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INVITED REVIEW

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Assessment of testicular function in boys and adolescents

Jimena Lopez Dacal[1] | Sebastián Castro[1] | Sofía Suco[1] | Lourdes Correa Brito[1] | Romina P. Grinspon[1] | Rodolfo A. Rey[1,2]

[1]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE), CONICET–FEI–División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina

[2]Departamento de Histología, Embriología, Biología Celular y Genética, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

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

Abstract

Objective: The hypothalamic-pituitary-testicular axis is characterised by the existence of major functional changes from its establishment in fetal life until the end of puberty. The assessment of serum testosterone and gonadotrophins and semen analysis, typically used in the adult male, is not applicable during most of infancy and childhood. On the other hand, the disorders of gonadal axis have different clinical consequences depending on the developmental stage at which the dysfunction is established. This review addresses the approaches to evaluate the hypothalamic-pituitary-testicular axis in the newborn, during childhood and at pubertal age.

Design: We focused on the hormonal laboratory and genetic studies as well as on the clinical signs and imaging studies that guide the aetiological diagnosis and the functional status of the gonads.

Results: Serum gonadotrophin and testosterone determination is useful in the first 3–6 months after birth and at pubertal age, whereas AMH and inhibin B are useful biomarkers of testis function from birth until the end of puberty. Clinical and imaging signs are helpful to appraise testicular hormone actions during fetal and postnatal life.

Conclusions: The interpretation of results derived from the assessment of hypothalamic-pituitary-testicular in paediatric patients requires a comprehensive knowledge of the developmental physiology of the axis to understand its pathophysiology and reach an accurate diagnosis of its disorders.

KEYWORDS

AMH, androgens, DSD, gonadotrophins, hypogonadism, inhibin B, puberty

1 | INTRODUCTION

1.1 | Scope of the review

The hypothalamic-pituitary-testicular axis shows significant functional changes from fetal life to adulthood. While its assessment in the adult most frequently relies on the determination of serum testosterone and gonadotrophins and on semen analysis, this diagnostic strategy is inappropriate in most paediatric patients, especially during infancy and childhood.[1] Furthermore, the impact of disorders of testicular function has different clinical consequences according to the stage of development at which the dysfunction is established.[2] In this review, we will address the different diagnostic approaches to evaluate testicular function, including the hormonal laboratory and genetic studies as well as the clinical and imaging signs to be sought for as the expected consequences of gonadal dysfunction at different stages of postnatal life.

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1.2 | Physiology of the prenatal and postnatal male gonadal axis

During early embryonic development, the undifferentiated gonadal ridges have the potential to develop into either testes or ovaries. The establishment of a complex gene interaction in the 46,XY fetus triggers the development of testes,[3] which start secreting anti-Müllerian hormone (AMH) and testosterone (Figure 1). AMH is produced by the immature Sertoli cells and causes the regression of the Müllerian ducts, which would otherwise form the internal female reproductive organs (uterus, fallopian tubes and upper vagina). Testosterone, synthesised by Leydig cells, leads to the development of the internal male reproductive system derived from the Wolffian ducts (epididymis, vas deferens and seminal vesicle) and, after peripheral 5α-reduction to dihydrotestosterone (DHT), virilises the urogenital sinus (prostate, urethra) and the external genitalia (penis and scrotum). During the first trimester of fetal life, androgen production is mainly regulated by placental human chorionic gonadotrophin (hCG). In the second and third trimesters, fetal pituitary gonadotrophins take over the regulation of testicular function. Luteinising hormone (LH) maintains androgen secretion, which is involved in testis descent to the scrotum and the enlargement of the penis. Follicle-stimulating hormone (FSH) promotes Sertoli cell proliferation and further increases AMH secretion, even if AMH action on Müllerian ducts has already finished. Sertoli cells also produce inhibin B in response to FSH. The germ cell population is composed of immature gonocytes that differentiate to spermatogonia. Germ cells proliferate by mitosis

