The prepubertal testis: biomarkers and functions

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Purpose of review
Biomarkers of prepubertal testicular function have become widely available only in recent years. The aim of this review is to update the knowledge on key biomarkers used to assess hypogonadism in boys.

Recent findings
Sertoli cells are the most representative cells of the prepubertal testis. Anti-Müllerian hormone and inhibin B are essential biomarkers of Sertoli cell function. Also, INSL3 arises as an additional marker of Leydig cell dysfunction.

Summary
The widespread use of these biomarkers has enhanced our knowledge on the pathophysiology and diagnosis of prepubertal male hypogonadism. Beyond their well known germ-cell toxicity, oncologic treatments may also affect Sertoli cell function. Pathophysiology is not the same in all aneuploidies leading to infertility: while hypogonadism is not evident until mid-puberty in Klinefelter syndrome, it is established in early infancy in Down syndrome. In Noonan syndrome, the occurrence of primary hypogonadism depends on the existence of cryptorchidism, and Prader–Willi syndrome may present with either primary or combined forms of hypogonadism. Prepubertal testicular markers have also provided insights into the effects of environmental disruptors on gonadal function from early life, and helped dissipate concerns about testicular function in boys born preterm or small for gestational age or conceived by assisted reproductive technique procedures.

Keywords
bisphenol A, disorders of sex development, gonadotropins, phthalates, testicular dysgenesis

INTRODUCTION

In the adult men, serum levels of gonadotropins, testosterone and inhibin B, and sperm count, morphology and motility are informative about interstitial and tubular testicular function. In paediatric patients, the appraisal should be made considering the developmental physiology context: basal testosterone and sperm count are not suitable biomarkers during childhood because the gonadotropes and testicular Leydig cells become quiescent, and sperm is not produced. This review will focus on the key biomarkers used to assess testicular function in paediatric patients. The underlying rationale will be introduced first, by reviewing the essentials of the hypothalamic–pituitary–testicular ontogeny.

ONTOGENY OF THE HYPOTHALAMIC–PITUITARY–TESTICULAR AXIS PHYSIOLOGY

The changing functional activity of the hypothalamic–pituitary–testicular axis throughout development depends on changes in hormone secretion and receptor expression.

Ontogeny of hormone production

In the foetus, the testis differentiates before the hypothalamic-pituitary axis is functional: in the seminiferous cords, Sertoli cells secrete anti-Müllerian hormone (AMH), responsible for the regression of the anlagen of the uterus and Fallopian tubes [1], and germ cells proliferate by mitosis, but do not enter meiosis. In the interstitial tissue, Leydig cells secrete androgens and the insulin-like peptide 3 (INSL3) (Fig. 1). Androgens drive internal and external genital virilization [2,3], while both testosterone and INSL3 are involved in testicular descent [4▪,5▪]. Foetal pituitary gonadotropins become

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KEY POINTS

- In the first 3–6 months of life, basal gonadotropin levels are useful markers of pituitary function, testosterone and INSL3 reflect testicular Leydig cell function (interstitial tissue) and AMH and inhibin B are indicative of testicular Sertoli cell function (seminiferous tubules).
- During the rest of infancy and childhood, basal gonadotropins, testosterone and INSL3 are very low or undetectable with routine methods in normal boys, so they are not useful markers of the pituitary–Leydig cell axis unless stimulation tests are used. Basal AMH and inhibin B are normally high, and represent excellent markers of Sertoli cell function.
- Low AMH and inhibin B, but not elevated FSH, should be sought for the diagnosis of primary hypogonadism in prepubertal boys.
- During childhood, AMH and inhibin B are low in congenital central hypogonadism, but normal in acquired central hypogonadism.
- At pubertal age, low testicular hormones associated with 'normal' gonadotropins are suggestive of combined hypogonadism.

