialed pretreatment with rhFSH before starting either GnRH therapy (85) or combined therapy with rhFSH/hCG (84, 99), with the rationale of inducing Sertoli cell proliferation before these cell numbers stabilized with the onset of androgen-induced cell maturation. Among CHH patients pretreated with rhFSH before starting combination therapy, almost all those who were able to provide semen samples had sperm cells in their ejaculate, which was an encouraging result considering their small initial TV (< 3 mL) (84). Subsequently, Dwyer et al. (85) reported that pretreatment with rhFSH before GnRH therapy led to a greater increase in TV, larger final TV, shorter time to spermatogenesis, greater numbers (100%) achieving spermatogenesis, and higher maximal sperm count, albeit all falling short of statistical significance. Nonetheless, they demonstrated an increase in the number of Sertoli cells and spermatogonia together with histological maturation of the testes and a doubling in TV during the initial phase of rhFSH monotherapy (85). Overall, these results suggest a possible beneficial effect of a rhFSH pretreatment, which indeed mimics the physiological pattern of gonadotropin activation in early puberty. CHH patients with history of cryptorchidism were excluded from these studies, but it is precisely these individuals with depleted Sertoli and germ cell mass who potentially stand to derive greatest advantage from an initial phase of FSH monotherapy. In all these studies, patients undergoing pretreatment with rhFSH necessarily delayed the beginning of hCG-induced virilization, which is physically and psychologically undesirable. However, another option would have been to deploy exogenous testosterone, of which relatively little would

diffuse into the interstitial fluid surrounding Sertoli cells. Overall, a simpler strategy might be to start combined FSH and hCG treatment contemporaneously; whether an initial treatment with hCG alone would be less effective than pretreating patients with FSH is not known.

Once spermatogenesis is induced with either GnRH or hCG/FSH treatment, it can be maintained with hCG alone for a variable amount of time (100, 101) (ie, once mature seminiferous tubules have arisen with combined gonadotropin treatment, Sertoli cells can continue to sustain spermatogenesis even with intermittent exposure to FSH). Following this rationale, Zhang et al. (91) recently compared the effects of continuous vs intermittent administration of uFSH in prepubertal CHH patients treated with combined therapy hCG/uFSH, reporting no significant differences between the 2 regimens in terms of either final TV, testosterone levels, virilization, timing of spermatogenesis, or sperm concentrations. Therefore, combination therapy with continual hCG and intermittent uFSH might represent a noninferior alternative regimen to induce virilization and spermatogenesis in prepubertal CHH patients at a considerably lower cost (91).

Finally, recent studies—including 1 randomized clinical trial—have reported that cryptorchidism (especially if bilateral), low initial TV, and a strong genetic background (all indicators of more severe GnRH deficiency since intrauterine life) represent the major negative predictors of response to gonadotropin treatment in terms of final TV and sperm concentration achieved (13, 34). Nevertheless, even among patients with these negative predictors, gonadotropin therapy can still be successful in achieving biological fatherhood (35, 84, 102).

In summary, despite the heterogeneity of the available data, some pillars of gonadotropin therapy can be distinguished. hCG promotes virilization, growth spurt, and sexual and psychosexual development, whereas the early addition of FSH grants a higher efficacy in terms of both TV increase and achievement of spermatogenesis and a much higher efficacy in achieving testicular descent (29). In this context, pretreatment with FSH for a few months may be the key to better maturation of testicular microarchitecture (85). Although no standard of care currently exists, some studies used fixed doses of FSH throughout (91, 96), and others allowed for increasing doses when initial TV increase or spermatogenesis were disappointing (36, 40). In pubertal induction, the hCG dose should start off low and then progressively increase during the course of treatment (34, 35, 40), according to regular testing of serum testosterone and estradiol and clinical assessment. However, patients already treated with testosterone might start treatment with higher initial doses compared with entirely prepubertal patients (34, 40).

Besides being able to induce pubertal development in hypogonadal boys with DP, gonadotropins can also be used to replace the so-called "mini-puberty" in male newborns with early signs of CHH: the term mini-puberty refers to the transient activation of the HPG axis that begins around 32 weeks of gestation and is sustained for the first 4 to 6 months of life in boys and the first 1 to 2 years in girls (29). This phenomenon contributes to the final stages of testicular descent into the scrotum and an increase in penile length; accordingly, the earliest neonatal signs of CHH are cryptorchidism and micropenis (103). The potential impact of mini-puberty on adult sexuality and fertility represents the strongest argument supporting gonadotropin replacement therapy in newborn boys with severe CHH (104). In this setting, early data are encouraging, albeit featuring small and heterogeneous samples of patients and different treatment regimens (105-110). In fact, neonatal mini-puberty replacement can have beneficial effects on testicular endocrine function and genital development, thus preventing psychological discomfort during adolescence. It also helps testes to descend to the scrotal position and/or to fix them there. Further prospective controlled trials are required to address the potential long-lasting advantages on adult reproduction health by assessing additional outcomes, such as sperm count after pubertal induction. Overall, we find it reasonable to offer this therapeutic option to newborn boys with severe CHH (micropenis and/or cryptorchidism), while explaining to their parents present uncertainties concerning long-term effectiveness and safety, as well as arranging to closely follow them up at the age of normal pubertal onset.