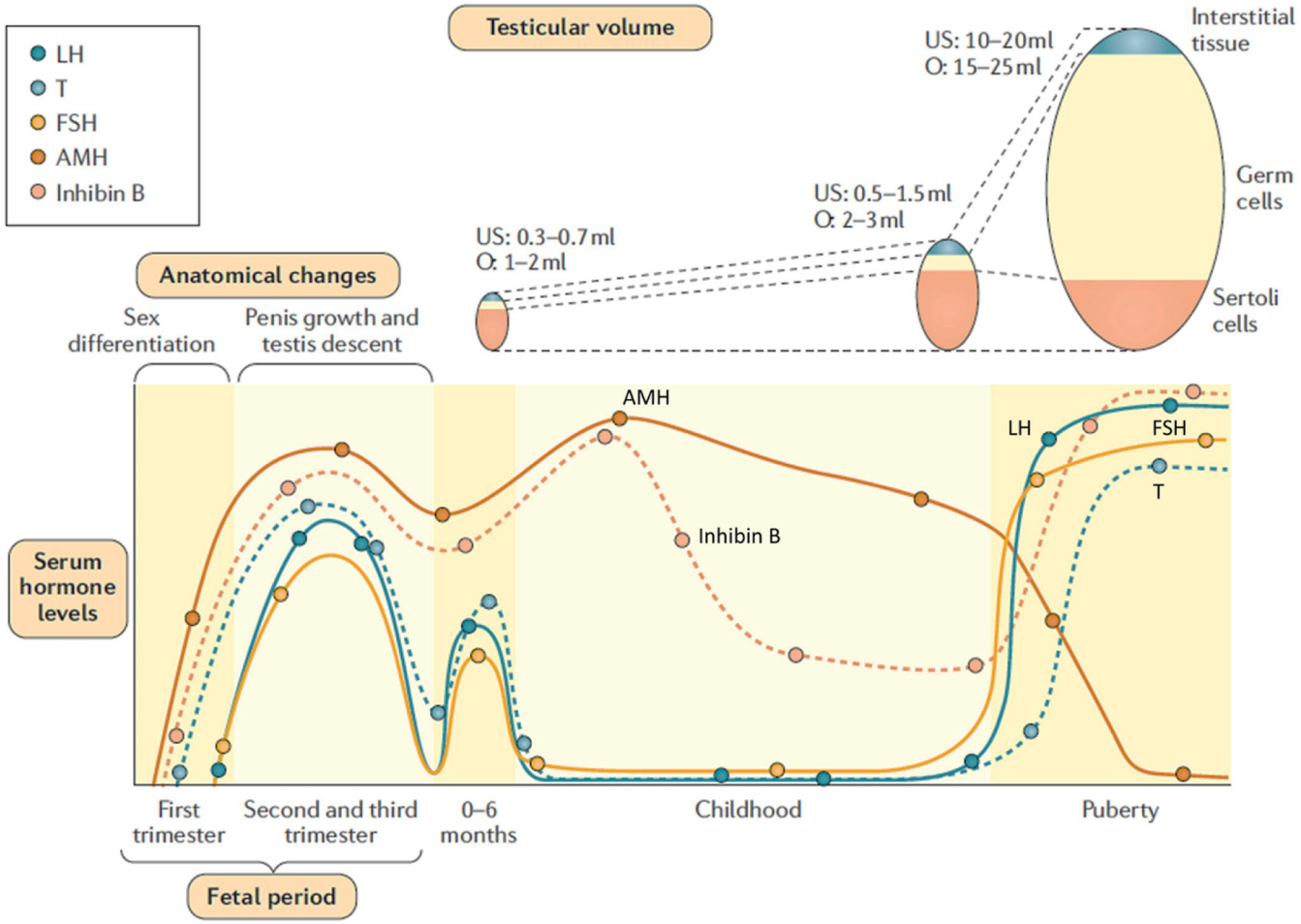

FIGURE 1 Developmental changes in serum levels of the pituitary-testicular axis and their impact on anatomical aspects. In the first trimester of fetal life, testicular hormones are responsible for the virilisation of the internal and external genitalia, independently of fetal gonadotrophins. Afterwards, luteinising hormone (LH) drives testosterone (T) secretion by Leydig cells, and follicle stimulating hormone (FSH) regulates Sertoli cell proliferation and anti-Müllerian hormone (AMH) and inhibin B levels. While T is needed for the descent of the testes to the scrotum and the enlargement of the penis, FSH provokes a modest increase in testicular size. All hormone levels increase after birth and remain high for 3–6 months. Thereafter, serum LH and T decline to undetectable levels during childhood, but AMH and inhibin B remain clearly detectable. Puberty is characterised by a reactivation of gonadotrophin and T secretion. T inhibits AMH production, whereas FSH and pubertal spermatogenesis upregulate inhibin B secretion. Testicular size increases dramatically, due to germ cell proliferation. Reprinted, with permission, from Salonia et al.[1] © 2019 Springer Nature Limited. O, orchidometer; US, ultrasonography. [Color figure can be viewed at wileyonlinelibrary.com]

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but do not enter meiosis; therefore, spermatocytes, spermatids and spermatozoa are absent. Impaired testis function results in ambiguous or female genitalia during the first trimester of gestation, but in male genitalia with micropenis and/or cryptorchidism when established later in fetal life (Figure 2).

At birth, the testis is composed mainly of immature Sertoli cells and spermatogonia within the seminiferous cords and Leydig cells in the interstitial tissue. Serum levels of gonadotrophins and testicular hormones are transiently low and increase within the first weeks of postnatal life, reaching levels similar to those observed at puberty (Figure 1). The postnatal activation of the axis, called 'minipuberty', wanes after 3–6 months. Thereafter, and until the onset of puberty, LH shows very low or undetectable levels in serum. Typical Leydig cells are no longer seen, and serum testosterone decline to undetectable levels. FSH also declines but remains clearly detectable, which may explain why immature Sertoli cells continue to proliferate resulting in a modest increase in testicular size, which can be detected only by using sensitive methods such as ultrasonography.[5] The secretion of AMH and inhibin B remains active, resulting in clearly detectable levels during childhood. While insufficient testicular hormone activity may result in a reduced penile enlargement during 'minipuberty', it may have no clinical impact during the rest of childhood (Figure 2).

