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PERSPECTIVE

Considerations when treating male pubertal delay pharmacologically

Rodolfo A. Rey [a,b]

[a]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE), CONICET – FEI – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina; [b]Facultad de Medicina, Departamento de Histología, Embriología, Biología Celular y Genética, Universidad de Buenos Aires, Buenos Aires, Argentina

ABSTRACT

Introduction: Delayed puberty , usually affects psychosocial well-being. Patients and their parents show concern about genital development and stature. The condition is transient in most of the patients; nonetheless, the opportunity should not be missed to diagnose an underlying illness.

Areas covered: The etiologies of pubertal delay in males and their specific pharmacological therapies are discussed in this review.

Expert opinion: High-quality evidence addressing the best pharmacological therapy approach for each etiology of delayed puberty in males is scarce, and most of the current practice is based on small case series or unpublished experience. Male teenagers seeking attention for pubertal delay most probably benefit from medical treatment to avoid psychosocial distress. While watchful waiting is appropriate in 12- to 14-year-old boys when constitutional delay of growth and puberty (CGDP) is suspected, hormone replacement should not be delayed beyond the age of 14 years . When hypogonadism is diagnosed, hormone replacement should be proposed by the age of 12 years . Testosterone replacement has been used for decades and is fairly standardized. Aromatase inhibitors have arisen as an interesting alternative . Gonadotrophin therapy seems more physiological in patients with central hypogonadism, but its efficacy and timing still need to be established.

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1. Introduction

Puberty is the most spectacularly changing period of postnatal development, characterized by the development of secondary sexual characteristics and the attainment of adult height and physiological features, including fertility [1–3]. Childhood shows a relatively constant height velocity, mainly regulated by the growth hormone (GH)–insulin-like growth factors (IGFs) axis. The reproductive axis shows a dissociated quiescence, characterized in males by a reduced activity of the gonadotrophin-releasing hormone (GnRH)–luteinizing hormone (LH)–Leydig cell axis responsible for androgen secretion and a relatively more active follicle-stimulating hormone (FSH)–Sertoli cell axis responsible for anti-Müllerian hormone (AMH) and inhibin B production (Figure 1).

Pubertal onset is characterized by the reactivation of the GnRH-secreting neurons [4]. Consequently, FSH and LH levels increase progressively. FSH impacts on the hitherto immature testicular Sertoli cells by inducing cell proliferation that results in an initial increase in testicular volume. LH promotes Leydig cell androgen secretion, thus increasing intratesticular testosterone concentration, which induces the maturation of Sertoli cells that cease to proliferate. Mature Sertoli cells dramatically decrease their AMH production, increase inhibin B secretion and become capable of supporting adult spermatogenesis; the latter process is responsible for the notorious enlargement of the testes [5,6]. Concomitantly, Leydig cell testosterone secretion continues to increase, resulting in an elevation in circulating levels that drive the development of secondary sexual characteristics and the pubertal growth spurt and bone mass accrual, either directly or after androgen conversion to estrogens by the enzyme aromatase [7,8].

From a clinical standpoint, the first sign of male puberty is testicular enlargement attaining a volume of 4 ml as measured by comparison to Prader's orchidometer, or 2.7 ml when measured by ultrasonography [9]. The clinical changes occurring during puberty, described in detail by Marshall and Tanner in 1970 [10], continue to be the milestones used in everyday practice for assessing pubertal disorders. Genital stage 1 (G1) reflects prepubertal features; stage G2 indicates the clinical onset of puberty, with 4-ml testes as its landmark; serum testosterone levels become detectable usually during stage G3 [11]; peak growth velocity occurs during stage G4, and stage G5 is the adult stage. Pubertal onset occurs in 95% of boys between the ages of 9.5 and 13.5 years [10,12–14]; therefore, consensus exists on defining pubertal delay in males when testicular volume has not attained 4 ml by the age of 14 years, i.e. 2 to 2.5 standard deviations beyond the mean for the population [1,10,15,16]. Once triggered, puberty progresses at approximately one Tanner stage per year [2,10,12–14]; failure to achieve completion of pubertal development, i.e. testicular volume <15 ml and genital stage <G5, within 5 years prompts the diagnosis of arrested puberty.

CONTACT Rodolfo A. Rey rodolforey@cedie.org.ar Centro de Investigaciones Endocrinológicas 'Dr. César Bergadá' (CEDIE), CONICET – FEI – División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Gallo 1330, Buenos Aires C1425EFD, Argentina

Article highlights

- Scarce high-quality evidence exists about the best pharmacological therapy approach for delayed puberty in males.
- Constitutional delay of puberty, an extreme of normal pubertal onset, is the most frequent cause of pubertal delay. Nevertheless, male teenagers seeking attention for pubertal delay most probably benefit from medical treatment to avoid psychosocial distress.
- In any case, testosterone replacement should be indicated by the age of 14 years in order to maximise height potential and peak bone mass.
- When primary or central hypogonadism is diagnosed, hormone replacement should be proposed by the age of 12 years, after functional hypogonadism has been ruled out.
- Testosterone replacement regimens have been used for decades and are fairly standardised.
- Aromatase inhibitors are an alternative for patients with constitutional delay of puberty and short stature. However, evidence based on robust clinical trials is lacking.
- In patients with central hypogonadism, gonadotrophin therapy would be the aetiological therapy, but its relative efficacy and most appropriate timing require clinical trials with long follow-up.

The prevalence of pubertal delay in males has not been exhaustively studied. Based on the above-mentioned definition of delayed puberty, which is statistical, approximately 2.5% of male adolescents should experience a delayed puberty. Nevertheless, the actual prevalence is likely higher because transient forms may remain undiagnosed [17]. Late pubertal onset usually affects psychosocial well-being in male teenagers. Patients and their parents are often concerned about stature and genital development. Even though the condition is benign and transient in approximately 2/3 of the cases, it represents an important opportunity to diagnose an underlying illness [16].