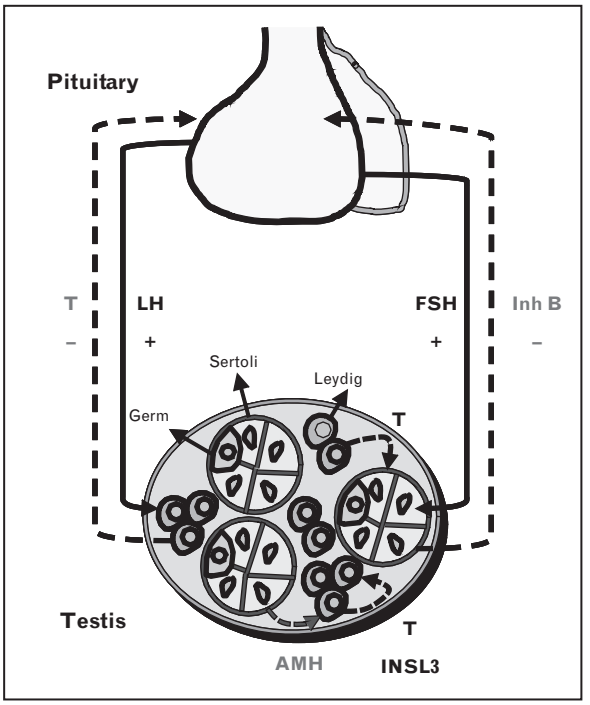

FIGURE 1. The pituitary–testicular axis. Gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are secreted by the pituitary gonadotropes. LH induces testosterone (T) synthesis by the Leydig cell of the testicular interstitial tissue. T acts within the testis as an autocrine regulator and as a paracrine factor on neighbouring Sertoli cells of the seminiferous tubules. T is also secreted to the circulation, having actions on many distant organs, including a negative feedback on LH secretion. Leydig cells also secrete insulin-like peptide 3 (INSL3). FSH acts on Sertoli cells, regulating inhibin B and anti-Müllerian hormone (AMH) secretion. Inhibin B is the main negative feedback signal for FSH at the pituitary level.

physiologically relevant in the second half of intrauterine life: luteinizing hormone (LH) takes over placental human chorionic gonadotropin (hCG) to regulate Leydig cell proliferation and androgen and INSL3 secretion, whereas follicle-stimulating hormone (FSH) plays a major role in Sertoli cell proliferation and AMH and inhibin B secretion [6,7].

After a transient perinatal decline (Fig. 2), all pituitary–testicular axis hormone levels increase during the first month of life [4▪,8]. FSH, LH, testosterone and INSL3 peak in the 2nd to 3rd months and subsequently decline to very low levels after the 6th month [4▪,9,10▪]. Conversely, Sertoli cells remain functionally active as reflected by the elevated serum levels of AMH [11,12▪] and inhibin B [13]. The decrease in gonadotrope activity during childhood does not seem to be dependent on a negative feedback by testicular factors, as it also occurs in anorchid boys [14▪].

Pubertal development of the testes occurs following the progressive increase in gonadotropin pulse amplitude and frequency. Androgen concentration increases within the testis long before it does in serum [15,16▪▪] and provokes Sertoli cell maturation. Germ cells enter meiosis, giving rise to sperm production. Spermatogenic development is the main responsible factor for testis volume increase during puberty. FSH and germ cells induce an increase in inhibin B, the major negative feedback regulator of pituitary FSH secretion (Fig. 1). INSL3 secretion also increases during puberty but becomes gonadotropin-independent once adult Leydig cells become fully differentiated [4▪,17].