Between the ages of 9 and 14 years, the hypothalamic-pituitary-testicular axis reactivates. FSH increases and stimulates an initial increment in testicular volume, from 1–3 to 4–5 mL approximately as measured by comparison with the Prader orchidometer. This corresponds to pubertal Tanner stage 2. Concomitantly, LH progressively increases and Leydig cells reappear in the interstitial tissue. Testosterone levels increase within the testis, but not yet in serum. Nonetheless, the high intratesticular testosterone concentration induces Sertoli cell maturation, resulting in a progressive decline in serum AMH and the initiation of pubertal spermatogenesis, that is, meiotic divisions leading to the progressive appearance of spermatocytes, spermatids and spermatozoa. FSH and spermatogenic cells drive an increase in inhibin B production. Between pubertal stages Tanner 3–5, the testicular volume enlarges dramatically due to germ cell proliferation, and testosterone levels increase finally reaching adult levels (Table 1). Hypogonadal states result in a lack of, a delay or an arrest in pubertal maturation (Figure 2).

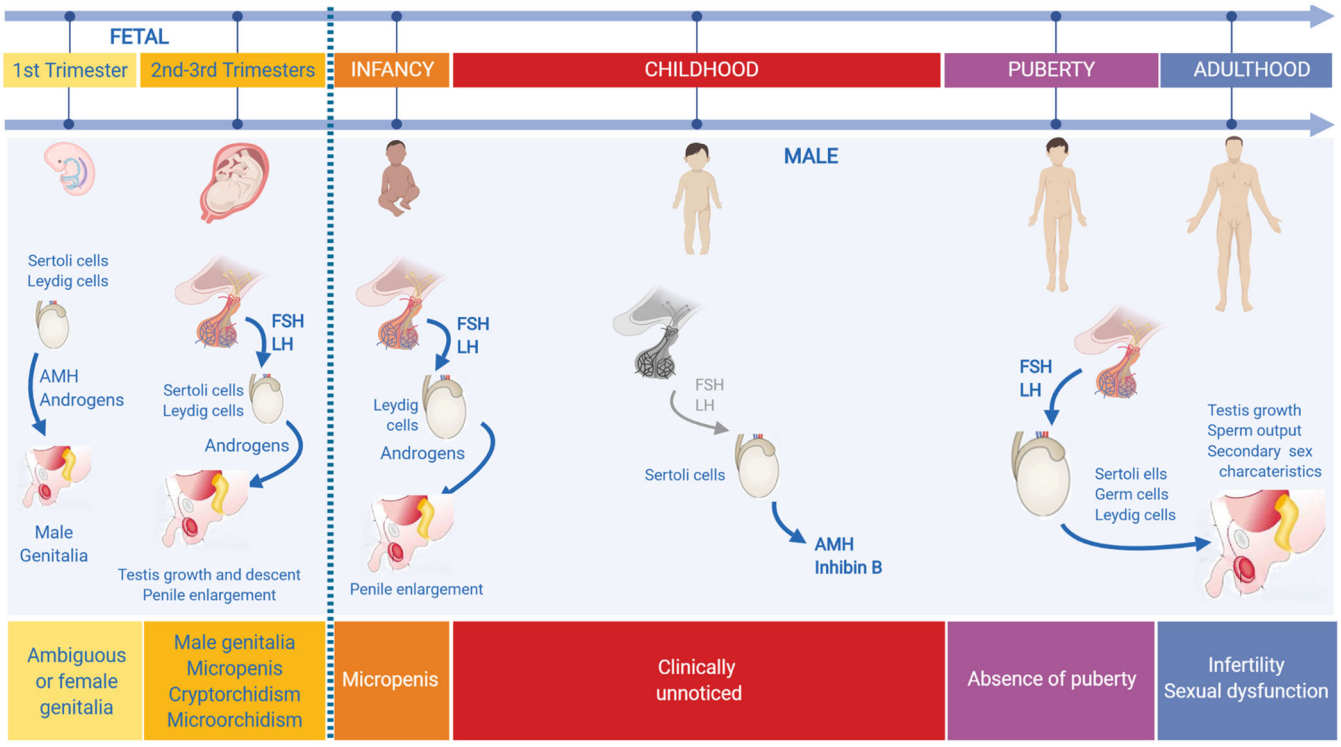

FIGURE 2 Ontogeny of the hypothalamic-pituitary-testicular axis, and the clinical impact of hypogonadism on clinical presentation. The testes differentiate in the first trimester of fetal life, independently of pituitary gonadotropins. Androgens and AMH drive male genital differentiation; in their absence, female differentiation of the genitalia occurs. Hypogonadism in this period leads to ambiguous or female genitalia in XY individuals. In the second and third trimesters, androgens stimulate testicular descent and penile enlargement. Hypogonadism, either primary or central, results in micropenis, cryptorchidism and/or microorchidism. In newborns, gonadotrophin and steroid secretion is active. Hypogonadism results in reduced penile enlargement. During childhood gonadotrophins and testosterone are low or undetectable. Hypogonadism established in this period does not result in clinically evident signs. During puberty, the gonadal axis is reactivated and results in the development of secondary sex characteristics. Hypogonadism results in absent or incomplete pubertal development. Reprinted, with permission, from Grinspon et al.[4] © 2019 Elsevier Limited. [Color figure can be viewed at wileyonlinelibrary.com]

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2 | ASSESSMENT OF TESTICULAR FUNCTION IN PREPUBERTY AND PUBERTAL AGE

2.1 | Patients with DSD

Testicular tissue may be present in all types of disorders of sex development (DSD): 46,XY DSD, 46,XX DSD and chromosomal DSD.[9] The amount and endocrine capacity of testicular tissue usually correlates with the degree of virilisation at birth as well as with serum hormone levels (Table 2).

2.1.1 | Physical examination and imaging

