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Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach

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SUMMARY

Normal testicular physiology results from the integrated function of the tubular and interstitial compartments. Serum markers of interstitial tissue function are testosterone and insulin-like factor 3 (INSL3), whereas tubular function can be assessed by sperm count, morphology and motility, and serum anti-Müllerian hormone (AMH) and inhibin B. The classical definition of male hypogonadism refers to testicular failure associated with androgen deficiency, without considering potential deficiencies in germ and Sertoli cells. Furthermore, the classical definition does not consider the fact that low basal serum testosterone cannot be equated to hypogonadism in childhood, because Leydig cells are normally quiescent. A broader clinical definition of hypogonadism that could be applied to male patients in different periods of life requires a comprehensive consideration of the physiology of the hypothalamic-pituitary-testicular axis and its disturbances along development. Here we propose an extended classification of male hypogonadism based on the pathophysiology of the hypothalamic-pituitary-testicular axis in different periods of life. The clinical and biochemical features of male hypogonadism vary according to the following: (i) the level of the hypothalamic-pituitary-testicular axis primarily affected: central, primary or combined; (ii) the testicular cell population initially impaired: whole testis dysfunction or dissociated testicular dysfunction, and: (iii) the period of life when the gonadal function begins to fail: foetal-onset or postnatal-onset. The evaluation of basal testicular function in infancy and childhood relies mainly on the assessment of Sertoli cell markers (AMH and inhibin B). Hypergonadotropism should not be considered a *sine qua non* condition for the diagnosis of primary hypogonadism in childhood. Finally, the lack of elevation of gonadotropins in adolescents or adults with primary gonadal failure is indicative of a combined hypogonadism involving the gonads and the hypothalamic-pituitary axis.

INTRODUCTION

Normal testicular physiology results from the integrated function of the tubular and interstitial compartments. From the early ages of male reproductive endocrinology, and despite the limited knowledge existing at that time on the hypothalamic-pituitary-gonadal axis, pioneering authors like Albright *et al.* (1941), Heller & Nelson (1948) and Hellinga (1957) have stressed the importance of considering both testicular compartments and the age at onset of the disorder for the correct diagnosis and treatment of male hypogonadism. Since then, substantial progress has been made on the physiology and pathophysiology of the hypothalamic-pituitary-gonadal axis, resulting in the development of useful tools for the diagnosis and treatment of male hypogonadism. The most widely used biomarkers of interstitial tissue function are serum testosterone and – more recently – insulin-like factor 3 (INSL3) levels, reflecting Leydig cell activity. Tubular function can be assessed by sperm count, morphology and motility, reflecting the germ cell population, and the determination of serum anti-Müllerian hormone (AMH) and inhibin B, as markers of Sertoli cell function. Nevertheless, the term male hypogonadism is most frequently applied in the adult to describe testicular failure associated with androgen deficiency, which reveals primary or secondary insufficient Leydig cell function, with less attention to potential deficiencies in the other testicular cell populations. Furthermore, so defined, the term hypogonadism does not consider either the normal

ontogeny of the hypothalamic-pituitary-gonadal axis. In fact, in infancy and childhood, low basal androgen production cannot be equated to hypogonadism as Leydig cells are quiescent and Sertoli cells are the most active testicular cell population. Therefore, a broader clinical definition of hypogonadism that could be applied to male patients in different periods of life requires a comprehensive consideration of the physiology of the hypothalamic-pituitary-testicular axis and its disturbances along development. In this review, we will discuss a classification of male hypogonadism based on the pathophysiology of the hypothalamic-pituitary-testicular axis in different periods of life, with a special focus on the foetal and pre-pubertal periods. We do not aim to make an exhaustive description of all the possible causes of hypogonadism, but rather to propose an extended view to classify these causes using a pathophysiological and developmental approach and giving illustrative examples.

FUNCTIONAL ONTOGENY OF THE PITUITARY-TESTICULAR AXIS

Foetal development

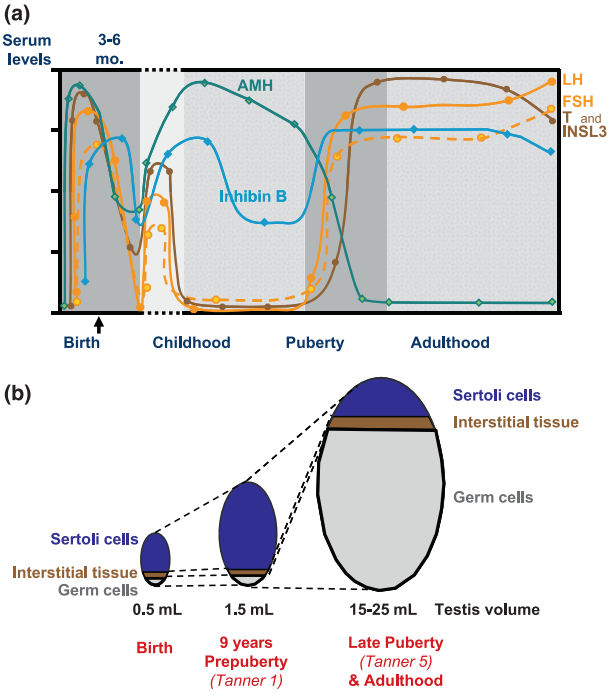
In the male foetus, testes differentiate by the end of the fifth embryonic week (7 weeks of amenorrhoea) before the gonadotropes are functionally active. Sertoli cells secrete AMH, responsible for the regression of Müllerian ducts. Leydig cells secrete testosterone and INSL3. Androgens induce the differentiation of the Wolffian ducts, and the virilization of the urogenital sinus and external genitalia. In this early stage of life, pituitary insufficiency does not affect male sexual differentiation. Conversely, in the second half of gestation, foetal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) become major regulators of testicular physiology (Petersen & Söder, 2006). LH controls Leydig cell function: androgens and INSL3 are responsible for testis descent (Ivell & Hartung, 2003) whereas androgens also have a trophic effect on the size of the penis and the scrotum. FSH induces Sertoli cell proliferation and AMH and inhibin B secretion. Altogether, these physiological events explain the occurrence of micropenis, microorchidism, cryptorchidism and hypoplastic scrotum in male newborns with gonadotropin deficiency. A decline in pituitary and testicular hormones is observed towards term [Fig. 1(A)].

Primordial germ cells are of extra-gonadal origin and migrate to enter the gonadal ridge and aggregate with the differentiating Sertoli and peritubular cells to form the seminiferous cords. Germ cell differentiation, migration and proliferation/apoptosis are regulated by a large number of factors (Wylie, 1999; Pentikäinen *et al.*, 2003; Matzuk & Lamb, 2008).