Patients' follow-up and life-long replacement therapy

During pubertal induction, careful clinical monitoring is required to evaluate genital maturation and virilization, growth rate, and potential side effects. Periodic reassessment of bone age may assist the physician in determining the adequacy of HRT in younger adolescents, but hold little clinical relevance in older patients who have already reached or are close to reaching final adult height. Patients' adherence and satisfaction with treatment results should also be taken into consideration (8).

The adverse events of hormone therapy are dose dependent, with the most concerning related to testosterone and hCG being erythrocytosis and premature epiphyseal closure, but also including aggressivity, mood swings, gynecomastia, and priapism when doses are greatly excessive. Older males may experience androgenic alopecia even at optimal doses. Local side effects of IM administration are pain and erythema at the injection site (1, 2). Despite the lack of strong evidence, we suggest monitoring hematocrit and serum testosterone levels to avoid overtreatment and

ensure a gradual rise of serum testosterone, ideally within the reference range for pubertal stage (111) for each laboratory. Moreover, because inhibin B and anti-Müllerian hormone are released from Sertoli cells in response to FSH, their levels both at baseline and after exogenous FSH administration might represent a useful marker to predict the spermatogenic response of the testicles to gonadotropin therapy (34). Measurement of BMD should also be considered at baseline in patients with risk factors for low BMD (112) and repeated once pubertal induction has been completed; in case it turns out to be low, it should be reassessed within 3 to 5 years.

TV should be periodically assessed; ultrasonography provides the most accurate measurement, whereas Prader orchidometer tends to overestimate TV, although it correlates well with ultrasonography and therefore can be a reliable surrogate in clinical practice (113). An increase of TV during testosterone therapy suggests that activation of the HPG axis has occurred. Because androgens have been suggested to have a positive priming effect on GnRH production and release (6, 33), testosterone treatment could lead to an activation of HPG axis in about 10% to 20% of patients affected with CHH, an event known as "reversal" (8). When reversal is clinically suspected (eg, through rising TV or gonadotropin levels, unexpected pregnancy), testosterone therapy should be withdrawn and levels of LH, FSH, and testosterone reassessed (2, 8). Moreover, a periodic reassessment of hormonal values off therapy should be considered, even if how often and up to what age remain open to question. However, because endogenous GnRH reactivation does not always persist indefinitely, these patients experiencing reversal need to be monitored clinically through a regular follow-up program in any case (8, 114).

With the exception of CHH men experiencing reversal, patients with DP resulting from hypogonadism continue to be treated with testosterone lifelong as for any hypogonadal man (115). Before switching to TRT, CHH patients previously treated with gonadotropins can perform a semen analysis to evaluate the response of spermatogenesis after treatment and to detect subjects at risk for inadequate sperm recovery later in adult life, giving them the possibility of sperm cryopreservation (despite the high costs) and a chance for future fertility (34). Otherwise, gonadotropin therapy can be simply reintroduced in later life when fertility is desired (2, 8).

Induction of Puberty in Females

In females, adequate maturation of secondary sex characteristics is achieved with estrogen alone, whereas the main role of progesterone is to prevent endometrial hyperplasia. Indeed, clinical experience suggests that premature treatment with a progestogen may be deleterious to both final breast and probably also final uterine maturation.

In girls with hypothalamic-pituitary disease, another treatment option is in principle represented by gonadotropins or pulsatile GnRH pump (in those with an intact pituitary gland). Although there are some anecdotal experiences with the use of the pulsatile GnRH pump for pubertal induction in females (116), there are no protocols for pubertal induction with gonadotropins. Stimulation with gonadotropins would be very complex because it would have to mimic the hormonal pattern characterized by an increasing amplitude of FSH and LH pulses, that in turn induce ovarian steroidogenesis, until it replicates the cyclical pattern that allows ovulation to occur in the late stages of puberty; such a strategy would also require close monitoring because of the risk of ovarian hyperstimulation. Unlike in males, the complexity and cost of this approach are not counterbalanced by any significant known advantage over the use of estrogen alone; therefore, GnRH and gonadotropins are deployed only in adulthood to induce ovulation when pregnancy is desired. Pulsatile GnRH treatment is an effective and appropriate method to achieve normal ovarian function, with the advantage of inducing monofollicular development, physiologic estrogen levels, and normal luteal phase function; however, it is not effective in women with pituitary damage, and most patients find it difficult to carry a pump continuously. Daily injections of gonadotropins are a better tolerated and more suitable treatment option for the induction of ovulation. In women with hypogonadotropic hypogonadism, differently from conventional treatment (FSH followed by hCG or LH to trigger ovulation), the additional administration of LH is required to stimulate local production of androgen substrates by theca cells, which facilitates sufficient secretion of estradiol by the dominant follicle. Protocols involve treatment with highly purified human menopausal gonadotropins or recombinant human gonadotropins (rhFSH and rhLH). This therapeutic option can be associated with increased risk of multiple follicular growth and ovarian hyperstimulation syndrome resulting from supraphysiologic follicular stimulus (117, 118).