Another issue that needs to be considered in the physiology of pubertal growth is that sex steroids exert an important influence on pituitary growth hormone (GH) secretion and its action in target organs. Indeed, estrogens – and testosterone through its aromatization – upregulate central GH secretion resulting in higher serum levels of insulin-like growth factor 1 (IGF1), mainly produced by the liver, and the main responsible for linear growth in postnatal life [18]. Interestingly, dihydrotestosterone (DHT), a more portent androgen that is not aromatisable, is unable to induce GH secretion [19,20]. Additionally, testosterone elicits a peripheral action by amplifying GH-mediated IGF1 secretion [20].

2. Etiologies of pubertal delay in males

Strictly, underlying the concept of delayed puberty in males is the assumption that puberty will begin spontaneously before the age of 18 years [15–17]. It comes out clearly that the diagnosis of pubertal

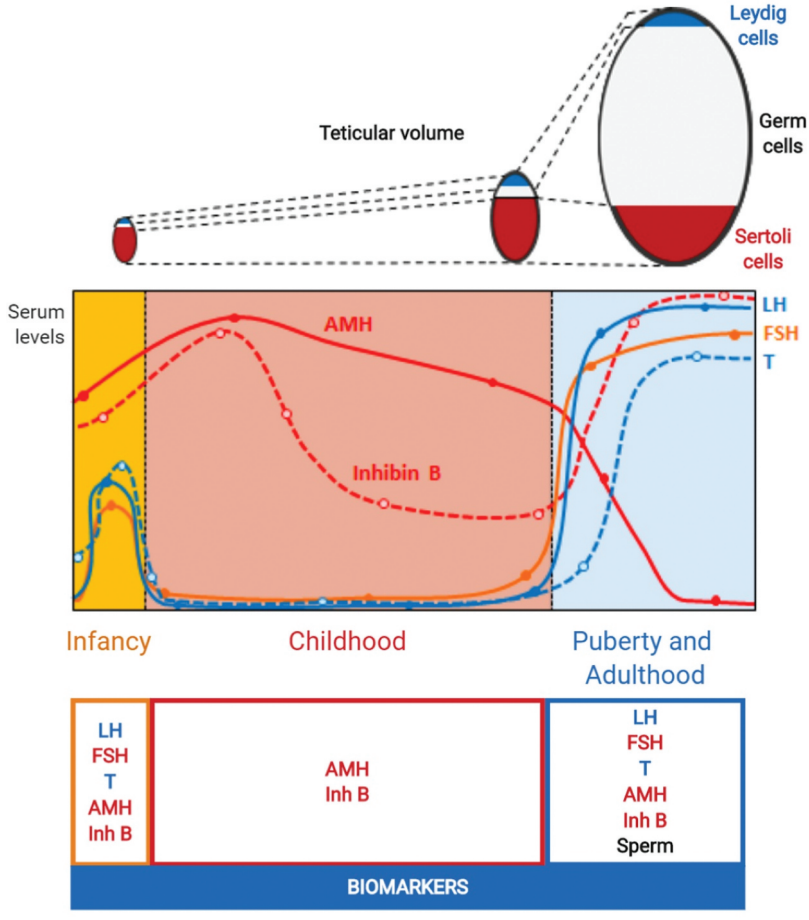

Figure 1. Ontogeny of reproductive serum hormone levels and testicular volume during postnatal life in the male. Reproduced with permission from: Grinspon RP, Freire AV, Rey RA. Hypogonadism in Pediatric Health: Adult Medicine Concepts Fail. Trends Endocrinol Metab 2019;30(12):879–890. © 2019 Elsevier Ltd.

delay is retrospective. In fact, when a boy seeks medical attention owing to the lack of pubertal signs, it is most frequently impossible on his first visit to predict whether puberty will occur spontaneously, and it often remains difficult even after performing the usual diagnostic tests [17,21,22]. Therefore, the term 'absence of pubertal signs' would be more adequate than 'pubertal delay.' However, since 'pubertal delay' and 'delayed puberty' are widely used in the literature to refer to the absence of pubertal signs, these expressions will be employed in this review for the sake of simplicity.

2.1. Constitutional delay of growth and puberty (CGDP)

Constitutional delay of puberty (also known as self-limited delayed puberty), associated with a constitutional delay of growth or not, is by far the main cause of delayed puberty in males evaluated at specialized centers, e.g. pediatric endocrinology services in tertiary hospitals (Table 1). It is defined as an extreme of the normal spectrum of pubertal timing, whereby boys enter puberty between the ages of 14 and 18 years and complete it spontaneously within 3–5 years. It represents approximately 60–70% of the cases [23,24]. The diagnosis of CGDP is reached by exclusion, once the other possible causes have been ruled out. There may however be some guiding clinical or laboratory signs: there is delayed maturation in many physical aspects during early childhood, such as tooth eruption, adrenarche and bone age. Consequently, these boys may be shorter than their peers and grow below their mid-parental height target all through childhood. Serum levels of gonadotrophins, testosterone, AMH and inhibin B are within the normal range for Tanner stage; however, especially LH and testosterone levels may not be informative, since they are very low or nondetectable in the majority of boys until Tanner stages 2 or 3 [11]. Genetic studies, based on massive parallel sequencing (or next-generation sequencing, NGS) usually do not find pathogenic variants in patients with CDGP, but this is not sufficient for a definitive diagnosis [25].

2.2. Primary hypogonadism

Primary hypogonadism – inadequately called hypergonadotrophic in pediatric patients [26] – rarely results in pubertal delay, but it could lead to an arrested puberty (Table 1). Indeed, most primary testicular disorders, such as Klinefelter syndrome, mild testicular dysgenesis, orchitis or cryptorchidism, show a more severe impairment of the seminiferous

Table 1. Etiologies of pubertal delay in males.