Infancy and childhood

At birth, pituitary and testicular hormones are transiently low, but their levels increase from the first week of life (Bergadá *et al.*, 2006); gonadotropins and testosterone remain high for 3–6 months, whereas AMH and inhibin B levels persist being elevated during infancy and childhood [Fig. 1(A)]. The hypothalamic-pituitary-testicular axis can be evaluated by measuring basal serum levels of gonadotropins, testosterone, INSL3, AMH and inhibin B. Although clinically unperceivable by palpation, testis size increases in early infancy (Goede *et al.*, 2011) mainly due to the proliferation of Sertoli cells (Nistal *et al.*, 1982; Müller & Skakkebæk, 1983) [Figs 1(B) and 2]. Interestingly, the elevated

Figure 1 A. Schematic ontogeny of circulating levels of gonadotropins and testicular hormones in male patients. Modified with permission from Grinspon RP, Rey RA. Anti-Mullerian hormone and Sertoli cell function in paediatric male hypogonadism. Horm Res Paediatr 2010;73:81–92. Copyright 2010, S Karger AG, Basel. B. Schematic ontogeny of the testicular volume. Seminiferous tubules (Sertoli + germ cells) are always the major components of the testis. From birth and during the whole pre-pubertal period (i.e., until ages 9–14 years, Tanner stage 1), seminiferous tubule volume depends mainly on Sertoli cells, whereas the significant increase in testicular volume during pubertal development (i.e., between Tanner stages 1 and 5) is mainly due to germ cell proliferation. Modified with permission from Rey R. Regulation of spermatogenesis. Endocr Dev 2003;5:38-55. Copyright 2003, S Karger AG, Basel.



levels of intratesticular testosterone cannot induce meiosis in the foetal and neonatal testis most probably due to the lack of androgen receptor expression in Sertoli cells at those developmental periods (Berensztein *et al.*, 2006; Chemes *et al.*, 2008; Boukari *et al.*, 2009; Rey *et al.*, 2009). Subsequently, the activity of the axis decreases substantially; however, AMH (Aksglæde *et al.*, 2010; Grinspon *et al.*, 2011) and inhibin B production (Bergadá *et al.*, 1999; Andersson & Skakkebæk, 2001) persists being active. Histologically, seminiferous cords do not have a lumen and are filled with Sertoli cells and spermatogonia that do not enter meiosis, whereas typical Leydig cells soon disappear from the interstitial tissue (Fig. 3).

In summary, during late infancy and childhood a physiological state of low gonadotropin and androgen secretion exists as compared to the foetal, early infantile, pubertal and adult periods. However, Sertoli cells remain active, clearly ruling out the classical view that childhood is characterized by a hypogonadal state (Grinspon & Rey, 2010). Therefore, the hypothalamic-pituitary-testicular axis function can be assessed by determining basal serum levels of AMH and inhibin B. Interestingly, in this period of life inhibin B levels reflect Sertoli cell activity even in the absence of germ cells (Andersson *et al.*, 1998). To evaluate Leydig cell function, stimulation with hCG is necessary.

Figure 2 Evolution of testicular volume, seminiferous tubule length and diameter and Sertoli and germ cell numbers in the pre-pubertal Cebus monkey testis. Reprinted with permission from Rey R. The pre-pubertal testis: a quiescent or a silently active organ? Histol Histopathol 1999;14:991–1000. Copyright 1999, Giménez-Godoy SA, Murcia.

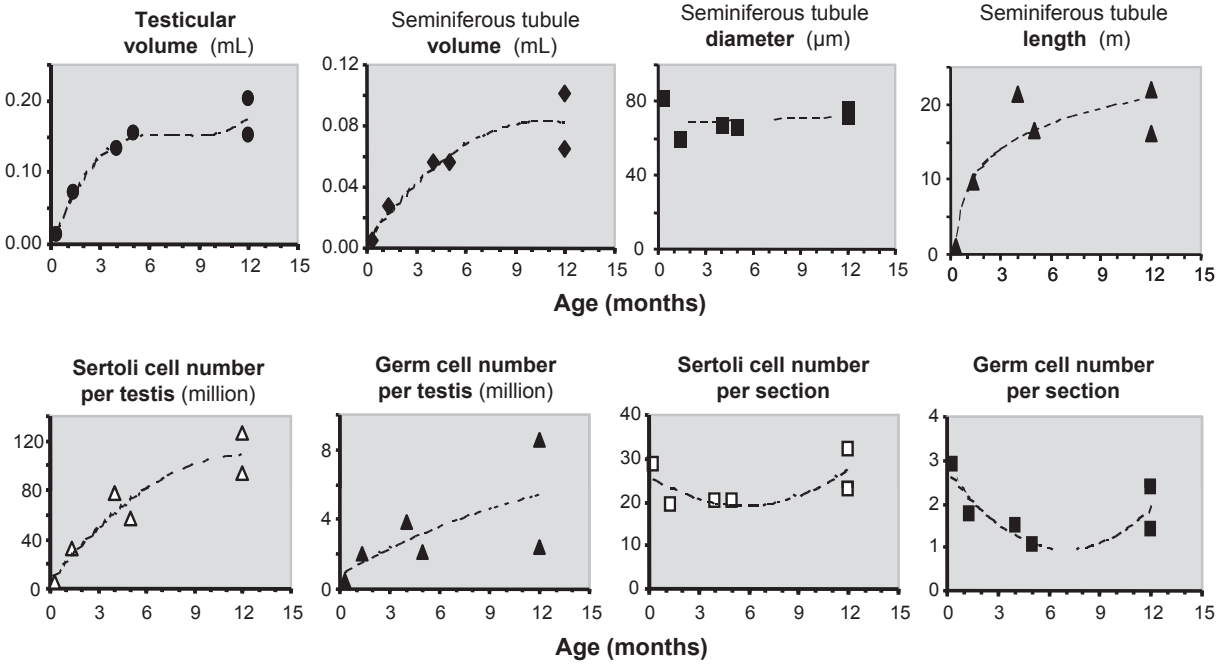
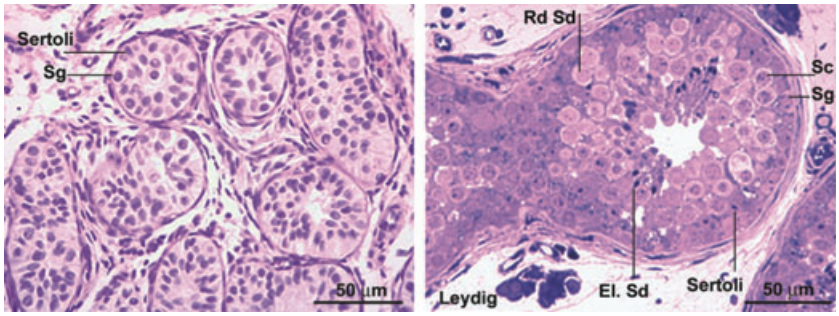


Figure 3 Testicular histology: in the pre-pubertal boy (left), seminiferous cords are solid and mainly composed of Sertoli cells; germ cells are limited to spermatogonia (Sg) and no typical Leydig cells are observed in the interstitial tissue. In the late pubertal and adult testis (right), the seminiferous tubules are larger, with a lumen and populated mainly by germ cells including spermatogonia (Sg), spermatocytes (Sc) and round (Rd Sd) and elongated (El Sd) spermatids; typical Leydig cells are observed in the interstitial tissue.