A bigger size of the phallus, a distal position of the urethral meatus, a higher degree of labioscrotal fusion and testis descent are anatomical signs indicative of androgen action during early fetal life. Normal testis function results in penile length >2.5 cm,[10] in full-term newborns.[11]

The use of imaging studies may be helpful, with certain limitations.[12] The presence of Müllerian remnants in ultrasound is indicative of insufficient Sertoli cell AMH secretion; however, the presence of intraabdominal testes is frequently missed. The presence

TABLE 1 Reference ranges of hormones of the pituitary-testicular axis according to age and pubertal stage.[a]

Age	LH (IU/L)	FSH (IU/L)	T (ng/dL)	AMH (pmol/L)	Inhibin B (ng/L)
1st week	<0.10–0.90	<0.10–1.00	15–150	160–800	20–190
2–4 weeks	0.40–7.60	0.25–4.10	20–300	280–900	30–510
1–5 moths	0.20–4.20	0.30–4.70	180–270	600–2400	280–430
6 moths–9 years	<0.10–0.30	0.30–1.70	<10	600–1800	120–380
>9 years, Tanner 1	<0.10–1.00	0.40–2.60	<10–60	300–1800	120–380
>9 years, Tanner 2	0.15–3.00	1.10–4.60	10–60	70–1000	180–440
>9 years, Tanner 3	0.70–5.20	1.30–6.50	40–550	40–400	220–490
>9 years, Tanner 4–5	1.40–6.00	1.50–7.00	130–660	30–200	230–500

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinising hormone.
[a]Data taken from Trigo et al.[6] Bergadá et al.[7] and Grinspon et al.[8]

TABLE 2 Clinical and laboratory consequences of fetal-onset male hypogonadism.

Type of hypogonadism	Aetiology	Genitalia	Infancy/childhood					Puberty				
			LH	FSH	T	AMH	Inh B	LH	FSH	T	AMH	Inh B
Primary hypogonadism	Gonadal dysgenesis	Female or ambiguous	N-H	N-H	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND
	Leydig cell hypoplasia/aplasia steroidogenic defects	Female or ambiguous	N-H	N	L-ND	N-H	N	H	H	L-ND	N-H	L-ND
	Testicular regression syndrome Testicular torsion	Micropenis empty scrotum	N-H	N-H	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND
	Klinefelter syndrome, XX male	Male	N	N	N	N	N	H	H	N-L	L-ND	L-ND
Central hypogonadism	Deficient GnRH production or action	Micropenis, cryptorchidism	L	L	L	L	L	L	L	L	L	L
	LHβ mutations	Micropenis, cryptorchidism	L	N	L	N	N	L	H	L	H	
	FSHβ mutations	Small testes	N	L	N			H	L	N		
Dual hypogonadism	Prader-Willi syndrome X-linked congenital adrenal hypoplasia	Micropenis, cryptorchidism	L-N	L-N	L-N	L	L	N	N	L	L	L

Abbreviations: FSH, follicle-stimulating hormone; L, N, H, low, normal, high with respect to reference range for age in males; LH, luteinising hormone; ND, nondetectable.

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of a vagina and a lower location of its opening into the urethra, as assessed by genitography and/or cystoscopy, are suggestive of insufficient androgen action on the urogenital sinus. Other imaging techniques, such as computed tomography scans or magnetic resonance are rarely used since they require patient sedation or anaesthesia but can provide more sensitive results.[13]

While incompletely virilised external genitalia are not indicative of the aetiology, the coexistence of Müllerian remnants is highly suggestive of testicular dysgenesis.