Ontogeny of hormone receptor expression in the testis

The FSH receptor is present in Sertoli cells, whereas the LH/chorionic gonadotropin receptor is expressed in Leydig cells. Steroid hormone receptors have a variable expression pattern in the different cell populations of the testes throughout development. The androgen receptor is expressed in peritubular cells and Leydig cells from the early foetal life through adulthood, but only after mid-infancy in Sertoli cells [18,19]. Oestrogen receptor α is expressed at low levels in Leydig and peritubular cells throughout life, whereas oestrogen receptor β is present in germ, Sertoli and peritubular cells, and in

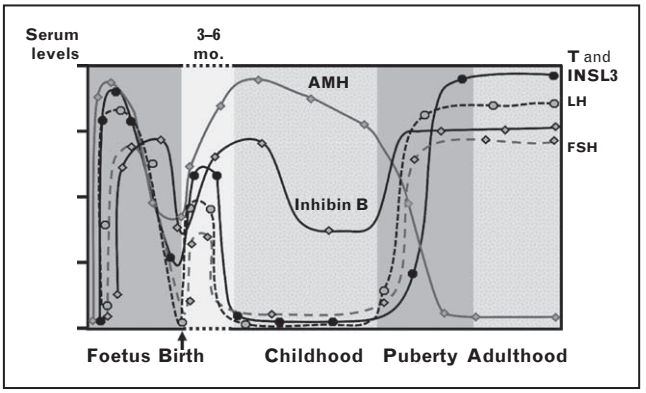

FIGURE 2. Schematic ontogeny of circulating levels of gonadotropins and testicular hormones in men. Modified with permission from [30]. Copyright 2010, S Karger AG, Basel.

a lesser amount in interstitial cells of the neonates [20]. The receptor for INSL3 is expressed in spermatocytes and spermatids and, to a lesser extent, in Leydig cells [21].

Paracrine regulation of testicular hormones: developmental aspects

High intratesticular testosterone levels and androgen receptor expression in Sertoli cells are necessary for Sertoli cell maturation, characterized by proliferation arrest [22], development of tight junctions involved in the formation of the blood-testis barrier [23,24▪,25▪] and a decrease in AMH production [26], as well as for germ cells to enter and achieve full meiotic divisions [27,28]. Interestingly, this does not occur in the foetal and neonatal periods owing to the lack of androgen receptor expression in Sertoli cells [18–20]. Serum testosterone is not always a good marker of intratesticular levels. For instance, at the beginning of puberty, intratesticular testosterone levels increase without being immediately reflected in serum, thus explaining why serum AMH decline is an early event in puberty [29]. Conversely, treatment with exogenous testosterone results in elevated serum levels but insufficient intratesticular androgen concentration, as revealed by lack of AMH downregulation and of adult spermatogenesis induction [30].

Oestrogen receptor signalling is required for normal spermatogenesis [16▪▪], but excessive oestradiol concentration is detrimental for Sertoli cells and spermatogenesis [16▪▪,31▪,32] and inhibits INSL3 expression in foetal Leydig cells [4▪].

BIOMARKERS OF PAEDIATRIC TESTICULAR ENDOCRINE DISORDERS

The assessment of the hypothalamic–pituitary–testicular axis depends on patient's age. During the first 3–6 postnatal months, basal levels of LH, testosterone and INSL3 are useful markers of the pituitary–Leydig cell axis, whereas FSH, AMH and inhibin B are useful markers of the pituitary–Sertoli cell axis (Fig. 1). For the rest of infancy and childhood, low or undetectable levels of LH, testosterone and INSL3 are uninformative (Fig. 2); dynamic tests involving stimulation with hCG or LH are required to evaluate Leydig cells. Conversely, Sertoli cells remain active: AMH and inhibin B are most useful markers of gonadal function in basal conditions (Fig. 2) [7] and in response to FSH treatment [33,34].

The elevation of serum gonadotropins may be a reliable sign of primary testicular failure during childhood; however, it is not always present, mainly if testicular failure is established after the age of 6 months [14▪]. After the age of 9 years, basal or stimulated gonadotropins [35], and basal testosterone and INSL3 become useful again. Yet, whereas testosterone is a marker of acute response to LH/hCG, INSL3 is no longer responsive to LH/hCG stimulation in the mature testis [4▪,5▪]. The tubular compartment function can be assessed by inhibin B levels, which reflect both FSH and germ-cell activities. AMH should decline to indicate adequate androgen action on Sertoli cells and spermatogenic development, it should be noted that the inhibitory effect of testosterone and germ cells prevails over the stimulatory effect of FSH [30].