Puberty and adulthood

The increase of gonadotropin pulse amplitude and frequency drives pubertal development of the gonads. FSH provokes a new wave of Sertoli cell proliferation and LH induces the appearance of mature Leydig cells again (Fig. 3). Testosterone concentration increases within the testis before it is reflected in circulation (Pasqualini *et al.*, 1981), and provokes Sertoli cell maturation, characterized by increase in cell size, proliferation arrest (Chemes *et al.*, 1979) and down-regulation of AMH (Josso *et al.*, 2006) [Fig. 1(A)]. Sertoli cell proliferation and the subsequent increase in cell size are probably responsible for the initial, modest enlargement of the testes that characterize the beginning of puberty in male adolescents. Clinically, the onset of puberty is defined by a testicular volume ≥ 4 mL. The subsequent increase of gonadal size to a final volume of 15–25 mL is dependent on normal spermatogenic development. The seminiferous tubules acquire a lumen and their diameter increase significantly (Fig. 3). Spermarche usually occurs in boys between 12 and 15 years of age, with a median testicular volume of 10–12 mL, at Tanner stages 2–3, preceding the age of pubertal peak height velocity by approximately 6 months (Nielsen *et al.*, 1986).

Inhibin B secretion increases during puberty [Fig. 1(A)], regulated by FSH and germ cells (Jensen *et al.*, 1997). Adult levels of circulating inhibin B are attained as early as Tanner stage 2 in boys (Andersson *et al.*, 1997; Trigo *et al.*, 2004), in coincidence with the increase in serum LH and intratesticular testosterone. Thereafter, inhibin B levels remain constant up to the end of puberty. Inhibin B is the major negative regulator of FSH secretion in the adult male.

The achievement of adequate sperm output by the testis implies a spermatogenic process that is normal both qualitatively and quantitatively. The total number of Sertoli cells

present in the testis has a direct effect on quantitative sperm production in adult life. Because Sertoli cell proliferation is dependent on FSH, testes of male patients with hypogonadotropic hypogonadism have less Sertoli cells resulting in reduced germ cell numbers and small gonads. However, FSH is not absolutely essential for the qualitative completion of spermatogenesis. Spermatozoa are produced, although in a lesser number, in the absence of FSH signalling (Huhtaniemi, 2002).

Testosterone levels and an adequate expression of the androgen receptor in Sertoli cells are necessary for meiosis (Rey *et al.*, 2009). Testosterone withdrawal induces an arrest at the pachytene stage of meiosis I (Woolveridge *et al.*, 1999). It is noteworthy that intratesticular – rather than serum – testosterone levels regulate spermatogenesis. In fact, the administration of exogenous testosterone results in elevated serum levels, but insufficient androgen concentration within the testis – which can be estimated by measuring serum AMH – to induce adult spermatogenesis (Young *et al.*, 1999).

Beyond hormonal regulation, full spermatogenesis requires a normal chromosomal number and the expression of a large series of genes, mapping to sex chromosomes and autosomes, involved in the control of germ cell proliferation and apoptosis, chromosome pairing and synapsis, homologous recombination, genomic integrity and DNA replication and repair during meiosis, and in cell remodelling, cytoplasmic extrusion, chromatin packaging and nuclear condensation of spermatids (Matzuk & Lamb, 2008).

A BROADER DEFINITION OF MALE HYPOGONADISM

Contrasting with the classical definition of hypogonadism, limited to describe inadequate androgen production and applicable only in adult endocrinology, a more comprehensive clinical definition of hypogonadism refers to decreased testicular function, as compared to what is expected for age, involving an impaired hormone secretion by Leydig cells (androgens, INSL3) and/or Sertoli cells (AMH, inhibin B) and/or a disorder of spermatogenesis.

It is not within the scope of this review to address disorders of the male reproductive tract in eugonadal males, e.g. obstructive azoospermia, non-endocrine erectile dysfunction, epididymitis, prostatitis or end-organ resistance to testicular hormones.

A DEVELOPMENTAL PHYSIOLOGY-BASED CLASSIFICATION OF MALE HYPOGONADISM

Male hypogonadism can be classified according to the following:

- the level of the hypothalamic-pituitary-testicular axis primarily affected,
- the testicular cell population initially impaired, and
- the period of life when the gonadal function begins to fail.

Hypothalamic-pituitary (central), testicular (primary), or combined hypogonadism

Hypothalamic-pituitary (or central) hypogonadism is characterized by testicular failure owing to a central disorder affecting the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator or the pituitary gonadotrope (Brioude *et al.*, 2010). It is usually called hypogonadotropic hypogonadism, but we will see that central hypogonadism is not always hypogonadotropic.

Testicular (or primary) hypogonadism refers to the condition where the testis is initially affected. Because the negative feedback to the hypothalamic-gonadotrope axis is usually not functional, resulting in an elevation of FSH and/or LH mainly after puberty, this condition is usually known as hypergonadotropic hypogonadism. However, this rule does not apply to all cases of testicular hypogonadism, as we will discuss below.

Finally, certain disorders may impair both the hypothalamic-pituitary axis and the testis concomitantly, and provoke a combined hypogonadism. Here, testicular failure is not exclusively a consequence of gonadotropin deficiency.

Whole or dissociated testicular failure

Primary, central or combined hypogonadism may reflect the concomitant impairment of all testicular cell populations, i.e. a 'whole testicular failure or hypogonadism'. Conversely, when only one testicular compartment (seminiferous tubules or interstitial tissue) or only one cell population (Leydig, Sertoli or germ cells) is primarily affected, there is a 'dissociated testicular failure or hypogonadism'.

Foetal-, childhood-, pubertal-, and adult-onset hypogonadism

The clinical consequences of male hypogonadism depend on the period of life in which the function of the testis begins to fail. Foetal-onset hypogonadism established in the first trimester of gestation results in disorders of sex development (DSD) presenting with ambiguous or female genitalia, due to the lack of sufficient levels of testis hormones during the critical window of male sex differentiation (Rey & Grinspon, 2011). Gonadal dysgenesis is an example of whole gonadal failure, whereas Leydig cell aplasia/hypoplasia and steroidogenic defects are dissociated forms of early foetal-onset hypogonadism. As discussed earlier, central hypogonadism cannot result in genital ambiguity, as Leydig cell function in the first trimester of foetal life is regulated by placental hCG. Foetal hypogonadism – primary, central or combined – established in the second half of gestation typically results in micropenis and cryptorchidism.

Because the hypothalamic-pituitary-testicular axis remains active for 3–6 months after birth (Forest *et al.*, 1974; Kuiri-Hanninen *et al.*, 2011), this period represents a window of opportunity to establish the diagnosis of hypogonadism (Grumbach, 2005). Thereafter, the hypothalamic-gonadotrope function normally declines in the boy. The androgenic activity of the testis becomes so low that is clinically unperceivable and the proliferative activity of Sertoli and germ cells do not elicit clinically evident changes in testicular volume, as explained above. Therefore, male hypogonadism may remain unapparent when established during infancy or childhood. The condition should be suspected and actively sought for by the physician (e.g. measuring serum AMH or inhibin B in basal conditions, or testosterone after stimulation with hCG); otherwise the diagnosis is delayed until the age of puberty.

At pubertal age, hypogonadism is characterized by the absence or the arrest of pubertal development. Owing to androgen insufficiency, secondary sex characteristics do not develop, body proportions are typically eunuchoidal (upper/lower body ratio <1 with an arm span 6 cm >standing height), voice remains high-pitched, bone age is delayed, and testicular volume does not increase, reflecting lack or arrest of spermatogenesis.

When the hypogonadal state is established in adulthood, the most common features that reflect androgen deficiency are decreased libido, impotence and oligo- or azoospermia. Other findings may be fatigue, loss of bone and muscle mass, increased fat mass and related metabolic disorders, as well as the impairment of cognitive functions. Some ageing men develop a mild testosterone deficiency, and present with symptoms reminiscent of hypogonadism in young men. The syndrome has been named late-onset hypogonadism (LOH) (Wang *et al.*, 2009).