In recent years, the discovery of novel hypothalamic neuropeptides and the description of their physiological mechanisms have enabled the development of pharmaceutical agonists and antagonists. In particular, the use of kisspeptin agonists has been evaluated in the treatment of pubertal delay associated with decreased LH secretion, such as hypothalamic amenorrhea and CHH resulting from mutations leading to loss of signalling of these neuropeptides (Kisspeptin and neurokinin B). Although further research is needed before kisspeptin analogs become incorporated

into clinical practice, these findings suggest a number of novel approaches to treat dysfunctions of the reproductive system (119, 120).

Estrogens are responsible for bone mass accrual and skeletal maturation, with a diphasic effect on long bone growth (inducing growth at low concentrations and epiphyseal closure at high concentrations), change in body composition, neurocognitive maturation and, of course, sexual development (121). Adequate uterine growth is also essential because poor development—which is unfortunately all too common in hypogonadal women who underwent pubertal induction—can adversely affect fertility and pregnancy outcomes.

Estrogens: types and routes of administration

Several types of compounds and routes of administration are available, with clinical differences in benefits and risks that have been extensively studied in postmenopausal and adult women; therefore, applying these studies to adolescents represents an extrapolation. Because adolescents with hypogonadism will need estrogen therapy for approximately 4 decades, the choice of medication is critical. However, as there are no licensed hormone preparations for pubertal induction, the off-label use of hormone formulations licensed for adult women (and in practice formulated for the suppression of postmenopausal vasomotor symptoms) is unavoidable.

Historically, some clinicians prescribed ethinylestradiol (EE—a synthetic estrogen) or conjugate equine estrogen (CEE—a xenoestrogen) to induce puberty in girls, but there has recently been a sensible reversion to physiological human 17β-estradiol (17β-E_2), whether transdermal or oral (32). Indeed, emerging data demonstrate greater safety and efficacy associated with 17β-E_2 use compared with EE or CEE (122-126), and many authors consequently discourage using anything but native 17β-E_2 (71, 127-129). The transdermal route bypasses the hepatic first-pass effect, whereas oral estrogens are metabolized by the liver before reaching the systemic circulation. Therefore, to achieve adequate systemic concentrations with oral estradiol, the liver is exposed to supraphysiologic levels, resulting in an increase in procoagulation factors, SHBG, and other binding proteins, triglycerides, and inflammation markers (130). This may explain why oral, but not transdermal estrogen delivery, is associated with a greater thromboembolic risk (131-138) and can have deleterious effects on body composition and lipid oxidation (139), glucose metabolism (140), and potentially induce peripheral resistance to GH in the liver by suppressing IGF-1 secretion/production (141, 142). However, an alternative explanation is that most of these studies compared transdermal 17β-E_2 with oral CEE or EE, so the key benefit may in fact reside in the choice of compound (17β-E_2) rather than with the transdermal route, something that had previously emerged in the transgender literature for induction and maintenance of feminization (143).

In young girls with hypogonadism, the clinical outcomes of pubertal induction with different formulations of estrogen were collected from isolated experiences, small underpowered observational studies, and small clinical trials. Most of these studies were conducted on girls with Turner syndrome (TS), a population with clinical peculiarities in terms of treatment goals and risk factors. Therefore, it cannot be assumed that the evidence on the outcomes of estrogen treatment is necessarily comparable for all DP-associated conditions.

Most studies (144-147) failed to demonstrate that different routes of administration result in different biochemical profiles (markers of inflammation, lipid metabolism, IGF1 and growth, insulin resistance, liver enzymes, and renin) as markers of metabolic outcomes, probably from the previously mentioned limitations. On the other hand, treatment with E_2 (144), especially in the transdermal form (145), resulted in higher estradiol levels and more effective feminization at the selected doses administered. These findings also suggest that dose equivalences based on clinical practice or extrapolation from published equivalences in adults (127, 148) may be incorrect. Therefore, measuring estrogen levels should now be considered core to both dosimetry and ascertainment of patient adherence. This is routinely feasible during treatment with 17β-E_2, albeit with certain limitations (see the following section), but cannot be done reliably for CEE and not at all for EE. Besides, even if the route of delivery of 17β-E_2 does not differentially affect body composition and metabolic parameters when E_2 concentrations are titrated to the normal range, total estrogen exposure (E_1, E_1S level, and total bioestrogen) is significantly higher with oral 17β-E_2, meaning that transdermal 17β-E_2 (TDE) achieves a more physiological estrogen milieu (147, 149). Moreover, Torres-Santiago et al. (147) show a tendency to lower IGF-1 and significantly higher levels of SHBG in the oral group as evidence of a stronger hepatic effect of oral estrogens. A follow-on study confirmed this finding and illustrated that common feminizing doses of oral 17β-E_2 result in substantial accumulation of unphysiological genotoxic metabolites compared with transdermal estradiol, although further studies are needed to determine whether any of this is clinically relevant (150). Finally, a more favorable impact of transdermal estrogens on some surrogate markers of cardiovascular risk (including fasting glucose, total cholesterol, and triglyceride concentrations) and BMD compared with oral estrogens was found in a systematic review and meta-analysis (151).