THE DIAGNOSIS OF HYPOGONADISM IN PAEDIATRIC PATIENTS

As mentioned, the search for low androgen levels is inadequate for diagnosing hypogonadism in prepubertal boys. A broader definition of male hypogonadism, applicable to paediatric patients, should consider decreased testicular function, as compared to what is expected for age (Tables 1 and 2), involving an impaired hormone secretion by Sertoli cells (AMH and inhibin B), and/or Leydig cells (androgens and INSL3 in 0–6 months and after pubertal onset) and/or a disorder of spermatogenesis (which can only be assessed by testicular biopsy in prepubertal boys). Three aspects need to be considered. First, the level of the hypothalamic–pituitary–gonadal axis primarily affected, which leads to the classical classification of primary hypogonadism (testicular damage), central (hypothalamic–pituitary disorder) or combined. A particular aspect that needs to be considered is that, as opposed to adulthood, primary hypogonadism is rarely hypergonadotropic during childhood. Second, according to the testicular cell population primarily affected, the patient may present with a whole testicular failure, when there is a concomitant impairment of all testicular cell

Table 1. Primary hypogonadism of known cause in prepubertal men

	Early infancy						Late infancy – childhood					
	LH	FSH	T	INSL3	AMH	Inh B	LH	FSH	T	INSL3	AMH	Inh B
Whole gonadal dysfunction												
Gonadal dysgenesis												
Chromosome defects, gene mutations	H	H	L-ND	L-ND	L-ND	L-ND	N-H	N-H	L-ND	L-ND	L-ND	L-ND
Testicular dysgenesis syndrome												
Endocrine disruptors	N	H	L-ND	n.a.	L-ND	L-ND	N	N	N-L	n.a.	n.a.	L
Vanishing testes	H	H	L-ND	L-ND	L-ND	L-ND	N	N	L-ND	L-ND	L-ND	L-ND
Klinefelter syndrome, XX male; Mulibrey syndrome	N	N	N	N	N	N	N	N	N	N	N	N
Prader–Willi syndrome	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	N	N	N	n.a.	L	L
Orchitis, testicular torsion or trauma, surgery (gonadal, inguinal)	H	H	L-ND	n.a.	L-ND	L-ND	N	N	L-ND	n.a.	L-ND	L-ND
Down syndrome	H	H	L	n.a.	L	L	N-H	N-H	N-L	n.a.	N-L	N-L
Chronic illnesses:												
Granulomatous diseases, renal failure, neurologic disorders, advanced cancer	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	N	N	N	n.a.	N-L	N-L
Dissociated gonadal dysfunction												
Leydig cells												
LH/CG receptor or steroidogenic protein mutations	H	H	L-ND	n.a.	N-H	L-ND	N	N	L-ND	n.a.	N-H	N
INSL3 mutations	N	N-H	N	ND		N-L	N	N	N	ND	N	N
Sertoli cells												
FSH-R mutation	N	H	N	n.a.	L	L	N	N	N	n.a.	L	L
AMH mutation	N	N	N	n.a.	ND	N	N	N	N	n.a.	ND	N
Chemotherapy												
Abdomino-pelvic radiotherapy	N-H	H	N-L	n.a.		L	N	N	N	n.a.	N-L	N-L

L, low; N, normal; H, high as compared to male reference range for age; T, testosterone. FSH, follicle-stimulating hormone; LH, leutenizing hormone; ND: nondetectable. n.a.: data not available.