FOETAL-ONSET HYPOGONADISM

Foetal-onset primary hypogonadism

Foetal-onset primary hypogonadism with 'whole testicular dysfunction'

Gonadal dysgenesis resulting in female or ambiguous genitalia: Early onset foetal hypogonadism with whole testicular dysfunction (Dysgenetic DSD) results from abnormal gonadal morphogenesis or differentiation in the first trimester of foetal life (Rey & Grinspon, 2011). Irrespective of its aetiology (Table 1), gonadal dysgenesis can be complete, partial or extremely mild. Patients with streak gonads and female internal and external genitalia represent the so-called pure or complete gonadal dysgenesis. The streak gonads do not secrete testicular hormones. These girls usually seek for medical assistance owing to the absence of pubertal development. Partial testicular dysgenesis may present with various degrees of undervirilization of the external genitalia and Wolffian derivatives as well as cryptorchidism – reflecting Leydig cell dysfunction – together with persistence of müllerian derivatives – reflecting Sertoli cell dysfunction. The severity of the phenotype depends on the amount of functional testicular tissue (Rey & Grinspon, 2011). Vanishing or regression of testicular tissue in the second half of foetal life, after virilization of the genitalia has already occurred, results in micropenis and hypoplastic scrotum. Serum AMH, inhibin B and testosterone are undetectable, associated with very high gonadotropins soon after birth or from pubertal age through adulthood (Kubini *et al.*, 2000; Grinspon & Rey, 2010; Grinspon *et al.*, 2012). However, LH and FSH may fall within the normal pre-pubertal range at mid-childhood (Grinspon *et al.*, 2012).

In boys with isolated hypospadias, but normal penis size and scrotum with descended testes, AMH, inhibin B and testosterone are normal, indicating that there is no hypogonadism (Rey *et al.*, 2005).

Mild gonadal dysgenesis: Klinefelter syndrome, XX male, Trisomy 21: Autosomal or sex-chromosome aneuploidies are characterized by the presence of mild testicular dysgenesis not affecting foetal virilization (Table 2). The most well known are Klinefelter syndrome and its variants, sex-chromosome aneuploidies characterized by the presence of two or more X chromosomes associated with one or more Y chromosomes. The earliest sign of testicular dysgenesis is the existence of a markedly reduced or even the absence of germ cells from the newborn period (Müller *et al.*, 1995; Aksglæde *et al.*, 2006). Increased germ cell degeneration is associated with sex-chromosome aneuploidy (Sciurano *et al.*, 2009). Whereas Leydig cell dysfunction in neonates remains controversial, Sertoli cells look normal during whole childhood (Rey *et al.*, 2011).

At the onset of puberty, germ cell degeneration is massive (Aksglæde *et al.*, 2006). Scarce foci of germ cells may undergo meiosis in non-mosaic Klinefelter patients; these germ cells have a normal karyotype, most probably coming from XY spermatogonia having lost one X chromosome (Sciurano *et al.*, 2009). Sperm retrieval can be successful and lead to paternity. Gonadotropin and testicular hormone levels remain normal in boys until pubertal Tanner stage 3 (Fig. 4). Subsequently, Sertoli cell function deteriorates, resulting in extremely low or undetectable AMH, in coincidence with undetectable inhibin B and very high FSH levels (Rey *et al.*, 2011). Testosterone levels may rise to the normal adult range, but LH is extremely elevated, probably indicating a primary Leydig cell insufficiency (Bastida *et al.*, 2007), which frequently progresses to an overt hypoandrogenism requiring testosterone supplementation (Lanfranco *et al.*, 2004).

The situation is similar in XX males, with serum hormone levels in the normal male range during childhood (Rey *et al.*, 1999). Then, germ cells fail to progress through meiosis and undergo apoptosis at puberty leading to reduced testis volume and azoospermia. Testosterone secretion is most frequently subnormal or clearly low, and gonadotropins are elevated in the adult (Vorona *et al.*, 2007; Aksglæde *et al.*, 2008).

Another aneuploidy with primary hypogonadism and whole testicular dysfunction is trisomy 21 (Down syndrome). However, the situation is different in this autosomal aneuploidy as both the tubular and interstitial compartments are affected in a high proportion of patients from early infancy, as shown by low serum AMH (Fig. 5) and testosterone with elevated FSH and LH. At puberty, testosterone reaches low-normal levels but with high LH indicating a compensated Leydig cell dysfunction (Grinspon *et al.*, 2011). Adults are infertile due to decreased spermatogenesis (Hsiang *et al.*, 1987).

Cryptorchidism: Rather than a pathology, cryptorchidism is a clinical sign with many possible aetiologies. It may reflect a primary hypogonadal disorder with 'whole testicular dysfunction' or with 'Leydig cell-specific dysfunction' (e.g. mutations of INSL3, (Ivell & Hartung, 2003), but also a central hypogonadism, or even result from anatomical defects of the inguinal region or the abdominal wall not associated with hypogonadism. In cryptorchid boys, AMH (Misra *et al.*, 2002) and inhibin B (Suomi *et al.*, 2006; Gaudino *et al.*, 2008) may be low, indicating Sertoli cell dysfunction. Gonadotropins may be in the upper normal range or slightly elevated above normal in the first months after birth (Suomi *et al.*, 2006; Grinspon *et al.*, 2012), but decrease to normal levels during childhood and may increase again over the normal range from the onset of puberty (Grinspon *et al.*, 2012). AMH and inhibin B are expected to be normal in infants with abdominal wall or inguinal region defects or with impaired INSL3 signalling. AMH and inhibin B levels are low in patients with central hypogonadism, as discussed below (Young *et al.*, 1999, 2005; Andersson, 2000; Bougnères *et al.*, 2008).

Bilateral cryptorchidism with non-palpable 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 pre-pubertal levels in many