The main formulations available are reported in Table 4, with some mention of their pharmacologic characteristics (130). Transdermal estradiol is usually delivered in patch form in pubertal induction, which makes it easier to

Table 4. Estrogens: types, routes of administration, and characteristics

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
17β-E_2	Oral tablets: 1 and 2 mg (0.5 mg available in some countries) Equivalent dose: 2-4 mg daily for a young hypogonadal adult woman	Bioidentical E_2, or esterified as hemihydrate or valerate It undergoes hepatic first-pass effect, after which its systemic bioavailability is about 5% Its levels rise rapidly, maintain a plateau for up to 12 h, and decrease slowly afterwards, reaching steady state within several days of administration E2 is metabolized to E_1 and E_1S that serve as a hormonally inert reservoir from which E_2 is continuously delivered after reconversion	Greater safety compared with EE (lower risk of thrombosis and hypertension) and CEE (lower risk of thrombosis) Greater efficacy compared with EE or CEE Levels are measurable for treatment monitoring by either immunoassay or LC-MS Well accepted by patients	The low doses required for the early phase of pubertal induction can only be achieved with careful use of a tablet cutter device and/or by resorting to alternate day dosing It undergoes first-pass effect
	Transdermal matrix patches: 25, 37.5, 50, 75, or 100 µg, typically changed twice weekly Equivalent dose: 100-200 mg for a young hypogonadal adult woman	Bioidentical E_2 It is absorbed into skin capillaries and continuously delivered into the bloodstream (depot effect in skin and subcutaneous fat) Estradiol plasma levels remain constant for the duration of patch life No accumulation of estrogens metabolites or conjugates in blood	Patches can be cut to administer precise fractional doses Greater safety than EE (lower risk of hypertension) and oral E_2 (lower risk of thrombosis) Levels are measurable for treatment monitoring by either immunoassay or LC-MS Better absorption and more physiological estrogen milieu	It may not be accepted by patients (visible patch, worn constantly). Poor adhesion of patches Skin reactions
	E2 hemihydrate sachets, or E2 0.06% multidose dispenser Equivalent dose: 1–2 g daily for a young hypogonadal adult woman		It bypasses first-pass effect; therefore, it has a neutral effect on lipids, coagulation profile, or other markers of hepatic metabolism Possible overall benefit over oral estrogens for long-term ERT	No published data for pubertal induction, but extensive clinical experience for inducing feminization of older transgender teenagers
EE	Oral tablets: 10, 20, or 30 µg Equivalent dose: 20-30 µg for a young hypogonadal adult woman	Synthetic E_2 analogue It binds estrogen receptors with high affinity (particularly in the central nervous system) 17α-ethinyl substitution prevents its inactivation in the liver, but it activates renin It is more stable and active in the bloodstream compared with E_2 It undergoes a more pronounced hepatic effect After administration, serum concentrations have both a rapid rise (1-3 h) and decline Oral bioavailability is 38%-48%	Wide clinical experience Well accepted by patients	Unphysiological type of estrogen Its first-pass effect leads to a more pronounced impact on estrogen-dependent hepatic serum parameters compared with E_2 The low doses required for the early phase of pubertal induction can only be achieved with careful use of a tablet cutter device and/or by resorting to alternate day dosing It is no longer easily available Possible suboptimal outcomes (uterine maturation) It is not measurable for treatment monitoring It is less safe than other options (risk of renin-induced hypertension and venous thrombosis)

Table 4. Continued

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
CEE	Oral tablets: 0.625 and 1.25 mg (0.3 and 0.9 mg available in some countries) Equivalent dose: 1.25-2.5 mg for a young hypogonadal adult woman	Extracted and purified from pregnant mares' urine and containing a large number of different estrogenic compounds of different potency Variable effects depending on the target tissue More pronounced effect than 17β-E_2 on the production of hepatic proteins, with higher ratio between hepatic and clinical effect	Wide clinical experience Well accepted by patients Levels are measurable for treatment monitoring by immunoassay, but not LC-MS	Unphysiological type of estrogen It is associated with wide variations in the biologic effects The low doses required for the early phase of pubertal induction can only be achieved with careful use of a tablet cutter device and/or by resorting to alternate day dosing First-pass effect is more pronounced than for oral E_2 (higher ratio between hepatic and clinical effects) Risk of venous thrombosis

CEE, conjugated equine estrogen; E_1, estrone; E_1S, estrone sulfate; E_2, estradiol; EE, ethinylestradiol; ERT, estrogen replacement therapy; LC-MS. liquid chromatography mass spectrometry.

administer specific doses of 17β-E_2 by cutting up a matrix patch (reservoir patches cannot be cut) to facilitate dose adjustment in the absence of low-dose products specifically designed for this purpose. Transdermal 17β-E_2 gel is not currently used for pubertal induction, both because of the difficulty of administering low doses with currently available formulations and because only one fairly dated paper proposing a gel induction scheme has been published (152). No data on dose equivalence between estradiol patches and gel are available in younger patients, although some authors have now begun to propose gel as an alternative in pubertal induction (153). As for other formulations of estradiol (injected estradiol valerate), there is very limited clinical experience (and even more limited published data), whereas vaginal rings are inappropriate for prepubertal/early pubertal girls.