Etiology	Relative frequency	Examples	Clinical features	Hormonal laboratory	Imaging
Constitutional delay of growth and puberty (CDGP)	60–70%		Prepubertal genitalia	AMH, inhibin B, testosterone, FSH and LH in normal prepubertal range	Delayed bone age
Primary hypogonadism	2–7%	Bilateral anorchia/Testicular regression syndrome	Absent testes, prepubertal genitalia	Undetectable serum AMH and inhibin B, normal/high FSH and LH	Normal bone age until ~14 years-old
		Mild testicular dysgenesis	Small testes, mild hypovirilisation (hypospadias, micropenis), some genital development	Low serum AMH and inhibin B, normal/high FSH and LH (for Tanner stage)	Normal bone age until ~14 years-old
		Klinefelter syndrome (47,XXY and variants)	Small testes, arrested puberty	Normal/low serum AMH and inhibin B, normal/high FSH and LH (for Tanner stage)	c
		Bilateral orchitis/chemotherapy	Small testes	Normal/low serum AMH and inhibin B, normal/high FSH and LH (for Tanner stage)	Normal bone age until ~14 years-old
Congenital central hypogonadism (genetic)	2%	Isolated central hypogonadism	Micropenis, cryptorchidism, micro-orchidism, prepubertal genitalia, with/without anosmia		Normal bone age until ~14 years-old Possible hypoplasia of olfactory tract
		Multiple pituitary hormone deficiency	Ibidem + hypothyroidism, cortisol deficiency, growth failure	Ibidem + low T4, TSH, cortisol, ACTH, IGF1 and GH	Delayed bone age
Acquired central hypogonadism	4–6%	Surgery of the sellar/suprasellar region Pituitary tumors Cranial trauma High dose cranial radiotherapy	Absent or arrested puberty	Low serum AMH and inhibin B (for Tanner stage), low testosterone, FSH and LH (for age) Other pituitary axes potentially affected	Brain mass or sequelae of surgical treatment
Functional central hypogonadism	16–20%	Systemic diseases (celiac or inflammatory bowel disease, diabetes, malnourishment, hypothyroidism, etc), emotional stress	Absent or arrested puberty	Low serum AMH and inhibin B (for Tanner stage), low testosterone, FSH and LH (for age)	According to etiology

AMH: anti-Müllerian hormone; FSH: Follicle-stimulating hormone; LH: luteinizing hormone.

tubules than of Leydig cells. Therefore, testosterone secretion is quite preserved, enough to trigger the development of secondary sex characteristics and linear growth. Bilateral anorchidism obviously presents with delayed puberty: the history of an empty scrotum, either congenital of following testicular regression or gonadectomy, with undetectable testicular hormones in serum during childhood [27], helps the diagnosis. Primary hypogonadism is responsible for 2–7% of cases of delayed puberty in males [23,24].

2.3. Central hypogonadism

Central (or hypogonadotrophic) hypogonadism refers to the insufficiency of the hypothalamic-pituitary axis to normally secrete gonadotrophins (Table 1). Physical maturation is normal during childhood. Consequently, these boys grow within their family target and lose height as compared to their peers only at pubertal age, due to the lack of steroid action needed for the growth spurt. Basal serum levels of LH and testosterone are low but uninformative, since they do not differ from those observed in boys with CDGP, and low FSH seems to be more specific [28]. Dynamic tests following GnRH administration might be helpful to diagnose severe forms of central hypogonadism when LH and FSH levels do not peak [17]. Other promising tests include the determination of LH after kisspeptin stimulation [29], or that of inhibin B following FSH stimulation [30].

2.3.1. Functional central hypogonadism

In these cases, the delay in the onset of puberty is due to an impaired function of the hypothalamic-pituitary axis secondary to a chronic disease (e.g. inflammatory bowel or celiac disease, malnourishment, hypothyroidism, etc.), excessive exercise or psychological or emotional stress (Table 1). Once the underlying cause is treated, pubertal development resumes spontaneously. It represents 16–20% of cases of delayed puberty in males [23,24].

2.3.2. Organic central hypogonadism

Here there is a genetic cause or an acquired anatomic lesion directly involving the hypothalamic-pituitary axis. As a whole, they are responsible for approximately 7–9% male cases with pubertal delay [23,24]. Cancer or cancer treatment directly affecting the hypothalamic-pituitary area is the underlying cause of pubertal delay in less than 2% of males [24]. Congenital multiple pituitary hormone deficiencies including a gonadotrophin deficit and other forms of central hypogonadism occurring as part of a broader syndrome (e.g. Bardet-Biedl, Noonan, Alagille, etc.) represent the cause of delayed puberty in approximately 4% of the cases [24]. Isolated central hypogonadism with anosmia (Kallmann syndrome) or without anosmia is responsible for approximately 2% of the cases [23,24]. A history of micropenis, micro-orchidism and cryptorchidism may guide the diagnosis (Box 1). Genetic testing using NGS technologies have helped to increase the diagnostic rate to almost half of the cases [31].

Box1. 'Red Flags' that make the diagnosis of congenital central hypogonadism significantly more likely than CDGP.

History of micropenis at birth
History of congenital cryptorchidism
Age >17 years
Anosmia
Deafness
Cleft palate/lip
Dental agenesis/digital anomaly
Bimanual synkinesis

3. Pharmacological therapy options

The aims of treatment in teenagers with delayed puberty are the development of the genitalia and of secondary sexual characteristics, the adequate attainment of the growth spurt and peak bone mass – resulting in normal linear growth, body composition, muscle mass and bone density – [32], as well as the promotion of psychosexual development, thus preventing low self-esteem and body image concerns resulting in social withdrawal and sexual inactivity in later life [33].