2.1.2 | Genetic studies

Karyotype is the basis for the initial classification into 46,XX, 46,XY or chromosomal DSD. G-banded karyotype has a resolution of approximately 5–10 Mb; microarrays, including array comparative genome hybridisation (aCGH) and single-nucleotide polymorphism (SNP) arrays, may detect deletions or insertions ≥10 kb.[14] Sequencing techniques are capable of detecting 1-bp changes; the progressive accessibility to high-throughput technologies, such as next-generation sequencing (NGS), has resulted in a clear increase in successful aetiologic diagnoses in recent years, not only in monogenic forms of DSD,[15] but also in the identification of potential oligogenic causes.[16] Their results may give a hint as to whether the function is affected in all testicular cell populations (e.g., genes associated with gonadal dysgenesis) or only specific cell types (e.g., genes involved in Leydig cell steroidogenesis). Whether NGS will become a first-line diagnostic test in patients with DSD remains a question of debate.[17] Nowadays, the diagnostic yield of standard NGS technologies ranges between 20% and 45%,[18] while a combined approach of genetic, hormonal and imaging studies increases the rate of aetiologic diagnosis up to approximately 80%.[19]

2.1.3 | Hormonal laboratory

As mentioned above, the occurrence of incompletely virilised genitalia indicates partial androgen activity. From the knowledge of the gonadal axis physiology, it can be deduced that serum gonadotrophin and testosterone measurement can be informative in the first 6 months of life.[20] It should be noted that all hormones of the gonadal axis are normally very low in the first 24–48 h of life[21]; therefore, assessment may need to be repeated later. The measurement of testosterone by mass spectrometry[22] and AMH may be sufficient in the initial workup.[21] Testosterone and AMH levels below the male and above the female range, together with elevated gonadotrophins in the first 6 months of life, are indicative of the existence of dysgenetic testicular tissue (Table 2). Low testosterone but male-range AMH suggest a Leydig cell disorder, for example, Leydig cell hypoplasia due to LHCG receptor mutations or steroidogenic enzyme defects. The coexistence of male-range testosterone and AMH suggests androgen insensitivity, due to androgen receptor signalling defects, 5α-reductase type 2 deficiency, or a malformative, nonendocrine DSD. An hCG test followed by measurement of all delta-4 and delta-5 steroids may be necessary to identify specific steroidogenic enzyme defects.[23] Other testicular peptide hormones, such as inhibin B and insulin-like 3 (INSL3), have been studied in research settings and may prove useful.[24,25]

After the first 6 months of life, serum AMH measurement is the most useful tool.[25] Testosterone and gonadotrophins are rarely informative since they are usually low, and can even be within the normal range in agonadal patients.[26] An hCG test is necessary when the assessment of Leydig cell function is sought during childhood.[27]

2.2 | Newborns/infants with micropenis and/or cryptorchidism

As discussed above, boys with completely virilised genitalia have necessarily been exposed to androgen action during the first trimester of gestation (Figure 1). However, deficient androgen secretion by the testes during the second half of fetal life may result in micropenis and/or cryptorchidism, that is, testes that have not completely descended to the scrotum (Figure 2). On the other hand, isolated micropenis or cryptorchidism might occur with no testicular dysfunction.[28]

2.2.1 | Physical examination and imaging

Stretched penile length, measured with a stiff ruler from the pubic ramus to the glans without involving the foreskin, correlates with serum testosterone during infancy[29] and is slightly decreased in cryptorchid boys.[30] The identification of testes and their position is of clinical importance. Although palpation and comparison to Prader's orchidometer is the most usual practice, ultrasound measurement of testicular volume is more accurate and sensitive, showing that gonadal size increases during infancy and childhood.[5] Testicular volume may be smaller in cryptorchidism; whether this reflects a primary gonadal defect that resulted in abnormal descent or an affected gonadal development due to the abnormal position may be difficult to ascertain. Ultrasonography and MRI have low sensitivity for the detection of intraabdominal testes.[31]

2.2.2 | Hormonal laboratory

When gonads are not palpable, the existence of abdominal testes can be demonstrated by detectable levels of AMH[32,33] or inhibin B[33] and testosterone (the latter, only during the first 3–6 months of postnatal life). In anorchid boys, gonadotrophins are high in the first years but may be within the normal range later, so they are not always good biomarkers.[26] Therefore, serum determination of AMH or inhibin B are the first choice, and they are even more informative than testosterone determination after hCG,[32] imaging studies or laparoscopy[33] when looking for the existence of testicular tissue in boys with nonpalpable

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testes. When gonads can be detected in the inguinal region, serum AMH[34] and inhibin B[35,36] levels are indicative of their functional status. The fact that AMH[34] and inhibin B[37] levels increase after orchiopexy indicates that, at least in some cases, cryptorchid position is affecting testicular function, which may recover. In boys with monorchism, serum AMH[38] and inhibin B[39] indicate the existence of compensatory function of the remaining testis.