In conclusion, transdermal 17β-E_2 should be considered as the preferred choice, as suggested by most authors (154). However, patients' preferences for oral estrogen should also be taken into account. The use of EE and CEE should nevertheless be avoided.

Progesterone: types and routes of administration

In addition to estrogen replacement therapy (ERT), progestogen is required to induce menstrual cycles, to prevent endometrial hyperplasia, and to minimize irregular bleeding. However, no data are currently available on either its use in young girls with hypogonadism or its metabolic effects, whereas several studies evaluated the effects of progestin therapy in adults (130, 155-161). Each progestin exerts a certain impact depending on their affinity for progesterone, glucocorticoid, mineralocorticoid, and androgen receptors, resulting in different properties that can translate to very different clinical effects (162).

Options for treatment mainly include micronized progesterone (which is bioidentical to endogenous progesterone), oral medroxyprogesterone acetate (MPA), norethisterone acetate, and dydrogesterone. MPA is the conventional progestin widely used in the past; however, micronized progesterone has demonstrated increased safety in several studies and clinical trials compared with MPA, concerning breast cancer risk, metabolic impact, and thromboembolic events, and gives good cycle control without significant side effects (157, 158). Norethisterone acetate is the most androgenic, and therefore, possibly the least suited progestogen for younger girls, whereas dydrogesterone is available in many countries only as part of combined HRT preparations. Therefore, micronized progesterone has been recommended in most of the recent expert consensus documents (8, 128, 154). The principal characteristics of commonly used progestogens are discussed in Table 5.

Progestogens are usually given for 12 to 14 consecutive days every month at the lowest dose that achieves complete

Table 5. Progestins: types, routes of administration, and characteristics

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
MPA	Oral tablets: 2.5, 5, or 10 mg Equivalent dose for a young hypogonadal adult woman: 7.5-12.5 mg daily (days 1-12/14 of calendar month) Or 2.5-5 mg daily (continuous)	Synthetic progestogen It has reduced inactivation rate and increased hormonal potency compared with native form After oral administration, its bioavailability is high It binds with high affinity the progesterone receptor, antagonizing the estrogen-induced endometrial proliferation Considerable glucocorticoid effects, which causes an upregulation of the thrombin receptor and stimulates the procoagulant activity It has weak androgenic properties	Wide experience Great endometrial safety	Its moderate androgen effects can affect patients both clinically and on lipidic and glucose metabolism Upregulation of thrombin receptor (prothrombotic effect) It may lead to weight gain and fluid retention
Micronized progesterone	Oral capsules; 100 or 200 mg. Equivalent dose for a young hypogonadal adult woman: 200-300 mg daily (days 1-12/14 of calendar month) Or 100-200 mg daily (continuous)	Bioidentical progesterone Progestogenic and antiestrogenic activities on the endometrium and cervix, anti-mineralocorticoid effect and "non-receptor"-mediated antiandrogenic effect It undergoes extensive metabolism in the gastrointestinal tract and liver, which leads to both low bioavailability and many circulating metabolites, some of which exert hormonal activities (eg, pregnanolone sedative effects via GABA-A receptor) Its micronized formulation, suspended in oil and packaged in a gelatin capsule, increases bioavailability Recommended doses (sequentially for 12-14 d/mo at 200 mg/d) proved to be effective in endometrial protection, despite low circulating levels of progesterone (as measured by reliable methods like LC-MS)	High selectivity and lack of glucocorticoid activity Substantial safety on breast, possibly with a more favorable effect on breast tissue than synthetic progestogens such as MPA Neutral in respect of cardiovascular risk and venous thromboembolism Neutral on body composition, blood pressure, glucose and lipid metabolism, and markers of endothelial function	Drowsiness and dizziness Worse cycle control than synthetic progestogens More frequent breakthrough bleeding than synthetic progestogens when used continuously Some concerns about less effective protection for uterine hyperplasia and cancer compared to synthetic progestogens such as MPA
Norethisterone acetate	Oral tablets: 0.5, 1 mg, 5 mg (lower doses only available as part of combined HRT) Equivalent doses for a young hypogonadal adult woman 1-2.5 mg daily (days 1-12 of calendar month or pack cycle) Or 0.5-1 mg daily (continuously). Transdermal patches: 170 µg, changed twice weekly Only available as part of combined HRT	Synthetic progestogen Highest potency on endometrium and cervix Highest androgenic activity Oral bioavailability approximately 64% Extensively metabolized primarily in the liver Steady-state concentrations achieved within 24 h	Neutral on venous thromboembolism and blood pressure Minimal glucocorticoid activity	Being the most androgenic progestogen, it affects patients both clinically and on lipids and glucose metabolism Highest risk of hepatotoxicity Poor adhesion Skin reactions