3.1. No diagnosis established: to treat or not to treat?

As already mentioned, differentiating between CGDP and central hypogonadism may be difficult owing to the many overlapping clinical and biochemical features. The managing physician should try the best to make the distinction, since these conditions differ significantly in their long-term outcomes, and avoid unnecessary delays in treating teenagers with central hypogonadism. Indeed, in patients with genetic forms of isolated central hypogonadism (often still called 'idiopathic'), linear growth is preserved, and delayed treatment initiation may lead to an increased adult stature as compared to mid-parental height [34] and eunuchoid habitus [35]. Conversely, in patients with functional hypogonadism or tumors of the sellar/parasellar region linear growth is impaired and delayed treatment may result in decreased adult height [36].

Intuitively, one would start treatment at the physiological age of pubertal onset, i.e. at about 12 years of age in boys. However, in the real-world situation the diagnosis is frequently achieved later, even in countries with high-quality health systems, especially in those cases where the etiological diagnosis is difficult to establish. Delays are frequently due to the inertia of clinical referral pathways and the time required to perform all necessary studies. On the other hand, in many cases, there might be an inadequate application of the concept of 'the most prevalent condition' for a default diagnosis [32]. Indeed, CGDP is by far the most likely diagnosis [23,24] but the misapplication of management principles established for teenagers with CDGP to patients with organic central hypogonadism leads to more undesired long-term effects than does the initiation of pharmacological therapy in boys with CDGP, as it will be discussed below.

A 'reassurance and watchful waiting' approach may be adequate under certain circumstances: a boy with a familial of CGDP, with no significant growth delay as compared to his peers and no signs of psychosocial affectation, or a boy with

Tanner stage G1 characteristics in whom testicular volume has passed from 2 ml to 3 ml predicting a prompt onset of pubertal signs [37].

Conversely, when it is not possible to establish a differential diagnosis between CDGP and central hypogonadism and there is concern of psychosocial distress or of the attainment of the growth and peak bone mass potentials, a low-dose testosterone therapy (also called 'priming') is frequently used [32,38–42]. This approach has more advantages than weaknesses. On one hand, it has been used as both a diagnostic test and a therapy. The hypothesis underlying its proposed used as a diagnostic test is that testosterone treatment for a few months triggers the activation of the hypothalamic-pituitary-gonadal axis in boys with CDGP [40,41,43]. The study design to test the hypothesis used does not allow to rule out the mere effect of time and ascribe the initiation of puberty to testosterone withdrawal. Indeed, the probability of entering puberty in the next year, without any treatment, for a boy with CDGP ranges between 38 ± 4% for a 13.5-year-old boy and 68 ± 22% for a 16-year-old boy [24]. Nonetheless, low-dose testosterone administration has a positive effect on psychosocial and psychosexual outcomes [33] as well as on linear growth and bone health [32,42,44], while it does not block spontaneous activation of the hypothalamic-pituitary-gonadal axis [42], impair spermatogenesis [45] or substantially advance bone age [42].

3.2. CDGP

3.2.1. Androgenic drugs

3.2.1.1. Testosterone.

Testosterone esters are the most frequently used formulations in boys with CDGP requiring treatment. They are oil-based compounds for slow-release IM administration. Testosterone enanthate is available in ampoules containing 200 or 250 mg; its pharmacokinetic half-life is 4.5 days in adults. Although the high levels observed in the first week decline to subphysiological levels in the 3rd and 4th weeks, some effects are still seen with those low levels as compared to the undetectable levels seen without treatment. This justifies the monthly administration in boys with CGDP. Testosterone cypionate comes in vials of 100 or 200 mg, and its half-life is 4 days in adults [46]. There are also ampoules of 250 mg containing 100 mg decanoate, 60 mg isocaproate, 60 mg phenylpropionate and 30 mg propionate [44,47,48]. Testosterone undecanoate for IM administration has only been used in patients older than 17 years [49,50]. Given its reasonable safety and beneficial effect, testosterone therapy is frequently initiated around the age of 12–14 years. Typical regimens use testosterone enanthate or cypionate IM 50 mg monthly for 6 months, with gradual increases of 50 mg every 6–12 months over 24 to 36 months until the full adult dose of 200–250 mg every 2–4 weeks is reached (Table 2). If testicular enlargement is observed – indicating spontaneous pubertal onset, especially during initial treatment with low doses of testosterone–, treatment is usually discontinued, and testicular volume progresses as a consequence of gonadotroph activation of LH and FSH secretion. This scheme is associated with increased growth, increased bone mineral density, development of secondary sexual characteristics and overall improved psychological well-being [38,39,42,44,51,52]. Replacement therapy is expected to mimic the normal pubertal tempo, with a progression of approximately one Tanner stage per year, so that a precocious early closure of the epiphyses is avoided. Amongst their limitations, these formulations show erratic pharmacokinetics, with serum testosterone attaining supraphysiological levels in the first week after IM injection and low levels in the fourth week [53,54], with interindividual variations [55,56]. This warrants a clinical follow-up every 3 months to monitor, with bone age assessment after 6 months of therapy start, which may lead to a personalized therapy scheme.

Oral formulations of testosterone that avoid liver metabolism and seem to be devoid of hepatotoxicity have been approved [57,58]. Testosterone undecanoate, available as 40-mg tablets, has been tested in adolescents at 20–160 mg daily for 3–15 months, with similar results to those obtained with IM formulations [59–63]. No experience exists in pediatric patients for mucoadhesive tablets providing sustained testosterone release [58] or with a testosterone gel for intranasal administration [57,58].

Transdermal testosterone formulations, popular in adults [58], have not yet been approved for use in pediatric patients. Satisfactory results have been observed with 10 mg daily of 2% testosterone gel for 3 months [64].