Table 2. Central and combined (primary + central) hypogonadism of known cause in prepubertal men

	Early infancy						Late infancy – childhood					
	LH	FSH	T	INSL3	AMH	Inh B	LH	FSH	T (*)	INSL3	AMH	Inh B
Congenital panhypopituitarism												
Isolated hypogonadotropic hypogonadism	L	L	L	L	L	L	N	N	L-N	L-N	L	L
Anosmic (Kallmann syndrome)/ normosmic												
Acquired panhypopituitarism												
CNS tumours, radiotherapy, surgery, trauma	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	N	N	N	n.a.	N	N
Prader–Willi syndrome												
X-linked adrenal hypoplasia congenita	L	L	L	n.a.	L	L	N	N	N	n.a.	L	L
Cranial radiotherapy + chemotherapy												
Total body irradiation												
Lead intoxication	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	N	N	N	n.a.	N	N

L, low; N, normal; H, high as compared to male reference range for age; T, testosterone. FSH, follicle-stimulating hormone; LH, leutenizing hormone; ND: nondetectable. n.a.: data not available.

populations, or with dissociated testicular failure, when only one testicular compartment (seminiferous tubules or interstitial tissue) is primarily involved, eventually affecting the other cell populations secondarily [36▪]. As Leydig cell function and spermatogenesis are mostly inactive during childhood, the Sertoli cell population needs to be particularly assessed. Finally, the period of life when hypogonadism is established leads to specific clinical features. For instance, foetal-onset hypogonadism results in disorders of sex development (DSD) presenting with ambiguous or female genitalia when established in the first trimester of gestation, but in micropenis and cryptorchidism with no genital ambiguity when established in the last half of gestation, childhood-onset hypogonadism may go underdiagnosed until pubertal age. A detailed review with an extended classification of male hypogonadism, including paediatric ages, has recently been published [36▪]. Here, we will first give a general overview of established issues concerning paediatric male hypogonadism, with special focus on the biomarkers used for diagnosis, and then address with more detail recent relevant findings related to biomarkers of the prepubertal testis.

Primary hypogonadism with whole gonadal dysfunction

Congenital primary hypogonadism with whole gonadal dysfunction is characterised by low Sertoli (AMH and inhibin B) and Leydig (testosterone and INSL3) cell hormone levels associated with elevated gonadotropins in the first months of life (Table 1). During the rest of childhood, AMH and inhibin B remain low, and represent the most conspicuous biomarkers. Well established examples are gonadal dysgenesis, leading to ambiguous genitalia [2], and late-foetal testicular regression resulting in micropenis and hypoplastic scrotum [30]. Although genetically determined, Klinefelter syndrome is characterized by normal gonadotropins, AMH, inhibin B, testosterone and INSL3 until mid-puberty. Then, Sertoli cells deteriorate, resulting in low or undetectable AMH and inhibin B and high FSH. Leydig cell dysfunction is milder [37,38▪]. The situation is similar in XX males [36▪,39,40].

Acquired primary hypogonadism may result from miscellaneous conditions [36▪]. Orchitis, testicular trauma or torsion and surgical treatment of the inguinal or genital regions (e.g. for cryptorchidism), granulomatous diseases, advanced cancer before chemotherapy, and renal failure can lead to reduced testicular hormones; an increase in serum gonadotropins is observed only after the age of puberty.

Primary hypogonadism with dissociated gonadal dysfunction

LH/CG receptor mutations or defects in steroidogenic proteins result in Leydig cell-specific primary insufficiency during foetal life leading to genital undervirilization (Table 1). However, Sertoli cell function is not impaired. Serum testosterone is low, but AMH is within the normal male range or higher [1,2]. Gonadotropin levels may be slightly elevated in the first months of life but they are usually normal during childhood and increase again at pubertal age [41], providing another example in which primary hypogonadism is not hypergonadotropic in childhood. In primary Sertoli cell-specific disorders, AMH and/or inhibin B are low. At pubertal age, androgen levels increase normally and FSH may reach abnormally high levels (Table 1).