Table 5. Continued

Drug	Formulations	Characteristics and pharmacokinetics	Advantages	Disadvantages
Dydrogesterone	Oral tablets: 2.5, 5, or 10 mg. Currently only available just as part of combined HRT Equivalent dose for a young hypogonadal adult woman: 10 mg daily (days 1-14 of calendar month or pack cycle) Or 5 mg daily taken continuously	Synthetic progestogen Highly selective progestogenic and antiestrogenic activities on the endometrium and cervix Weak anti-mineralocorticoid properties No androgenic effects Rapidly absorbed, it has a bioavailability of 28% Completely metabolized to 20-dihydrodydrogesterone, resulting in increased half-life (14-17 h)	High selectivity and lack of glucocorticoid/androgenic activity Neutral in respect of cardiovascular risk and venous thromboembolism Neutral on body composition, blood pressure, glucose and lipid metabolism, and markers of endothelial function	Headaches Not available as a separate tablet

Abbreviations: HRT, hormonal replacement therapy; LC-MS, liquid chromatography mass spectrometry; MPA, medroxyprogesterone acetate.

shedding of the endometrium, but the frequency of withdrawal bleeding may be adjusted according to the patient's wishes, as long as progestogen is administered at least every 2 to 3 months to avoid endometrial hypertrophy. Continuous administration of progestins for those who do not desire to have menstrual bleeding can be also considered. In practice, many postmenopausal women choose to insert capsules of micronized progesterone vaginally rather than taking them orally, to mitigate gastrointestinal side effects, but the product licence does not reflect this.

Proposed therapeutic schemes

Review of the literature highlights several relevant studies over the past 2 decades (144, 145, 152, 163-166), presenting schemes of ERT for pubertal induction, difficult to compare because of different types and routes of administration, populations studied, and age at ERT start.

Overall, a progressive increase in the dose is suggested, both to recapitulate normal puberty as far as possible and because excessive doses might lead to premature epiphyseal fusion and a reduction of adult height, impaired bone mineralization, and poor uterine and breast maturation (prominent and underdeveloped breasts).

Several years ago, some authors (127, 163, 167) proposed to start induction with a low dose of transdermal 17β-E_2, corresponding to about 0.1 µg TDE/kg, to be applied only at night, and then to be carefully increased before mid-pubertal levels are reached, to mimic the spontaneous estrogenic levels in the early pubertal range (peak value between 10 and 40 pmol/L) as well as the diurnal pattern of serum 17β-E_2. The rationale of this cautious approach is that maintaining these low levels of 17β-E_2 probably not only promotes breast maturation, but also increases growth velocity (as it is well known that peak height velocity in girls is observed in early puberty). The induction regimen consisting in low growth-promoting TDE doses for 18 to 24 months (starting from 0.1 µg/kg to doubling every 6 months) based on body weight with initial overnight ERT, and adjusting the patch size to achieve target serum estradiol levels, as proposed by Davenport (127), is still one of the most widely used regimens to date and has been advocated by several authors in recent publications (2, 8, 154). However, no clinical study has yet demonstrated any actual superiority of the initial overnight treatment. In addition, early studies in TS were primarily focused on maximizing adult height, with the opportunity to initiate age-appropriate estrogen replacement all too frequently overlooked. Therefore, although a very slow approach may allow patients to achieve a greater stature through a more delayed epiphyseal fusion, it cannot be assumed that the clinical concerns and outcomes of estrogen treatment are identical for all conditions associated with

Table 6. Clinical approach in pubertal induction

Regimen proposed	Follow-up			
	Clinical examination		**Laboratory and instrumental tests**	
Male				
Testosterone	· Genital development, TV (less useful in stated hypergonadotropic hypogonadism [eg, Klinefelter]), Prader orchidometer, and virilization	Every 4-6 mo (or at any change of dose)	· Serum testosterone and hematocrit	Every 4-6 mo (or at any change of dose)
Testosterone esters:			· AMH and Inhibin B	Baseline and after gonadotropin stimulation in HH
initial dose of testosterone enanthate 50 mg IM/4 weeks, to escalate every ~6 mo up to adult dose (~250 mg IM/2-4 wk) in 24-36 mo (even 18 mo in older patients)			· Testicular US	When indicated by physical examination
Testosterone gel:	· Height, weight, and growth rate			
initial dose 10 mg (e.g. testosterone gel 2% 1 puff) every second day to escalate every ≈6 months up to adult dose (≈40-60 mg daily) in 24-36 months (even 18 months in older patients)	· Blood pressure		· Wrist RX for bone age	Annually up to final height achievement
Gonadotropins (for HH patients)	· Compliance and satisfaction		· DXA for BMD	At baseline (selected cases) and at least 2 y after treatment initiation
hCG:	· Side effects appearance			
Initial dose 250 IU SC twice weekly to escalate every ~6 mo up to adult dose (~1500 IU 3 times weekly) in 24-36 mo (even 18 mo in older patients)			· Semen analysis	At conclusion in HH patients treated with gonadotropins
+				
FSH:				
75-150 IU SC 3 times weekly (consider pretreatment before hCG for 3-6 mo)				
Female				
Transdermal 17β-E_2:	· Breast development and femininization	Every 4-6 mo (or at any change of dose)	· Serum estradiol	Every 4-6 mo (or at any change of dose)
initial dose ⅛-¼ 25 µg patch (based on weight) only at nighttime for the first 6 mo, then escalate every ~6 mo up to adult dose (50-100 µg patch) in 24-36 mo (even 18 mo in older patients)	· Height, weight, and growth rate		· Uterine US	After the completion of induction or periodically
Oral 17β-E_2:	· Blood pressure			to evaluate the endometrial thickness
initial dose 0.5 mg every second day to escalate every ~6 mo up to adult dose (2 mg daily) in 24-36 mo (even 18 mo in older patients)	· Compliance and satisfaction		· Wrist RX for bone age	Annually up to final height achievement
+	· Side effects appearance		· DXA for BMD	At baseline (selected cases) and at least 2 y after treatment initiation
Progestin:				
Start 200-300 mg micronized oral progesterone for 12-14 consecutive days/mo at reaching of adult dose either after about 2 y of unopposed estrogen or earlier if breakthrough bleeding occurs				