Endocrine assessment of the gonadal axis can also help distinguish between primary and central forms of hypogonadism in newborns with micropenis and/or cryptorchidism.[28] The coexistence of low gonadotrophins, testosterone, AMH and inhibin is indicative of central (hypogonadotrophic) hypogonadism (Table 2). Gonadotrophin replacement in these boys is followed by testicular hormone elevation, testis descent and penile enlargement.[40] Conversely, low testicular hormones with normal or elevated gonadotrophins are highly suggestive of primary hypogonadism.[41] In older children before adolescence, the differential diagnosis between primary and central hypogonadism may be challenging.

2.2.3 | Genetic studies

The growing availability of high-throughput sequencing technologies has helped in the identification of the aetiologies in boys with micropenis and/or cryptorchidism. The finding of a genetic cause is helpful to guide or to avoid the stimulation tests (e.g., a GnRH or an hCG test) when the causal relationship is well established,[42,43] including primary or central hypogonadism and syndromic disorders. However, the diagnostic efficacy still needs to be improved, and many nonsyndromic cases of cryptorchidism remain unsolved, especially in prepubertal age.[42]

2.3 | Patients born preterm or small for gestational age

2.3.1 | Physical examination and imaging

Cryptorchidism is more frequently observed in preterm boys because the testes usually complete their descent about normal term. On the other hand, neonates born small for gestational age (SGA) present a higher incidence of hypospadias and cryptorchidism, probably reflecting a placental dysfunction resulting in reduced hCG stimulation of testis androgen secretion in fetal life.[44]

2.3.2 | Hormonal laboratory

The postnatal activation of the gonadal axis ('minipuberty') is prolonged in preterm neonates as compared to boys born at term, resulting in higher serum testosterone levels associated with faster testicular and penile growth. However, the waning of minipuberty occurs at a similar age when corrected for the age of prematurity,[45] and testicular function does not seem to differ in young males born preterm.[46]

In boys born SGA there are conflicting results about postnatal testicular function.[47] This may be explained by the fact that testicular volume is smaller in some of these boys, who present with lower inhibin B and slightly higher FSH suggesting a mild primary hypogonadism.[48] In boys born SGA presenting with hypospadias, LH is high and testosterone is low during minipuberty, also supporting a mild hypogonadism.[44]

2.4 | Boys with precocious pubertal maturation

2.4.1 | Physical examination and imaging

Precocious puberty, defined as the appearance of pubertal signs before the age of 9 years, is a rare condition in boys. It may be due to an early reactivation of the GnRH neuron, a condition called central precocious puberty. The aetiologies are central nervous system lesions, including brain hamartomas or malignant tumours,[49,50] or genetic imprinting disorders in familial forms,[51,52] resulting in a pubertal maturation process that is normal, except for the age at onset. Alternatively, testicular function may be precociously activated by LH or hCG produced by tumours (e.g., pituitary gonadotrophinomas, hepatoblastomas, etc.) or by activating mutations of the LH receptor ('testotoxicosis' or 'familial male-limited precocious puberty') or the $G\alpha_s$ subunit coupled to the LH and FSH receptors (McCune-Albright syndrome). All of these cases are characterised by an initial increase of testicular volume (≥4 mL as measured by the orchidometer or ≥25 mm in length by ultrasound), followed by other secondary sex characteristics, such as pubic hair and growth and bone age acceleration. These conditions should be distinguished, on one hand, from isolated prepubertal macroorchidism (e.g., Fragile X syndrome) and, on the other, from conditions characterised by the occurrence of pubic hair development and acceleration of growth and bone age due to early androgen production but with prepubertal testicular volume (known as 'pseudo' or 'peripheral' precocious puberty).[50] Although rarely seen nowadays, macroorchidism may also result from long-standing primary hypothyroidism.[53]

Clinical examination red flags are a Tanner stage 2 or higher, stature above mid-parental height and a recent increase in height velocity, together with an advanced bone age. MRI of the brain or the hypothalamic-pituitary region is useful to detect expansive lesions. The existence of café-au-lait skin pigmentation should prompt the search of polyostotic fibrous dysplasia by X-ray, typical of McCune-Albright syndrome. Testis asymmetry may be indicative of a gonadal tumour, which could be supported by ultrasonography.

2.4.2 | Hormonal laboratory

Endocrine laboratory assessment is essential for ascertaining the existence of precocious pubertal maturation and the differential

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TABLE 3 Hormonal laboratory assessment in boys with suspicion of precocious puberty.