Central hypogonadism

Congenital central hypogonadism can be detected in the critical first 3–6 months of life [42]. Gonadotropins, testosterone, AMH and inhibin B are low [34]. During childhood, basal gonadotropins and testosterone are no longer useful. However, the persistently low levels of serum AMH and inhibin B are helpful for the diagnosis (Table 2). These patients fail to enter puberty by 14 years of age. Very low basal FSH is diagnostic of central hypogonadism; when FSH is borderline a gonadotropin-releasing hormone (GnRH) infusion test is helpful [35]. The association of low gonadotropins with low testosterone, AMH and inhibin B is also helpful to distinguish these patients from those with constitutional delay of puberty [43,44].

Acquired central hypogonadism, usually associated with other pituitary hormone deficiencies, may result from tumours or infiltrative lesions disrupting the hypothalamic–pituitary axis, surgical or radiant therapy of the primary lesions or cranial trauma (Table 2). AMH and inhibin B are not significantly affected, and the diagnosis of central hypogonadism is usually delayed until pubertal age.

Combined hypogonadism

Certain patients with low serum AMH and inhibin B and defective response to hCG, indicative of a primary testicular failure, do not show an elevation of gonadotropins at pubertal age, indicating a concomitantly impaired function of the gonadotrope (Table 2). An example is the X-linked form of adrenal hypoplasia congenita associated with combined hypogonadism resulting from mutations in the DAX1 gene, a transcription factor acting at several levels of the pituitary–gonadal and adrenal axes [45].

BIOMARKERS OF THE PREPUBERTAL TESTICULAR FUNCTION: RECENT ADVANCES

The most recent widespread use of Sertoli cell markers has broadened our knowledge on the pathophysiology and diagnosis of prepubertal male hypogonadism.

Effect of oncologic treatment on prepubertal testicular function

Pituitary-testicular hormone levels are normal in untreated patients recently diagnosed with leukaemia or solid tumours in prepuberty and early puberty, whereas low inhibin B with slightly increased FSH can be observed in patients diagnosed at advanced puberty, indicating a tubular dysfunction [46]. Seminiferous tubules are more sensitive than Leydig cells to most oncologic treatments, although Leydig cell function may also be impaired with high doses [47▪]. Serum AMH and inhibin B may be low-to-normal, and testosterone is normal after pubertal onset. The differences may be due to the type of radio/chemotherapy used. Gonadotropins are within the normal range in prepubertal boys and increase above the normal range only in those pubertal patients with permanent testicular damage [47▪,48,49▪]. Conditioning for haematopoietic cell transplantation, using total body irradiation and cyclophosphamide, busulphan or melphalan, affects whole gonadal function in approximately 1/3 of the patients and only tubular function, that is decrease in inhibin B and AMH, in almost half of the cases. A minor proportion of boys do not show testicular impairment [50▪].

Genetic syndromes

Unlike Klinefelter syndrome, the somatic aneuploidy of Trisomy 21 (Down syndrome) results in early-onset primary hypogonadism in a large proportion of cases. Serum AMH is low from infancy; at puberty, testosterone reaches low-to-normal levels but with high LH indicating a compensated Leydig cell dysfunction [12▪].

Mulibrey nanism is caused by mutations in the TRIM37 gene, mapping to 17q22–23. Like in Klinefelter syndrome, boys have normal FSH, LH, testosterone and inhibin B until mid-puberty when inhibin B and testosterone levels decrease and FSH – and to a lesser extent LH – progressively increase to hypergonadotropic levels [51].

In Noonan syndrome, cryptorchidism occurs in approximately two out of three cases. During childhood, reproductive hormones are within the expected range. Pubertal onset is delayed, but gonadotropins, inhibin B, testosterone and oestradiol increase during early puberty. By mid-to-late puberty, gonadotropin levels increase over the normal range and AMH and inhibin B decline to subnormal levels in patients with a history of cryptorchidism but remain within normal levels in those with descended testes [52].