Abbreviations: 17β-E_2: 17β-estradiol; AMH, anti-Müllerian hormone; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; RX, radiography; TV, testicular volume; US, ultrasound.

DP. One recent study (164) investigated faster protocols of induction in TS (first 2 months 12.5 µg/24 hours, thereafter 25.0 µg/24 hours until breakthrough bleeding), devised especially for girls with delayed diagnosis and/or initiation of estrogen treatment, considering that in such conditions a 2- to 3-year model for induction of puberty may not be optimal: the authors found a satisfactory rate of progression through pubertal stages, no influence on growth potential, and a satisfactory increase in uterine volume that did not correlate with the duration of treatment or the dose of estradiol per kilogram of the initial body weight. Two other, different protocols were adopted in clinical studies comparing TDE with oral ERT (144, 145), with an initial dose of TDE of 12.5 µg and 25 µg, respectively, showing that treatment with TDE resulted in higher estradiol levels, more effective feminization (144), faster bone accrual at the spine, and increased uterine growth compared with CEE regimens (145). Last, a more simplified regimen (168) proposed to start with one-quarter of a 25-µg patch, slowly increasing the dosage up to adult dose over 2 to 2.5 years (when progestogen is added), whereas other authors suggest starting with one-quarter of a 25-µg patch applied mid-week (128) to reduce the initial doses while avoiding having to cut the patch into small fractions.

Concerning oral 17β-E_2, a well-established regimen proposed by Delemarre (52) suggests starting from 5 µg/kg of oral 17β-E_2 per day, doubling every 6 months until an adult dose of 2 mg per day in 2 years. However, a therapeutic regimen based on body weight is laborious and complex to deliver, especially for oral delivery. In this regard, a study by Labarta et al. (166) shows that a simplified incremental fixed dose can provide satisfactory pubertal development not inferior to individualized dose depending on weight and, therefore, it might be preferable because it is simpler. Indeed, a regimen proposed most recently by Zacharin et al. (168) aims at an easier administration, starting at 0.5 mg every second day for about 3 months, increasing to 0.5 mg daily for 6 to 9 months, then 1 mg/d for about 12 months, and finally increasing to an adult dose of 2 mg/d.

The main studies that evaluated the effects of different estrogen formulations and regimens for pubertal induction are listed in Supplementary Table 3 (83) (https://zenodo.org/record/5572710#.YWm24RpBxPZ).

On the whole, despite the different regimens used, the dynamics of breast maturation is quite uniform in the cited studies, with the achievement of stage B2 during the first 6 months and B4 after approximately 2 years, which is comparable to spontaneous puberty (154, 164). Data on uterine maturation are less reassuring: a recent study (169) assessing uterine parameters in hypogonadal women who had undergone pubertal induction found that uterine growth is often compromised regardless of diagnosis, despite standard estrogen therapy. This observation could be due to inadequate estrogen replacement doses, as suggested by an association between serum estradiol levels and uterine dimensions. However, neither the type of ERT nor its dose were linked to uterine size parameters. Moreover, no significant correlation between the age of initiation of therapy or menarcheal age and uterine size was found. It has also been reported that treatment with nonphysiological estro-progestin regimes gives worse results in terms of uterine development (170).

Considering the wide inhomogeneities of type and dose of therapy, age of initiation, and population treated, carefully designed studies are required to confirm these data and to indicate the most effective induction scheme to ensure optimal uterine development. It has been hypothesized that there might be a "critical window" for uterine development during adolescence, outside of which reduced size cannot subsequently be recovered. Although this is not clearly substantiated, the existence of precise timing in uterine maturation might be suggested by the evidence that only during pubertal development is there a significant correlation between uterine volume and estradiol concentration and the changes in uterine size are more striking at Tanner stage B3 and B4 (171-173).

In summary, treatment should be initiated with a starting dose of approximately 10% of the adult replacement dose and increased (by increments of 1.5- to 2-fold) every 6 months over a 2- to 3-year period (or less in older adolescents or young women). After at least 2 years of unopposed estrogen, or if more than 1 episode of significant breakthrough bleeding occurs, it is necessary to consider a progestin to induce withdrawal bleeding, but only if adult breast and uterine conformation has been achieved. We recommend that, if symptoms of endometrial hyperplasia develop when breasts or the uterus are not yet fully developed, then a slight reduction in 17β-E_2 dose should be considered instead of introducing the progestin, although we acknowledge the lack of direct evidence. However, in recognition that physiologically progesterone levels only rise substantially in the late stages of puberty (174), when ovulatory cycling becomes effective, and that it plays a role in both the breast and the uterus (175), it seems to us that following this approach that aims to recapitulate normal physiology represents the path of lowest risk.