Table 1 Fetal-onset hypogonadism in the male

		Genitalia	Childhood					Puberty-Adulthood					
			LH	FSH	T	AMH	Inh B	LH	FSH	T	AMH	Inh B	Sperm
Primary hypogonadism	**Whole gonadal dysfunction**												
	Gonadal dysgenesis: Chromosome defects: Deletions of *Yp, 9p XX/XY, XY/X,* other mosaicisms Gene mutations: *SRY, CBX2, MAMLD1, SF1, WT1, SOX9, DHH, XH2, DHCR, TSPYL1,* etc. Endocrine disruptors	Female or undervirilised	N-H	N-H	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND	Azoosp.
	Vanishing testes, Testicular torsion	Micropenis, empty scrotum	N-H	N-H	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND	Azoosp.
	Klinefelter syndrome, XX male	Male	N	N	N	N	N	H	H	N-L	L-ND	L-ND	Azoosp.
	Dissociated gonadal dysfunction												
	Leydig cells												
	Hypoplasia/aplasia: *LH/CG-R* mutation Steroidogenic defects: *CYP11A1, CYP17A1, HSD3B2, POR, CYB5A, or HSD17B3* mutation	Female or undervirilised	N-H	N	L-ND	N-H	N	H	H	L-ND	N-H	L-ND	Azoosp.
	INSL3 mutations	Cryptorchidism	N	N	N	N	N	N	N-H	N		N-L	Oligosp.
	Sertoli cells												
	FSH-R mutation	Small testes	N	N	N	L	L	N	H	N	L	L	Oligosp.
	AMH mutation	PMDS	N	N	N	ND	N	N	N	N	ND	N	N
Central hypogonadism	**Whole gonadal dysfunction**												
	Multiple pituitary hormone deficiency: Pituitary development defects: *HESX1, LHX3, LHX4 or PROP1 mutation, Septo-optic dysplasia,* etc.	Micropenis, cryptorchdism	L	L	L	L	L	L	L	L	L	L	Oligosp./ azoosp.
	Isolated central hypogonadism: Normosmic: *GnRH, GnRH-R, Kiss1, GPR54, TAC3 or TACR3 mutation* Anosmic: *KAL1, PROK2, PROKR2 or NELF mutation* Syndromic: *FGF8, FGFR1 or CHD7 mutation*	Micropenis, cryptorchdism	L	L	L	L	L	L	L	L	L	L	Oligosp./ azoosp.
	Dissociated gonadal dysfunction												
	Multiple pituitary hormone deficiency: Pituitary development defects: *LHX4* mutation	Small testes	N	L	N	L	L	N	L	N		L	
	Isolated central hypogonadism: Normosmic: *TAC3 or TACR3 mutation*	Micropenis, cryptorchdism	L	N	L	N	N	L	N	L		L	Oligosp./ azoosp.
	LHβ mutation	Micropenis, cryptorchdism	L	N	L	N	N	L	H	L	H		Oligosp./ azoosp.
	FSHβ mutation	Small testes	N	L	N			H	L	N			Oligosp./ azoosp.
Combined hypogonadism	**Whole gonadal dysfunction**												
	Prader-Willi syndrome X-linked adrenal hypoplasia congenita	Micropenis, cryptorchdism	L-N	L-N	L-N	L	L	N	N	L	L	L	Oligosp./ azoosp.

L, N, H: Low, Normal, High as compared to male reference range for age. ND: non-detectable, PMDS: persistent Müllerian-duct syndrome.

Table 2 Postnatal-onset hypogonadism in the male

		Childhood					Puberty-Adulthood					
		LH	FSH	T	AMH	Inh B	LH	FSH	T	AMH	Inh B	Sperm
Primary hypogonadism	**Whole gonadal dysfunction**											
	Orchitis Testicular torsion or trauma	N	N	L-ND	L-ND	L-ND	H	H	L-ND	L-ND	L-ND	Oligosp./azoosp.
	Down syndrome	N-H	N-H	N-L	N-L	N-L	H	H	L	L	L	Azoosp.
	Varicocele	N	N	N	N	N	N-H	N	N-L	N	N	Teratozoosp./ asthenozoosp.
	Chronic illnesses: e.g. granulomatous diseases, amyloidosis, cystic fibrosis, chronic pulmonary disease, renal failure, neurologic disorders						H	H	L-ND	L-ND	L-ND	Oligosp./azoosp.
	Late-onset hypogonadism	Not applicable					N-H	N-H	L		L	
	Dissociated gonadal dysfunction											
	Y chromosome deletions and microdeletions: *AZF*	N	N	N	N	N	N	H	N		L	Oligosp./azoosp.
	Gene mutations: *CILD1*, *USP9Y*, etc.											
	Chemotherapy Abdominopelvic radiotherapy	N	N	N-L		N-L	N-H	H	N-L		L	Oligosp./azoosp.
	Pharmacological treatments: spironolactone, ketoconazole	N	N	N-L			N-H	N-H	L			Oligospermia
Central hypogonadism	**Whole gonadal dysfunction**											
	CNS and pituitary lesions: Tumours, histiocytosis, trauma, etc.	L	L	L	L	L	L	L	L	L	L	Oligosp./azoosp.
	Functional central hypogonadism: Impaired general health Acromegaly, Hypothyroidism Alcohol and drug abuse						L-N	L-N	L		L	Oligosp./azoosp.
Combined hypogonadism	**Whole gonadal dysfunction**											
	Cranial radiotherapy + chemotherapy Lead intoxication Marijuana consumption Total body irradiation	L-N	L-N	L-N	L	L	L-N	L-N	L	L	L	Oligosp./azoosp.

L, N, H: Low, Normal, High as compared to male reference range for age. ND: non-detectable.

cases – before increasing to extremely high levels at puberty (Grinspon *et al.*, 2012). In acquired anorchia, gonadotropin levels are usually within normal levels in childhood, but an increase response to GnRH stimulation may be observed.

Foetal-onset primary hypogonadism with Leydig cell-specific dysfunction: androgen deficiency

When foetal gonadal dysfunction primarily affects only Leydig cells, e.g. LH/hCG receptor mutations or defects in steroidogenic enzymes (Table 1), insufficient androgen production results in undervirilization and cryptorchidism. However, Müllerian ducts fully regress owing to normal Sertoli cell AMH secretion, and the patient has no uterus and a short blind-end vagina. Like for complete gonadal dysgenesis, these female patients usually seek medical attention because of the absence of pubertal development and primary amenorrhoea. Serum testosterone is undetectable (Mendonça *et al.*, 2010), but AMH is within the male range or higher (Rey *et al.*, 1999). Less severe Leydig cell defects may present with minimal undervirilization (micropenis and cryptorchidism). Serum testosterone may be detectable (Mendonça *et al.*, 2010), but AMH is always in the male range or higher (Rey *et al.*, 1999). Gonadotropin levels may be somewhat elevated in the first months of life, but they are usually normal during childhood. This is another example where primary hypogonadism is not hypergonadotropic before puberty. In the rare cases reported where the gonads are still present after pubertal onset, gonadotropins are usually elevated with a clear predominance of LH levels (Mendonça *et al.*, 2010).

Foetal-onset primary hypogonadism with Sertoli cell-specific dysfunction

AMH deficiency: Defects in AMH production result in the persistent Müllerian duct syndrome (PMDS) or male with uterus (Table 1). Patients are otherwise normally virilized, reflecting normal Leydig cell function. The persistence of the uterus and Fallopian tubes is an unpredicted finding at surgery for hernia or cryptorchidism. AMH is undetectable (Josso *et al.*, 2006), but inhibin B and androgens are in the normal male range (Kubini *et al.*, 2000). In the absence of longstanding cryptorchidism, testes contain germ cells but fertility is infrequent. PMDS should not be confused with testicular dysgenesis, where persistence of Müllerian derivatives is associated with external sexual ambiguity reflecting both AMH and androgen deficiency, i.e. an early onset foetal hypogonadism with whole gonadal dysfunction.

Figure 4 Circulating levels of gonadotropins, Leydig cell hormone (T, testosterone) and Sertoli cell hormones (AMH and inhibin B) in paediatric male patients with Klinefelter syndrome grouped by pubertal development (Tanner stage). Shaded areas represent normal ranges for Tanner stage. Red dots indicate patients with hormone levels indicating gonadal dysfunction. Reprinted, with John Wiley & Sons' permission, from Bastida MG, Rey RA, Bergadá I, Bedecarrás P, Andreone L, Del Rey G, Boywitt A, Ropelato MG, Cassinelli H, Arcari A, Campo S and Gottlieb S. Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. Clin Endocrinol 2007;67:863–70. Copyright 2007, Blackwell Publishing Ltd.