Side effects seen with the use of androgenic drugs include acne, priapism, irritability, erythrocytosis and gynecomastia due to aromatization to estrogens [65]. Special concern raises the potential acceleration of bone age, which could compromise adult height. However, these effects are rarely observed with the low doses typically used in adolescents Exogenous androgen administration in full adult dose results in the inhibition of pituitary LH secretion, which leads to a reduction in intratesticular androgen concentration and spermatogenic arrest, explaining the persistence of small testicular volume [5,66].

3.2.1.2. Oxandrolone.

Oxandrolone cannot be aromatized and has a predominant anabolic action [65,67]. It exists as oral tablets of 2.5 and 10 mg. In boys with CGDP, short-term treatment with oxandrolone 2.5 mg daily has demonstrated to be as effective as testosterone to induce growth acceleration and secondary sexual characteristics, without negative impact on bone age progression [61,68]. Mild hepatotoxicity, reversible with treatment discontinuation, has been reported [65].

3.2.2. Aromatase inhibitors

Because treatment with low-dose testosterone might initially suppress the hypothalamic–pituitary–gonadal axis and the inhibition of P450 aromatase – the enzyme responsible for androgen conversion to estrogens – enhances gonadotrophin secretion at the age of puberty in boys [69], a recent clinical trial tested the hypothesis that therapy with the aromatase inhibitor letrozole would be more efficacious than low-dose testosterone to induce pubertal onset in boys with CDGP [47]. Using a randomized, controlled study design, the authors showed that oral letrozole 2.5 mg/day for six months (Table 2) induced a significantly greater gonadotrophin secretion and testicular growth than low-dose testosterone. Letrozole also results in accelerated height growth, although slightly less than testosterone

[47], despite delaying bone maturation [70], finally increasing adult height in comparison with its prediction before treatment [71]. For details on clinical trials with different aromatase inhibitors in boys with delayed puberty and short stature the reader is referred to two recently published reviews [72,73].

Letrozole is well-tolerated in short-term regimens, with mild, nonspecific adverse events reported in clinical trials [47]. Longer regimens used for many years in boys with short stature may affect bone mineral density, lipid metabolism, insulin sensitivity and cognitive performance [73].

3.3. Central hypogonadism

In boys with a confirmed diagnosis of central hypogonadism, there is no reason for watchful waiting. Induction of puberty is indicated from the age of approximately 11.5–12 years, i.e. the mean age of pubertal onset in the general male population [74,75]. Beyond inducing secondary sexual characteristics, accelerating height velocity and promoting bone health, therapy should also address future fertility.

3.3.1. Testosterone

In the United States [76], and most probably elsewhere [32,44,74], testosterone therapy has been the most widely used pharmacological treatment in males with central hypogonadism to stimulate the development of secondary sexual characteristics and maximize adult height, promoting skeletal maturation and inducing normal muscle and bone mass. To maintain these changes, androgen replacement needs to be continued through adulthood and is only replaced temporarily by gonadotrophin therapy when sperm production is sought. Indeed, testosterone therapy aims at attaining physiological serum levels, but intratesticular testosterone concentrations remain far below the range achieved in eugonadal males in whom testicular Leydig cells are the main source of androgens. Consequently, Sertoli cells do not mature [77] and spermatogenesis is arrested at a prepubertal stage, with no sperm production [66,78,79].

For approval of a testosterone replacement therapy, the US FDA has required evidence on safety and on pharmacokinetics but not on benefit by clinically efficacy (pharmacodynamic) measure indicating that testosterone improves hypogonadal signs or symptoms. Typical clinical trials have undergone 3

Table 2. Indications of hormones/endocrine medicines used in male teenagers with delayed puberty.

Condition	Agent	Formulation	Regimen	Side effects
Constitutional delay of growth and puberty (CDGP)	Testosterone enanthate	Vial 200 or 250 mg (IM)	50–100 mg monthly for 3–6 months	Pain and swelling at injection site Erythrocytosis, Persistent erections, Gynecomastia, Acne, Accelerated bone maturation, Nervousness, irritability
	Testosterone undecanoate	Capsule 40 mg (oral)	20–160 mg daily for 3–15 months	Erythrocytosis, Persistent erections, Gynecomastia, Acne, Accelerated bone maturation, Nervousness, irritability
	Testosterone	2% gel/cream (transdermal)	10 mg of gel/cream daily for 3 months	Skin irritation, Transfer to other persons Erythrocytosis, Persistent erections, Gynecomastia, Acne, Accelerated bone maturation, Nervousness, irritability
	Letrozole	Tablet 2.5 mg (oral)	2.5 mg daily for 6 months	Decreased bone mineral density, and disturbed lipid metabolism, insulin sensitivity and cognitive performance (long-term therapy)
Primary or central hypogonadism	Testosterone enanthate	Vial 200 or 250 mg (IM)	50 mg/month, increasing of 50 mg/month every 6–12 months to attain a full adult dose of 250 mg/month after 2–3 years of treatment initiation	Pain and swelling at injection site Erythrocytosis, Persistent erections, Gynecomastia, Acne, Accelerated bone maturation, Nervousness, irritability
	Testosterone undecanoate	Vial 1000 mg (IM)	1000 mg/12 weeks once final height attained	Pain and swelling at injection site Erythrocytosis, Persistent erections, Gynecomastia, Acne, Accelerated bone maturation, Nervousness, irritability
	Crystalline testosterone	Subdermal implant 12.5, 25, 37.5, 50 or 75 mg	8–10 mg/kg, replaced every 6 month for 18 months	Local surgical procedure, pellet extrusion, local fibrosis
Central hypogonadism	r-FSH	Vials/cartridges 50–975 IU (SC or IM)	75–150 IU daily or every other day to attain target serum FSH levels of 7–9 IU/L for 2–6 months, followed by combined r-FSH plus hCG	Pain and swelling at injection site
	hMG	Vials 75 IU FSH + 75 IU LH (IM)	75–150 IU 2–3 times per week	Pain and swelling at injection site
	hCG	Vials 75 IU FSH + 75 IU LH (IM)	After initial r-FSH for 2–6 months, r-FSH + hCG for another 6 months, and finally hCG for one year. hCG is started at 250–500 IU once/twice weekly and progressively increased by 500 IU every 6 months to finally attain 1500 IU 3 times a week	Pain and swelling at injection site
	GnRH	Vials 50–100 µg	Mini-infusion pump that delivers GnRH at a rate of 25 ng/kg every 120 min	Pain and swelling at injection site