Aetiology	Testis volume	Gonadotrophins	Testosterone	AMH	Inhibin B
Central precocious puberty	Pubertal	Pubertal	Pubertal	Pubertal	Pubertal
LH-secreting Gonadotrophinoma	Pubertal, but small for Tanner stage	Pubertal LH, prepubertal FSH	Pubertal	Pubertal	Pubertal
hCG-secreting tumour	Pubertal, but small for Tanner stage	Prepubertal/completely inhibited	Pubertal	Pubertal	Pubertal
Testotoxicosis	Pubertal, but small for Tanner stage	Prepubertal/completely inhibited	Pubertal	Pubertal	Pubertal
McCune Albright syndrome	Pubertal	Prepubertal/completely inhibited	Pubertal	Pubertal	Pubertal
Leydig cell tumour	Asymmetric	Prepubertal/completely inhibited	Pubertal	High for pubertal stage	Low for pubertal stage
Nongonadal androgen source	Prepubertal	Prepubertal/completely inhibited	Pubertal	Prepubertal	Prepubertal
Prepubertal macroorchidism	Prepubertal	Prepubertal	Prepubertal	Prepubertal/high	Prepubertal/high

aetiological diagnoses (Table 3). In a boy presenting with 'pubertal' testis volume, the finding of prepubertal AMH, inhibin B, testosterone and LH are indicative of prepubertal macroorchidism. Undetectable LH and FSH associated with elevated testosterone rule out central precocious puberty, but may not distinguish between testotoxicosis, McCune-Albright syndrome and other forms of 'pseudoprecocious' puberty. Sertoli cell maturation is a process driven by intratesticular androgen concentrations; therefore, AMH decline and inhibin B increase in boys with testotoxicosis, McCune-Albright syndrome or steroid-producing Leydig cell tumours.[54] Conversely, when prepubertal AMH and inhibin B coexist with pubertal serum testosterone levels, a nongonadal source of androgens should be sought, for example, simple virilising forms of congenital adrenal hyperplasia, adrenal tumours or exposure to exogenous androgens. In boys complaining for the appearance of pubertal signs, central precocious puberty almost always presents with pubertal levels of all pituitary-testicular axis hormones,[55] and a GnRH test is not necessary to certify the diagnosis. Very high TSH levels support the diagnosis of primary hypothyroidism as a cause of macroorchidism.[53]

2.4.3 | Genetic studies

Genetic and epigenetic aetiologies of central precocious puberty can be confirmed by the finding of rare gene variants that disrupt specific hypothalamic-pituitary regulatory pathways.[52] Peripheral forms of precocious puberty may be familial, and the genetic diagnosis is directed to variants in the *LHCGR* gene, or caused by somatic mutations in the *GNAS1* gene, which are not passed on to offspring.[56]

2.5 | Adolescents with delayed or arrested puberty

The function of the pituitary-testicular axis needs to be assessed in boys with delayed puberty, that is, ≥14-year-old and showing no signs of pubertal maturation (especially testicular volume <4 mL or testis length <2.5 cm) as well as in those who initiated puberty at a normal age but in whom pubertal progression is too low or arrested (e.g., more than 1 year in the same Tanner stage).[57] Although constitutional, self-limited delay of puberty is the most frequent cause of pubertal delay in males, functional or persistent hypogonadism[1] should be ruled out.

2.5.1 | Physical examination and imaging

The absence of pubertal signs is key. A recently developed puberty nomogram helps assessing delayed pubertal progression.[58] Since the growth spurt reflects androgen action and bone age maturation is mainly dependent on oestrogen activity on the bone plate, in boys with constitutional delay of puberty, stature is short for chronological age but adequate for bone age, which is delayed already during childhood. After the age of 14, bone age also delays in boys with hypogonadism. Adrenarche is usually delayed in boys with constitutional delay of puberty but occurs at a normal age in boys with hypogonadism; therefore, the existence of pubic hair is more frequently observed in hypogonadal patients and does not necessarily reflect testicular activity. Any sign of chronic disease, including malnutrition or psychosocial conditions, or the use of medication may orient the diagnosis to functional hypogonadism.[59] On the other hand, diverse phenotypic features typical of genetic disorders points the diagnosis to permanent forms of primary or central hypogonadism.

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2.5.2 | Hormonal laboratory

Primary hypogonadism rarely results in absence of pubertal signs but may be the cause of slow progressive or arrested puberty. The diagnosis can be easily supported by the elevation of gonadotrophins associated with low levels of testosterone, inhibin B and AMH (Table 2).[1,59,60]

On the contrary, the differential diagnosis between constitutional delay of puberty and central hypogonadism may prove difficult. Basal serum gonadotrophins and testosterone are more useful to rule out than to confirm central hypogonadism.[61] A large number of dynamic tests with GnRH or its agonists have been described, but none has proved sufficiently accurate.[62] Low basal levels of inhibin B and AMH are suggestive of congenital hypogonadism.[63–65] Recently, the measurement of serum inhibin B after stimulation with FSH has appeared as a promising test, as shown in a small series of boys with delayed puberty.[66] However, a larger study is needed for a confirmatory conclusion.