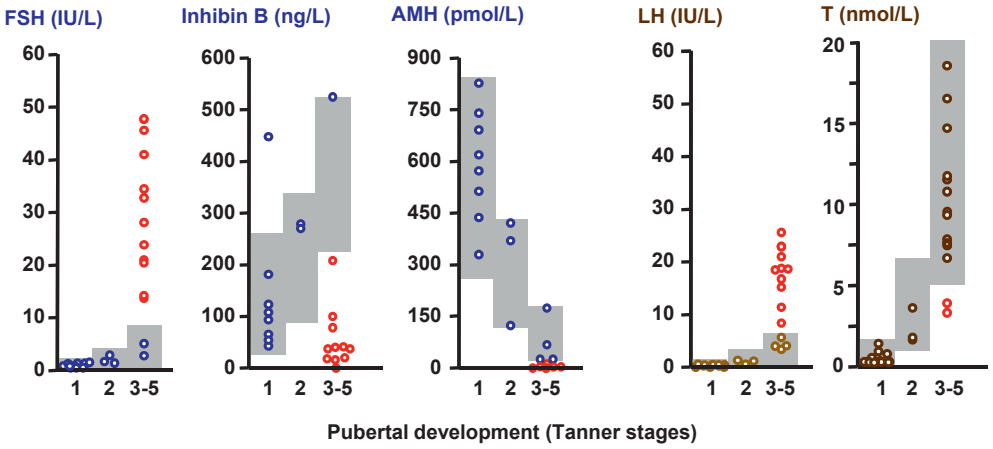
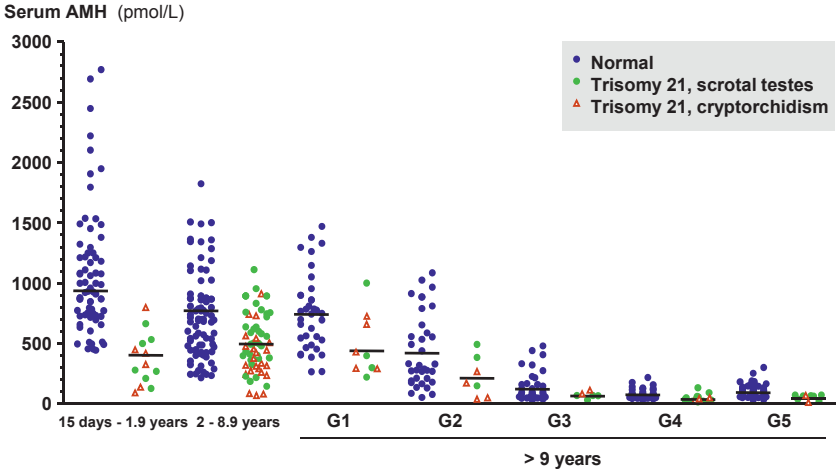


Figure 5 Serum AMH levels in male patients with trisomy 21 compared with age-matched normal males. The black lines represent the medians. Reprinted with permission from: Grinspon RP, Bedecarrás P, Ballerini MG, Iñiguez G, Rocha A, Resende EAMR, Brito VN, Milani C, Figueroa Gacitúa V, Chiesa A, Keselman A, Gottlieb S, Borges MF, Ropelato MG, Picard JY, Codner E, and Rey RA for the LAREP Group. Early onset of primary hypogonadism revealed by serum anti-Müllerian hormone determination during infancy and childhood in trisomy 21. International Journal of Andrology 2011, 34:e487–e498. Copyright 2011 European Academy of Andrology and the authors.



FSH receptor mutations: Because Sertoli cell differentiation in early foetal life is not dependent on FSH, male patients with FSH receptor mutations are normally virilized, but have small testes owing to reduced Sertoli cell proliferation secondarily resulting in low sperm count, low inhibin B and moderately elevated FSH (Tapanainen *et al.*, 1997).

Foetal-onset central hypogonadism

Foetal-onset central hypogonadism with 'whole testicular dysfunction'

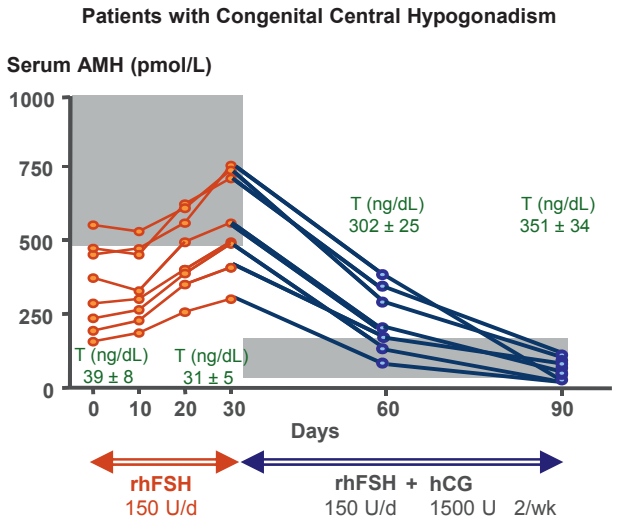
Multiple pituitary hormone deficiency: In male infants with congenital multiple pituitary hormone deficiency (Table 1), the presence of micropenis, cryptorchidism and/or microorchidism is usually associated to low levels of gonadotropins, gonadal hormones and hormones of the GH-IGF axis (Jensen *et al.*, 2005; Bougnères *et al.*, 2008). Early treatment with FSH results in testicular growth – owing to Sertoli cell proliferation – and serum AMH and inhibin B elevation (Bougnères *et al.*, 2008).

Isolated central (hypogonadotropic) hypogonadism: Isolated central hypogonadism – as opposed to multiple pituitary hormone deficiency – comprises a group of rare disorders characterized by gonadotropin deficiencies, which can present as the only manifestation (normosmic hypogonadotropic hypogonadism), or be associated with partial or complete loss of olfaction and other neurological abnormalities (Kallmann syndrome or anosmic hypogonadotropic hypogonadism) or with syndromic endocrine, metabolic or neurological manifestations, with or without olfactory defects (syndromic hypogonadotropic hypogonadism) (Bouvattier *et al.*, 2012). Like in multiple pituitary hormone deficiency, isolated central hypogonadism results in low gonadotropins and testicular hormones in the newborn (Table 1). The first

3–6 months of life are a critical period for the diagnosis of central hypogonadism (Grumbach, 2005). Thereafter, the determination of basal gonadotropins and testosterone is no longer useful – because they are normally low or undetectable. However, the persistently low levels of serum AMH and inhibin B are helpful for the diagnosis during childhood. At pubertal age, serum AMH is elevated for age – because serum testosterone remains at pre-pubertal levels and does not inhibit AMH – but lower than expected for patient's Tanner stage and serum testosterone (Young *et al.*, 1999, 2005) – reflecting the lack of FSH stimulus (Fig. 6). FSH treatment provokes an increase in serum AMH. Further treatment with hCG results in an elevation of intratesticular androgen levels, which inhibit AMH (Fig. 6) and trigger adult spermatogenesis resulting in testis enlargement. Conversely, exogenous testosterone treatment only results in the development of secondary sex characteristics, without inducing spermatogenesis or AMH down-regulation, probably due to the lower intratesticular androgen levels obtained with this treatment (Young *et al.*, 2005). Furthermore, testosterone may even curtail the residual endogenous LH secretion, resulting in a more severe depletion of intratesticular testosterone concentration.