GnRH: gonadotrophin releasing hormone; hMG: human menopausal gonadotrophin (FSH+LH) obtained from urine; IM: intramuscular; IU: international units; r-FSH: recombinant human FSH; SC: subcutaneous.

phases: a dose-finding period, a 'stable dose' period and a safety 'extension' period, with an overall duration of up to one year, and have only been performed in hypogonadal adults with the aim of increasing serum testosterone to the normal range between ~300–1000 ng/dl [76]. Traditionally, the US FDA has waived the requirement of clinical trials to approve testosterone replacement formulations in teenagers, based on the fact that the number of boys under treatment was too low, rendering clinical trials highly impracticable. Two products, testosterone enanthate for intramuscular use and testosterone pellets for subcutaneous implant, are approved to treat 'carefully-selected' adolescent males with 'clearly delayed puberty.' No testosterone formulation is approved for long-term treatment in male teenagers [76]. No testosterone product is licensed for use in children in Europe [44].

Few case series have been published where patients with delayed puberty due to central hypogonadism have received testosterone pellets subcutaneously at 8–10 mg/kg, replaced every 6 month for 18 months [80], oral testosterone undecanoate 120 mg/day for 3 months followed by long-acting intramuscular testosterone undecanoate 1000 mg every 12 weeks for 2 years [49], or intramuscular testosterone undecanoate 1000 mg every 3–4 months with no previous scaling [50,81]. All of them include teenagers older than 16 years.

Despite the astonishing lack of published evidence, testosterone enanthate is largely the most frequently formulation to induce pubertal changes in boys, with an initial dose of ~50 mg every 4 weeks and an increase of 50 mg every 6–12 months to attain a full adult dose of 250 mg every 4 weeks after 2–3 years of treatment initiation (Table 2) [2,32,42,44,52,74,82,83]. Monitoring of the effectiveness of androgen therapy relies on the clinical assessment of the progression of genital maturation, height velocity and changes in body composition every 3–6 months. The aim is to mimic the physiological progression of puberty, avoiding abrupt virilization and premature epiphyseal fusion that would compromise adult height. When the diagnosis is delayed until an age at which the patient has reached an acceptable stature compared to his mid-parental height target, scaling may not be needed and a full dose of testosterone enanthate 250 mg every 4 weeks or testosterone undecanoate 1000 mg every 12 weeks could be used directly from the beginning in order to accelerate body changes [50,81]. Prolonged testosterone therapy may cause erythrocytosis, i.e. hemoglobin >18.5 g/dl or hematocrit >52%, in adult males. Although there is no clear evidence of erythrocytosis in adolescents receiving testosterone replacement, the need for an assessment of total blood count has recently been proposed basally and during treatment [44].

3.3.2. Gonadotrophins

Gonadotrophin therapy is essential to address infertility in patients with central hypogonadism. FSH administration promotes the proliferation of immature Sertoli cells. The latter remain immature until they are exposed to sufficient intratesticular testosterone concentration; therefore, they are immature in patients with pubertal delay due to complete central hypogonadism, i.e. with testicular volume <3 ml and no secondary sex characteristics, independently of their age. LH, or more frequently hCG, administration triggers Leydig cell maturation and testosterone secretion, with paracrine and endocrine effects. The most conspicuous paracrine effect of testosterone within the testis is Sertoli cell maturation and the onset of adult spermatogenesis, the main factor responsible for adult testicular volume. Continued hCG treatment results in the elevation of serum testosterone, responsible for the development of adult characteristics in the genitalia, the growth spurt and bone and muscle mass acquisition. hCG alone induces spermatogenesis and testicular enlargement in adult patients with partial or post-pubertal onset of central hypogonadism, characterized by a basal testicular volume >4 ml [84,85]. However, FSH pre-treatment results in significantly better results in patients with testicular volume <4 ml: more patients achieve complete spermatogenesis with higher sperm concentration [86–88] and bigger final testicular size [45,86,88].

Several preparations containing FSH, LH and/or hCG exist, either obtained from urine or as recombinant products [52,89]. Formulations used in pediatric patients with hypogonadism are briefly reviewed here. Human menopausal gonadotrophin or menotropin (hMG), contains FSH and LH, or predominantly FSH after immunological purification from urine. However, conclusive published experience in male patients with delayed puberty is lacking. hCG, obtained from the urine of pregnant women, is available for intramuscular or subcutaneous injection in vials containing 500–10,000 IU. LH and hCG bind the same receptor on Leydig cells, but hCG has a longer half-life [89] allowing it to be administered at 1000 IU twice a week in adolescents with central hypogonadism. Recombinant gonadotrophin formulations have been developed in the last two decades. They may be given as intramuscular or subcutaneous injections, but subcutaneous administration is preferred by patients, resulting in improved long-term adherence [74]. Recombinant hCG and LH (lutropin alfa) are produced in CHO cells [90]. Recombinant hCG has shown similar pharmacokinetic and pharmacodynamic profiles to those of urinary hCG in adult women, but it has not been used in boys with delayed puberty. Lutropin has a similar pharmacokinetic profile to that of the LH component of hMG. It has been used subcutaneously at 50 IU per day in a small series in male newborns with hypogonadotrophic hypogonadism [91] but there is no experience in patients with pubertal delay. Recombinant FSH (follitropin) exists as several different formulations. Follitropins obtained in Chinese hamster ovary (CHO) cells differ from natural FSH in their glycosylation status, resulting in a dissimilar bioactivity [89]. Follitropins alfa and beta are very similar, differing slightly in posttranslational changes resulting from the technical processes used to produce them [90]. Follitropin delta was generated using a human cell line to circumvent this limitation [90,92]. Corifollitropin alfa consists of r-FSH fused to the carboxyl-terminal peptide of the beta-subunit of hCG, resulting in a longer half-life [90,93,94].