It should be highlighted that the approach must be individualized, depending on the specific characteristic, circumstances, and desires of patients. When hypogonadism is diagnosed late, which is very common especially in patients with CHH, or it develops after spontaneous pubertal start, estrogen dosing regimens can progress more rapidly, especially for those in whom adult height is not a concern.

Patients' follow-up and life-long replacement therapy

For both transdermal and oral 17β-E_2 induction regimens, we suggest checking estradiol levels after starting treatment and after dose changes, ideally with an ultrasensitive assay (although, unfortunately, it is not widely available). To date, there are no general recommendations regarding the timing of blood sampling in relation to 17β-E_2 administration and measurements of its levels can be influenced by absorption, metabolism, and user-related discrepancies. Despite these limitations, monitoring blood concentrations of estradiol is a useful complementary tool to clinical objectivity for assessing compliance and guiding treatment choices in titration. Even if the treating physician has no access to a laboratory with a sensitive method for E_2 measurements, some authors suggest that a value above 40 pmol/L should be considered as a marker of an excessive starting dose in a 12 to 14 year old (163). Ideally, serum estradiol levels should progressively increase with each dose change and remain <180 pmol/L until full dose is reached (eg, 50 µg of TDE) to accelerate linear growth without rapidly advancing bone maturation (127). For girls completing puberty, an adult target 17β-E_2 concentration around 350 pmol/L should be aimed for during therapy (149), similar to what is sensibly recommended in transgender women (143).

Pelvic ultrasound should be performed during pubertal induction and upon completion to document the uterine size and shape and to evaluate the endometrial thickness and hence the optimal timing for progestin introduction. For uterine length, a cutoff of 65 mm is commonly used for maturity, based on the normative data by Griffin et al. (176). It is important to appreciate uterine size in its entirety rather than relying on a single measurement because this may provide false reassurance in terms of adequate development; however, there is variation in the literature as to the criteria comprising uterine maturity. In fact, during pubertal development the uterus also changes from tubular to "pear" shaped and the ratio of the corpus to the cervix changes from being approximately 1:1 before puberty, to between 2:1 and 3:1 after puberty. A normative age-matched model of uterine volume was reported by Kelsey et al., with a predicted postpubertal uterine volume of 25.8 cm^3 (68% prediction limit, 25.8-77.8 cm^3) (173), whereas Burt et al. provided references based on a eugonadal control population for 3-plane diameters (total uterine length, anteroposterior, transverse), volume and fundal-cervical ratio (169).

Screening for thromboembolic risk should be performed only in girls with a personal or family history, considering that an increased risk does not preclude estrogen therapy, but requires a careful monitoring of hematological parameters (154). However, even if no other routine screening or monitoring tests are required, they may be prompted by specific symptoms or concerns (177). As for BMD monitoring, the same approach discussed about male patients can be followed.

ERT must be maintained until at least the average age of physiological menopause (51-52 years of age), but in practice many hypogonadal women will have received inadequate treatment in earlier life or had prolonged breaks in therapy, and so should be encouraged to continue longer. Progestogens, which can be added either cyclically or continuously, are available either as single agents or in combined formulation with estrogen. European consensus guidelines for CHH do not recommend the combined oral contraceptive pill (COCP) (8) even though these continue to be prescribed through force of habit. Emerging data show that COCP is less favorable in BMD improvement, uterine parameters, and long-term cardiometabolic health compared with a physiological estrogen replacement, particularly in older females. Nevertheless, it is clearly better to prescribe COCP than nothing at all, although packs should generally be taken back-to-back to avoid the risk of reexposure to hypogonadism for 1 week in 4 (124, 129, 178, 179).

Conclusions

Pubertal induction needs to be performed within a physiological timeframe to guarantee harmonious growth and sexual and psychological development. Hitherto, mainly studies with small case series or isolated experiences have been conducted for pubertal induction, often recruiting patients with different etiologies of hypogonadism or within broad age ranges at the start of treatment. It is hopeful to have carefully designed studies performed among specific and uniform populations to establish which treatment protocol is the most effective in each clinical condition, considering the heterogeneity of the populations to treat (180). Furthermore, as some treatments are still off-label, despite being already validated in clinical practice (such as gonadotropin treatment), multicenter randomized controlled trials would allow to officially extend the indication of these drugs to pubertal induction in young patients. These will need to be funded by national agencies, given that sex hormones are by and large low-cost drugs for which the costs of applying for a variation in product licence are excessive compared with the additional earnings that would accrue to the relevant pharmaceutical company.

Hitherto, no standard of care exists for pubertal induction; however, some treatment strategies have consolidated in clinical practice. Useful tips for clinical practice in terms of both treatment strategies and clinical, laboratory, and instrumental assessments during follow-up are listed in Table 6.

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