Isolated central hypogonadism should be distinguished from constitutional delay of puberty in boy with lack of pubertal signs. Constitutional delay of puberty represents a prolongation of the quiescence of the gonadotrope. In these patients, basal levels of FSH, AMH and inhibin B are within the normal pre-pubertal range, i.e. higher than in male patients with central hypogonadism (Adan *et al.*, 2010; Grinspon *et al.*, 2010), and LH response to GnRH is normal (Grinspon *et al.*, 2010).

Figure 6 Effect of recombinant human FSH (rhFSH) and hCG treatment on testicular AMH production by the testis in patients with previously untreated central hypogonadism. Initial treatment with rhFSH for 30 days resulted in an elevation of serum AMH in all eight patients, while testosterone (T) remained at pre-pubertal levels. Shaded area represents normal AMH for Tanner stage 1, according to T levels observed in these patients. Subsequent addition of hCG treatment resulted in an elevation of T, which provoked a decline in serum AMH. Shaded area represents AMH values for Tanner stages 4–5, according to T levels observed in the treated patients. Reprinted with permission from Young J, Chanson P, Salenave S, Noël M, Brailly S, O'Flaherty M, Schaison G and Rey R. Testicular anti-Müllerian hormone (AMH) secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2005;90:724-8. Copyright 2005, The Endocrine Society.



Foetal-onset central hypogonadism with 'dissociated testicular dysfunction'

Multiple pituitary hormone deficiency and isolated central hypogonadism: In a few cases, male infants with congenital multiple pituitary hormone deficiency or with isolated central hypogonadism may present with a deficiency of only one gonadotropin, in certain genetic aetiologies affecting factors that regulate the GnRH neuron – e.g. TAC3, LHX4 – LH deficiency with normal FSH secretion has been described (Table 1) (Topaloglu *et al.*, 2009; Brioude *et al.*, 2010).

Defects of LHβ and FSHβ subunits: Central hypogonadism with 'dissociated testicular dysfunction' may be the consequence of isolated LH or FSH deficiencies, respectively, due to mutations of the LHβ- or FSH β-subunit genes (Table 1). Isolated LH deficiency results in failure of Leydig cell differentiation and testosterone secretion in late foetal life and pubertal age (Weiss *et al.*, 1992; Valdes-Socin *et al.*, 2004). Secondarily, the seminiferous tubule development is impaired, with no maturation of Sertoli cells and spermatogenic arrest at meiotic entry. Interestingly, this central form of hypogonadism can even be hypergonadotropic, as observed in a young with delayed puberty, who had a functionally inactive, but immunoreactive LH resulting in elevated serum levels associated with low testosterone (Huhtaniemi, 2002). Less severe mutations may result in the 'fertile eunuch syndrome' (Shiraishi & Naito, 2003). In mice, FSH levels are usually elevated and Sertoli cells remain immature, producing high AMH levels (Ma *et al.*, 2004).

Isolated FSH deficiency is associated with low Sertoli cell number, oligo- or azoospermia and normal androgen levels with high LH after puberty (Huhtaniemi, 2002). Inhibin B is low in adults (Lindstedt *et al.*, 1998); no reports exist for inhibin B or AMH in childhood.

Foetal-onset combined (primary and central) hypogonadism

Foetal-onset combined hypogonadism with 'whole testicular dysfunction'

DAX1 is an essential transcription factor at several levels of the pituitary-gonadal and adrenal axes. DAX1 mutations result in an X-linked disorder characterized by adrenal hypoplasia associated with combined hypogonadism (Table 1). Serum AMH and inhibin B are low from early pubertal stages, indicating the existence of a primary failure of testicular function. Unexpectedly, this primary hypogonadism is not followed by an elevation of gonadotropins at pubertal age, owing to a concomitantly impaired function of the gonadotrope (Bergadá *et al.*, 2008).

Prader-Willi syndrome is another form of combined central and primary hypogonadism. Boys and adolescents present with low inhibin B and testosterone and inadequately normal FSH and LH (Eiholzer *et al.*, 2006).

POSTNATAL-ONSET HYPOGONADISM

Postnatal-onset primary hypogonadism

Postnatal-onset primary hypogonadism with 'whole testicular dysfunction'

Varicocoele: The dilation of the pampiniform plexus veins is frequently associated with infertility, although there is no clear-

cut causal relationship. According to its grade, varicocoele can be associated with defective sperm motility and morphology, and abnormal testicular hormone production (Paduch & Skoog, 2004) which may be already present during pubertal development (Trigo *et al.*, 2004).

Infectious or traumatic aetiologies: Mumps orchitis after puberty, testicular trauma or torsion and surgical treatment of the inguinal or genital regions, e.g. for cryptorchidism, can disrupt testicular function. If both gonads are affected testicular hormone secretion may be reduced and sperm production, impaired, resulting in oligo- or azoospermia. An increase in serum gonadotropins is observed, but only after the age of puberty (Table 2).

Chronic illnesses: Granulomatous diseases, like leprosy, amyloidosis, advanced cancer before chemotherapy, cystic fibrosis, chronic pulmonary disease and renal failure may result in primary testicular dysfunction. Oligo- or azoospermia with low testosterone and inhibin B and mildly elevated gonadotropins are found (Matsumoto & Bremner, 2011).

Neurologic Disorders: Myotonic dystrophy can be associated with small testes, low testosterone levels and elevated plasma LH and FSH levels. Spinal cord lesions resulting in quadriplegia or paraplegia may provoke primary hypogonadism with low testosterone levels and oligozoospermia (Matsumoto & Bremner, 2011).

Postnatal-onset primary hypogonadism with 'dissociated testicular dysfunction'

Oncological treatments: Chemotherapy and abdomino-pelvic radiotherapy affect primarily germ cells, yet Leydig cell function may also be impaired with higher treatment doses (Jahnukainen *et al.*, 2011). Results from recent studies have modified the previous concept that germ cells of younger males were less susceptible to chemotherapy compared to older boys and young adults (Jahnukainen *et al.*, 2011). In paediatric patients (Table 2), AMH or inhibin B values have been reported in few cases with variable results, from decreased to normal values. Testosterone levels were normal after pubertal onset (Cuny *et al.*, 2011). The differences may be due to the type of radio/chemotherapy used. Leydig cells seem to be more resistant than Sertoli cells. Gonadotropins are within the normal range in pre-pubertal boys with low inhibin B and AMH, as another example that primary hypogonadism is not always hypergonadotropic before pubertal onset.

In adults (Table 2), low doses of chemotherapy or abdomino-pelvic radiotherapy can deplete differentiated spermatogonia, whereas less sensitive spermatogonial stem cells, spermatocytes and spermatids survive, leading to temporary oligozoospermia or azoospermia. In high-dose treatments, all germ cells are damaged, and Sertoli cells may also lose their supporting capacity, which leads permanent azoospermia. Testes become smaller and softer, inhibin B levels decline dramatically and FSH increases. Impairment of Leydig cell function is rarer. The sensitivity of the different testicular cell populations to chemotherapy and radiotherapy and its consequences also vary according to the agent used (Jahnukainen *et al.*, 2011).