Patients with Prader–Willi syndrome have hypogonadism leading to small genitalia and arrested pubertal development, classically attributed to hypothalamic dysfunction. However, recent investigations have demonstrated that the disorder may follow different patterns in prepubertal and pubertal males: central hypogonadism with low LH and testosterone associated with low FSH and inhibin B [53▪▪]; primary hypogonadism, with low inhibin B, AMH and testosterone levels associated with normal to moderately elevated gonadotropin [53▪▪,54,55▪▪]; or a combined form of hypogonadism, with low testicular hormones and inadequately normal gonadotropins [53▪▪,56].

Boys born preterm or small for gestational age

Although preterm birth and being born small for gestational age have been associated with impaired reproduction rates, recent studies could not evidence abnormalities in serum levels of gonadotropins, testosterone, AMH, or inhibin B [57,58], although the neonatal axis activation seems somewhat delayed and exaggerated [10▪].

Boys conceived by assisted reproductive technology

Another issue of concern has been the testicular function of boys conceived by assisted reproduction technologies, mainly when used to overcome infertility because of a male factor. Except for a subtle decrease in serum testosterone observed during the neonatal activation period [59], no other abnormalities have been observed in testicular hormones or pubertal development [60–62].

Cryptorchidism

Cryptorchidism is a sign that can be present in many disorders of different causes, most of which remain elusive [63,64]. Mutations in INSL3 or its receptor explain less than 1% of the cases [63].

Dissociated testicular dysfunction primarily affecting the tubular compartment seems to be the underlying pathophysiology in cases presenting with low AMH [65] and inhibin B [66,67] but with normal testosterone and INSL3 [68] during early

infancy and childhood. FSH may be in the upper normal range or slightly elevated above normal in the first months after birth [14▪,66], but decrease to normal levels during childhood and may increase again over the normal range from the onset of puberty [14▪]. This situation is frequent but the underlying causes have not been identified. Leydig cells seem to be less sensitive, but low testosterone levels associated with elevated LH, has also been reported in a proportion of cryptorchid infants [66]. Finally, others could not detect significant changes in hormone levels of cryptorchid boys [69▪▪]. The apparently contradictory results are most probably because of the heterogeneity of the cryptorchid patients with underlying conditions of different causes and prognoses [70]. Although more invasive, germ count in testicular biopsies may be a better predictor. Recent studies have highlighted that cryptorchid testes with higher germ-cell counts have better prognosis than those with reduced cell counts [71] and that germ-cell numbers decline with the persistence of the gonad in cryptorchid position [69▪▪].

Bilateral cryptorchidism with nonpalpable gonads should be distinguished from anorchia. Newborns with congenital anorchia may have micropenis, reflecting the lack of testosterone in late foetal life. AMH, inhibin B, INSL3 and testosterone are undetectable, and do not respond to gonadotropin stimulation. FSH and LH are high in the first months or years of life, but then decrease – even to normal prepubertal levels in many cases – before increasing to extremely high levels at puberty [14▪]. In acquired anorchia, gonadotropin levels are usually within normal levels in childhood, but an increase response to GnRH stimulation may be observed.

Testicular dysgenesis syndrome and endocrine disruptors

Increasing rates of cryptorchidism, hypospadias and testis cancer, together with a decline in sperm concentration are thought to be associated with each other. The association, named 'testicular dysgenesis syndrome', is considered to originate from a disturbed testicular development in foetal life possibly due to exposure to environmental chemicals with endocrine disrupting properties, including oestrogenic and/or antiandrogenic activities [72].

The original 'oestrogen hypothesis' [73] pointed to an increased oestrogen exposure of the human foetus or neonate. Although many new environmental oestrogens have been identified in the last two decades, the vast majority seem to have weak oestrogenicity, thus arguing against the possibility that they could induce the claimed disorders. Furthermore, men exposed to massive doses of the orally active synthetic oestrogen diethystilbestrol (DES) *in utero* in the 1950s, though showing a three-fold increased risk of genital malformations, were as fertile as other men [74]. The issue remains controversial, with detractors underscoring the extremely high doses of the potential endocrine disruptors used to induce gonadal lesions in experimental conditions, and supporters insisting on the additive effects of mixtures of environmental chemicals, present each in low concentrations [75]. We summarize hereafter those recent findings in which an effect on testicular biomarkers has been reported in paediatric patients.