Several treatment protocols using gonadotrophins have been reported. Initially, hCG therapy showed a comparable effect to IM testosterone on secondary sex characteristics in teenagers with central hypogonadism [95]. Nonetheless, hCG provokes local testosterone production within the testis, thus

inducing Sertoli cell maturation and spermatogenesis, while exogenous testosterone administration does not seem to result in sufficient intratesticular testosterone concentration to provoke similar changes. Therefore, testis volume increases upon gonadotrophin treatment, but remains small in boys treated with testosterone. Furthermore, subsequent studies showed the beneficial effect of pre-treatment with hMG [96] or r-FSH [45,86,97], as reflected in enhanced testicular growth and increased serum levels of the Sertoli cell markers AMH [98] and inhibin B [98,99]. Subsequent addition of hCG induces testosterone secretion by Leydig cells and Sertoli cell maturation, as reflected in AMH decline [98]. Increasing inhibin B levels are useful to monitor sperm production [99]. Typically, r-FSH is given 75–150 IU daily or every other day to attain target serum FSH levels of 7–9 IU/L for 2–6 months, followed by combined r-FSH plus hCG for another 6 months and hCG alone thereafter for one year (Table 2). hCG is started at 250–500 IU once/twice weekly and progressively increased by 500 IU every 6 months to finally attain 1500 IU 3 times a week [82,100]. Alternatively, hMG is given instead of r-FSH at 75–150 IU 2–3 times per week [96]. Once full development has been obtained, treatment may be switched to testosterone administration for maintenance until the patient desires paternity.

3.3.3. GnRH

GnRH is available in only a few countries, initially administered in a pulsatile manner using a mini-infusion pump that delivers GnRH at a rate of 25 ng/kg every 120 min (Table 2) [82,100,101]. Subsequently, a dose titration is performed to attain target serum testosterone [82,100]. A recent meta-analysis comparing GnRH versus gonadotrophin therapies in males with central hypogonadism (most of them in patients of adult age, and treated with hCG+hMG not sequentially) found that GnRH treatment induced a slightly greater increase in testicular volume (0.3–2.5 ml), earlier spermatogenesis (1.7–8.9 months) and less estrogen-related adverse reactions (e.g. gynecomastia); however, no significant differences in sperm concentration and pregnancy rate were found [102]. A clinical trial in adult patients showed that pre-treatment with r-FSH before starting GnRH led to a greater enlargement of testis size, shorter time to achieve adult spermatogenesis and higher sperm count [100], highlighting the importance of mimicking physiology with FSH action on Sertoli cells before triggering the paracrine effect of androgens [103]. Given its complex way of administration, GnRH treatment does not seem to be practical in adolescents.

3.3.4. Selective estrogen receptor modulators

With the aim of preserving fertility in patients with central hypogonadism, selective estrogen receptor modulators (SERMs) have also been used 'off-label,' especially in patients with functional hypogonadism during adulthood. Clomiphene citrate and tamoxifen are SERMs antagonizing the estrogen feedback on the estrogen receptor in the hypothalamic-pituitary axis. This results in an increased gonadotrophin release, finally leading to improved testicular stimulation. Clomiphene citrate is available as tablets of 50 or 100 mg and used at dosages between 25 mg every other day and 50 mg per day. A recent meta-analysis has concluded that clomiphene citrate is an effective therapy for hypogonadism in males, with few side effects and good safety [104]. Tamoxifen, available as tablets of 10 or 20 mg, is generally used at 20–30 mg/day. Evidence from a systematic review indicates that, like clomiphene citrate, tamoxifen is an effective and safe therapy in adult males with infertility [105]. The advantages of SERMs, as compared to testosterone treatment, includes the maintenance of fertility, the avoidance of supra-physiological testosterone levels and the lower risk of erythrocytosis [106]. However, they require a functional hypothalamic-pituitary-testicular axis and are not effective in males with organic etiologies [107]. Therefore, SERMs could only rarely be used in adolescents.

3.4. Primary hypogonadism

Testosterone replacement is the only feasible therapy in patients with primary hypogonadism. In teenagers with a previously established diagnosis of bilateral anorchia, testosterone therapy can be started at the age of pubertal onset in the general male population, i.e. approximately 11.5–12 years [56]. The aims are similar to those mentioned for patients with central hypogonadism, except for fertility. The treatment regimens and cautions are those already discussed above. In boys with primarily impaired gonadal function, e.g. Klinefelter syndrome [108,109], testicular dysgenesis [11,109], history of cryptorchidism [110], chemotherapy [111] or orchitis [76], Leydig cell androgen production is usually better preserved, and testosterone therapy may not be needed to induce the initial pubertal changes. However, a hypoandrogenic state may be present, requiring testosterone replacement to achieve adult height and full bone and muscle mass development. The dosage should be individualized according to bone age: a progressive increase from 100–125 mg to 250 mg every 4 weeks of testosterone enanthate may be needed in boys with bone ages of 12–14 years, whereas a full dose of testosterone enanthate 250 mg every 4 weeks or testosterone undecanoate 1000 mg every 12 weeks could be used directly in boys having attained their near-adult height (Table 2).