Other pharmacological treatments: Spironolactone and ketoconazole interfere with steroidogenesis, impairing CYP17 activity Plasma testosterone levels may show a transient decrease after a single dose, with the nadir occurring within 4–8 h. However, chronic treatment may provoke a sustained hypoandrogenism resulting in decreased libido and erectile dysfunction in adults (Matsumoto & Bremner, 2011).

Primary defects of spermatogenesis: Although spermatogenesis is regulated by a large number of genes directly expressed in the germ cells (Matzuk & Lamb, 2008), and theoretically defects in any of these genes may cause primary spermatogenic failure, only a few genetic alterations have clearly been shown to be the cause of germ cell depletion (Matzuk & Lamb, 2008; Visser & Repping, 2010; Krausz, 2011). The low number of genetic defects identified may be due to the fact that many patients undergo assisted reproductive technology treatments without a detailed workup to identify the aetiology of the condition. Chromosomal aberrations, either numeric (e.g. aneuploidies) or structural (e.g. Robertsonian translocations), are usually associated with meiotic failure driving to oligozoospermia or azoospermia. Deletions occurring on the long arm of the Y chromosome (AZF regions, classically divided into AZFa, AZFb and AZFc) are the most frequently identified cause of spermatogenic defects (Table 2). Finally, single gene mutations have been identified in the Y chromosome or autosomes that are responsible for defects in the process of spermatogenesis at its different levels, from spermatogonial renewal (Matzuk & Lamb, 2008), through meiosis (Sciurano *et al.*, 2007; Matzuk & Lamb, 2008; Visser & Repping, 2010; Krausz, 2011) to sperm differentiation and release (Chemes & Rawe, 2010). Sperm analysis reveals the abnormality in sperm number (oligo- or azoospermia), morphology (teratozoospermia) and/or motility (asthenozoospermia). Testis volume is reduced when germ cell number is impaired, but unaffected in the other cases. FSH levels are usually increased above the normal range in association with low or undetectable inhibin B, which reflects Sertoli cell dysfunction and germ cell depletion (Jensen *et al.*, 1997; Jørgensen *et al.*, 2010). Androgenic function is usually unaffected (Krausz, 2011).

Postnatal-onset central hypogonadism

Postnatal-onset central hypogonadism with 'whole testicular dysfunction'

Organic aetiologies lying in the central nervous system: Tumours or infiltrative lesions (e.g. Langerhans histiocytosis) of the central nervous system may directly disrupt the hypothalamic-pituitary axis resulting in hypogonadotropic hypogonadism associated with other pituitary hormone deficiencies (Table 2). Prolactinomas may affect gonadotropin secretion due to the anti-gonadotropic effect of prolactin, independently of the size of the tumour. Alternatively, the surgical or radiant therapy of the primary lesions may provoke the pituitary dysfunction. Cranial traumatic lesions may also result in stalk disruption. In paediatric patients, the diagnosis is usually suspected by the defects in the growth, thyroid and adrenal axes. Gonadotropin and testosterone levels are low, but may be undistinguishable from those observed in normal boys. AMH and inhibin B are usually not significantly affected when the disruption of the pituitary-testicular axis is established after the age of 6 months, i.e. when the gonadotrope is already quiescent. At pubertal age, the lack or the arrest of pubertal development is indicative of gonadotropin insufficiency. In adulthood, the signs and symptoms are similar to those resulting from primary hypogonadism. Low serum gonadotropin levels help to establish the diagnosis of central hypogonadism.

Functional central hypogonadism: general and endocrine disorders and drugs: Disorders that affect the general health status – e.g. severe anaemia and hypoproteinaemic states, anorexia nervosa, etc. – may impact the hypothalamic-pituitary function and result in hypogonadotropic hypogonadism (Table 2). Similarly, endocrine disorders like acromegaly (Katznelson *et al.*, 2001) and long-lasting hypothyroidism (Meikle, 2004) may result in central hypogonadism independently of a mass effect. Estrogen excess due to gain-of-function mutations of the CYP19 gene-encoding aromatase results also in an inhibition of the gonadotrope function (Shozu *et al.*, 2003). Similar effects are observed in excess of circulating steroids due to anabolic drug consumption (Rogol, 2010). Acute and chronic alcohol abuse also is associated with an impairment of the pituitary-testicular axis (Diamond *et al.*, 1986).

Postnatal-onset combined (primary and central) hypogonadism

Cranial radiotherapy combined with chemotherapy affects the hypothalamic-pituitary-gonadal axis at different levels simultaneously (Table 2). The effect of chemotherapy provoking primary gonadal failure has already been discussed. Radiotherapy affects the GnRH neuron resulting in central hypogonadism. Radiotoxicity depends on the total dose: low doses do not impact on the axis, whereas doses >35–40 Gy result in central hypogonadism with high frequency (Constine *et al.*, 1993; Schmiegelow *et al.*, 2001; Darzy & Shalet, 2005).

Total body irradiation used before bone marrow transplantation may also have a simultaneous deleterious effect on the gonad and the hypothalamic-pituitary axis depending on the dose. In the gonad, the seminiferous epithelium is more sensitive than Leydig cells. Although small doses may provoke a transient hypospermatogenesis with relatively rapid recovery, doses >24 Gy induce permanent germ cell depletion and may affect Leydig cell function with consequent hypoandrogenism (Jahnukainen *et al.*, 2011).

In pre-pubertal boys, the effect of cranial radiotherapy alone, or in combination with chemotherapy, on the reproductive axis is difficult to assess because gonadotropins and testosterone are normally low and germ cells cannot be studied without invasive procedures (e.g. a biopsy). The effect of radiotherapy on pre-pubertal AMH and inhibin B secretion has not been reported. In adolescence and adulthood, testicular hormones are low, yet not associated with gonadotropin elevation, which is indicative of the GnRH neuron and gonadotrope insufficiency. Leydig cell response to LH or hCG is impaired, thus reflecting the primary gonadal dysfunction.

Chronic intoxication with low amounts of lead is deleterious for Leydig cell and gonadotrope function concomitantly, resulting in low testosterone and inadequately normal LH (Ng *et al.*, 1991).

Marijuana consumption may be associated with low testosterone production and inadequately normal LH, suggesting combined hypothalamic-pituitary and testicular defects (Kolodny *et al.*, 1974).

CONCLUDING REMARKS: PRACTICE POINTS

The classical definition of hypogonadism, limited to describe impaired androgen production, is inadequate to cover all aspects of the hypothalamic-pituitary-testicular pathology. A more comprehensive definition of hypogonadism includes an impaired hormone secretion by Sertoli cells (AMH, inhibin B) and/or Leydig cells (androgens, INSL3) and/or a disorder of spermatogenesis, as compared to what is expected for age.

The clinical and biochemical features of male hypogonadism vary according to the level of the hypothalamic-pituitary-testicular axis primarily affected, the testicular cell population initially impaired, and the period of life when the gonadal function begins to fail.

The evaluation of basal testicular function in infancy and childhood relies mainly on the assessment of Sertoli cell markers (AMH and inhibin B) because gonadotropin and testosterone levels are normally very low until the onset of puberty. To evaluate gonadotrope or Leydig cell function, dynamic tests are necessary.

Hypergonadotropism should not be considered a sine qua non condition for the diagnosis of primary hypogonadism in childhood. Finally, the lack of elevation of gonadotropins in adolescents or adults with primary gonadal failure is indicative of a combined hypogonadism involving the gonads and the hypothalamic-pituitary axis.

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