2.5.3 | Genetic studies

In patients with low gonadotrophins, genetic testing may help identify the aetiology: approximately half of males with congenital central hypogonadism carry gene variants in one or more of about 50 genes.[67] Genetic variants have also been identified in a different set of genes in a low proportion of males with constitutional delay of puberty.[68]

2.6 | Boys and adolescents with miscellaneous conditions affecting testicular function

Testicular function may be affected by a number of endogenous or exogenous causes during childhood and adolescence (Tables 2 and 4).[69]

Diverse chromosomal aberrations have been shown to impact on testicular function from paediatric ages.[28] The most prevalent are Down and Klinefelter syndromes, characterised by the existence of a trisomy. Although the presence of an extra autosome or sexual chromosome is known to block germ cell meiosis, which only initiates during puberty in the testis, an earlier impairment of gonadal function occurs. In boys with Down syndrome, a primary hypogonadism is observed from infancy even in those cases without cryptorchidism, as revealed by low serum AMH.[8] In Klinefelter syndrome, germ cell apoptosis is increased already at birth[70]; however, Sertoli and Leydig cell function is preserved until mid-puberty, as shown by normal serum levels of AMH, inhibin B, testosterone and gonadotrophins.[71]

Varicocele is another endogenous disorder affecting testicular function during pubertal age. Patients with varicocele show decreased serum levels of inhibin B and a smaller testis size on the affected side,[72] frequently associated with impaired semen parameters in young adults.[69]

Diabetes mellitus type 1 does not seem to impair gonadal function in males,[69,73] while adolescents with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) may present with primary hypogonadism and require testosterone replacement.[74]

TABLE 4 Laboratory consequences of postnatal-onset male hypogonadism.

		Infancy/childhood					Puberty				
Type of hypogonadism	**Aetiology**	**LH**	**FSH**	**T**	**AMH**	**Inh B**	**LH**	**FSH**	**T**	**AMH**	**Inh B**
Primary hypogonadism	Orchitis Testicular torsion or trauma	N-H	N-H	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND
	Down syndrome	N-H	N-H	L-N	L-N	L-N	H	H	L	L	L
	Varicocele	N	N	N	N	N	N-H	N	L-N	N	N
	Chronic diseases: *Diabetes mellitus, APECED*	N	N	N	N	N	N-L	N-L	N-L	N-L	N-L
	Chemotherapy Abdomino-pelvic radiotherapy	N	N	N-L	L	L	N-H	N-H	N-L	N-L	N-L
	Drug therapies: *Ketoconazole*	-	-	-	-	-	N-H	N-H	L	H	-
Central hypogonadism	Pituitary and CNS damage: *Tumours, histiocytosis, trauma and so forth*	L	L	L	N	N	L	L	L	N	L
	Functional central hypogonadism: *Chronic diseases, drug/alcohol abuse and so forth*	N	N	N	N	N	L-N	L-N	L		L
Dual hypogonadism	Brain radiotherapy + chemotherapy	N	N	N	L	L	L-N	L-N	L	L	L

Note: Modified with permission, from: Rey and colleagues © 2012 American Society of Andrology and European Academy of Andrology.
Abbreviations: L, N, H, low, normal, high with respect to reference range for age in male;s ND, nondetectable.

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Oncologic diseases and their treatments may affect gonadal function in boys and adolescents. Since long-term survival has significantly improved in children due to optimized treatments, studies initially focused on the side effects of chemotherapy and radiotherapy. However, recent studies have shown that endocrine testicular function is acutely affected at diagnosis. In prepubertal boys with haematologic malignancies[75,76] or solid cancers,[75] serum inhibin B and/or AMH were low before the initiation of treatment. Similarly, testosterone was also decreased in pubertal boys. Unexpectedly, testicular biomarkers normalised within the first 3 months of chemotherapy together with an improvement of the general health status.[76] Subsequently, serum hormone levels decreased during treatment,[77] but recovered in most cases and remained stable 1 year after the end of chemotherapy.[77,78] The impact of oncologic treatment may persist through adulthood when intensive chemotherapy, radiotherapy and stem cell transplantation are used. In these cases, there is a severe impairment of the Sertoli, germ and Leydig cells.

3 | CONCLUDING REMARKS

The assessment of the gonadal function in boys and adolescents differs significantly from the usually done in the adult male. The accurate knowledge of the developmental physiology of the axis is essential for the understanding of its pathophysiology and the diagnosis of the diverse conditions affecting it. While serum gonadotrophins and testosterone may be informative in the first 3–6 months after birth and at pubertal age, serum AMH and inhibin B are the most useful biomarkers during childhood. Both reflect Sertoli cell activity, the most relevant cell population in the prepubertal testis. At the age of puberty, the decline in serum AMH reflects a normal (or precocious) elevation of intratesticular androgen concentration, while the increase in serum inhibin is indicative of spermatogenic development in the seminiferous tubules.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Jimena Lopez Dacal http://orcid.org/0000-0003-0116-8705
Sebastián Castro http://orcid.org/0000-0002-8073-2046
Sofía Suco http://orcid.org/0009-0000-4061-8700
Lourdes Correa Brito http://orcid.org/0000-0002-1583-1988
Romina P. Grinspon http://orcid.org/0000-0002-6291-1518
Rodolfo A. Rey http://orcid.org/0000-0002-1100-3843

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