4. Conclusion

Testosterone replacement therapy has been used for decades to induce secondary sexual characteristics and pubertal growth spurt in male teenagers with delayed puberty for short periods in patients with CDGP and as a permanent treatment in those with primary or central hypogonadism. The longstanding experience indicates that low-dose starting treatments with progressive scaling to attain serum testosterone levels in the adult range within 2–3 years are safe and efficacious. Aromatase inhibitors have arisen as an alternative to induce pubertal androgenisation while preserving the growth cartilage at the epiphyseal plate, thus specially interesting when short stature is a concern. Finally, pulsatile GnRH treatment using special pumps and gonadotrophin replacement therapies, starting with FSH and subsequently adding hCG or LH, are needed to induce spermatogenesis in patients

with central hypogonadism. These treatments have only been used under research protocols. Whether these complex regimens should be used to induce pubertal changes, or only later in adulthood when fertility is sought, is still matter of debate.

5. Expert opinion

Unfortunately, there is little high-quality evidence published addressing the best approach in the pharmacological therapy of delayed puberty in males. Most of the current practice is based upon unpublished experience or limited cases series. Notwithstanding, a wide consensus exists that male teenagers seeking medical attention for pubertal delay would most probably need pharmacologic therapy to avoid psychosocial distress, and that hormone replacement should not be delayed beyond the age of 14 years in order to harness the height potential and maximize the peak bone mass. While watchful waiting is appropriate between the ages of 12 and 14 years when no definitive etiological diagnosis could be reached – thus probably indicating that CGDP is the reason for the absence of pubertal signs – hormone replacement should be readily proposed by the age of 12 years in boys with a clearly established diagnosis of primary or central hypogonadism. The search for a general illness should not be overlooked in order to rule out a functional central hypogonadism not needing hormonal replacement.

Pharmacological therapy may also be offered for a short term (3–6 months) to boys with a likely diagnosis of CDGP between 12 and 14 years of age, when a clear lack of psychosocial adjustment is noticed. Nevertheless, the endocrinologist should be careful when indicating testosterone therapy in these cases. The psychosocial benefit needs to be better substantiated. Most of the conclusions are drawn from observational studies describing impaired self-esteem, and more prevalent psychopathology and risky behavior in teenagers with pubertal delay. Although time-series studies have shown some beneficial effects after testosterone therapy, quality of life scores remained significantly lower than in boys with normal pubertal timing in many aspects, e.g. emotional role difficulty and satisfaction with general health and social function [33]. The body changes produced by the low testosterone doses used in these cases may result insufficient as compared to those expected by the adolescent, and professional psychological support is certainly warranted.

Whether low-dose testosterone therapy 'induces' puberty remains elusive. It certainly induces physical changes in genitals, skeleton and muscle. However, there is not sufficient evidence to prove that it triggers the reactivation of the hypothalamic-gonadotroph axis. The existing time-series (or 'before and after') studies show that after 6 months of testosterone treatment, there is an increased prevalence of higher gonadotropin levels and testicular enlargement as compared to before treatment. This could be due to the intervention, but also to the elapsed time. To test the hypothesis adequately, the study design would certainly be too complex and the sample size too big to justify the initiative.

No doubt exists that testosterone therapy is the only possibility in boys with delayed puberty due to primary hypogonadism, and that treatment should be started with no delay when chronological age is 11.5–12 years and bone age is >11 years. If pubertal signs develop spontaneously, but the tempo of progression is too slow or puberty is arrested, testosterone therapy also seems warranted even though no clinical studies have specifically addressed this issue. The question is how long the attending physician should wait until starting testosterone replacement. Once again, there are no studies that could provide evidence, and the decision is mainly based on the knowledge of physiology: if a genital stage or serum testosterone concentrations persists with no significant change for more than 1 year, hormone replacement should be considered. This is the art of medicine and requires clinical judgment.

A hitherto unsolved question is whether gonadotrophin or GnRH treatment should be preferred over testosterone therapy for the induction of pubertal changes in teenagers with central hypogonadism. A physiology-based approach would support the use of GnRH or gonadotrophins, with FSH preceding LH or hCG, in order to obtain a more efficient spermatogenesis. However, the practical answer should be given by controlled clinical trials with a sufficient sample size in the adequate population, i.e. teenagers aged 12–14 years (the available studies generally include older teenagers and young adults). One arm should receive GnRH/gonadotrophins for some time (androgenisation and induction of spermatogenesis) before switching to testosterone (maintenance of androgenic and anabolic effects), while the other arm should be treated with testosterone all the time (no spermatogenesis, only androgenic and anabolic effects); then both groups should receive GnRH or gonadotrophins to (re) induce spermatogenesis and the primary fertility outcome should be compared in both groups. Many further questions arise. For how long should GnRH or gonadotrophin treatment be maintained both in the induction and the (re) induction phases? Which is the most adequate primary outcome: sperm count, pregnancy rate, time to pregnancy, other? Once again, the necessary studies may prove too complex/long. A more pragmatic approach, based on the observation that previous testosterone therapy does not hamper the induction of spermatogenesis by gonadotrophin treatment during adolescence [45], would be to start by testosterone therapy and maintain it until the patient seeks fertility. Nonetheless, when gonadotrophin treatment is considered, attention should be driven to the negative predictors of response to treatment: a worse prognosis in terms of final testicular volume and sperm count is observed in patients with a history of bilateral cryptorchidism, low initial testicular volume or a genetic etiology associated with severe GnRH deficiency since fetal life [35,45,86,99].

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ORCID

Rodolfo A. Rey http://orcid.org/0000-0002-1100-3843

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