Phthalates are used as plasticizers and found in personal care products (e.g. hair sprays), and certain dietary supplements, medications, food packaging, home furnishings and medical equipment [76]. Phtalates affect Sertoli cell [77,78] and Leydig cell function – including androgen [79,80▪] and INSL3 production [79] – as well as germ-cell survival [81]. Cryptorchidism, associated with biomarkers of insufficient foetal androgenization like reduced anogenital distance and penile length, have been found in infants in relation to several phthalate metabolites measured in third-trimester maternal urine [82,83▪▪]. In line with the potential role of reduced androgen action as the underlying pathogenesis in phthalate-associated defects in male reproductive organs, significant decreases in serum-free testosterone and Leydig cell function have been reported in infants exposed to phthalate-bearing maternal breast milk [84].

Bisphenol A is used in the manufacture of polycarbonate plastics (e.g. water bottles), epoxy resins (e.g. food cans) and thermal paper. It has a weak oestrogenic activity [76], inhibits androgen biosynthesis in Leydig cells [85▪] and has a deleterious effect on Sertoli cells [86]. Another link between bisphenol A and testicular disorders may be explained by recent findings indicating that the nonclassical membrane G-protein coupled estrogen receptor (GPER/GPR30) mediates bisphenol effects in testicular germ cells [87] and that Sertoli cell gap junctions involved in the formation of the blood–testis barrier at puberty are perturbed by exposure to bisphenol A or ethynyloestradiol [25▪].

Contemporary used pesticides are nonpersistent, that is designed to break down in hours or days; however, many of these pesticides can remain for years if applied in indoor environments protected from sunlight, moisture and other degradation mechanisms [76]. Pesticides have been shown to exert both oestrogenic [88] and antiandrogenic [89] activities. Sons of women occupationally exposed to

nonpersistent pesticides in early pregnancy have an increased prevalence of congenital cryptorchidism, lower serum testosterone in the neonatal activation period in line with smaller penile size, and Sertoli cell dysfunction markers like lower inhibin B and higher FSH than boys of unexposed mothers. Low inhibin B levels persist through prepuberty [90▪▪].

CONCLUSION

The evaluation of the hypothalamic–pituitary–testicular axis function should be performed taking into account its normal ontogeny. The first 3–6 months of life are precious for an early diagnosis. Thereafter, basal gonadotropins and Leydig cell hormones are normally very low until the onset of puberty. Therefore, direct biomarkers of Sertoli cells, that is serum AMH and inhibin B, are essential tools in infancy and childhood. When gonadotrope or Leydig cell function evaluation is required, dynamic tests are necessary.

The use of prepubertal testicular markers became widely available in the last decade, and has produced a substantial increase in our knowledge of the pathophysiology and diagnosis of many congenital and acquired conditions. For instance, beyond the well known effect of oncologic treatments on pubertal spermatogenesis, new biomarkers have shown that Sertoli cell function may also be affected. In the sex-chromosome, aneuploidy-related Klinefelter syndrome primary hypogonadism is not evident until mid-puberty, whereas in the autosomal aneuploidy-derived Down syndrome primary hypogonadism is present from early infancy. In Noonan syndrome, the occurrence of primary hypogonadism depends on the existence of cryptorchidism, and Prader–Willi syndrome may result in diverse forms of male hypogonadism. Prepubertal testicular markers have also provided insights into the effects of environmental disruptors on gonadal function from early life, and has also helped dissipate concerns about testicular function in boys born preterm or small for gestational age or conceived by assisted reproductive technique procedures.

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Conflicts of interest

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