Review Article

Sexual Development

Sex Dev
DOI: 10.1159/000479572

Published online: August 30, 2017

Importance of Serum Testicular Protein Hormone Measurement in the Assessment of Disorders of Sex Development

Analía V. Freire[a] Romina P. Grinspon[a] Rodolfo A. Rey[a, b]

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

Keywords
Anti-müllerian hormone · Inhibin B · Insulin-like factor 3 · Leydig cells · Sertoli cells

Abstract
Commonly known for testosterone secretion, the testes also produce the protein hormones anti-müllerian hormone (AMH), inhibin B, and insulin-like factor 3 (INSL3). AMH and inhibin B are secreted by Sertoli cells, whereas INSL3 is a Leydig cell product. AMH is involved in fetal sex differentiation and induces the regression of the anlagen of the uterus and fallopian tubes. INSL3 participates in fetal testicular descent. Serum testicular protein hormone assessment can be very useful and complementary to testosterone measurements in patients with DSD. AMH and inhibin B determination is extremely helpful during childhood, when basal testosterone is normally low. Serum AMH and inhibin B above the female range are indicative of the presence of testicular tissue, and their circulating levels reflect the amount of functional Sertoli cells. In DSD patients with normal male levels of AMH and inhibin B, the diagnosis of gonadal dysgenesis can be ruled out, and isolated androgen secretion deficiency or androgen insensitivity should be suspected. In externally virilized XY patients with persistent müllerian ducts, serum AMH levels determine the diagnosis to AMH deficiency or resistance. At pubertal age, inhibin B levels serve to predict spermatogenic development.
© 2017 S. Karger AG, Basel

In this review, we will address the usefulness of serum testicular protein hormone determination and the interpretation of the results in patients with disorders of sex development (DSD). To this end, we will first discuss the role of these testicular peptides in normal fetal sex differentiation and the ontogeny of their expression and circulating levels, primarily focused in human, in order to set the background for the understanding of their clinical utility.

Testicular Protein Hormones in Fetal Sex Differentiation

The idea that testicular secretions drive male-specific genital changes during fetal development dates from the beginning of the 20th century, when the French research-

KARGER
© 2017 S. Karger AG, Basel

E-Mail karger@karger.com
www.karger.com/sxd

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

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

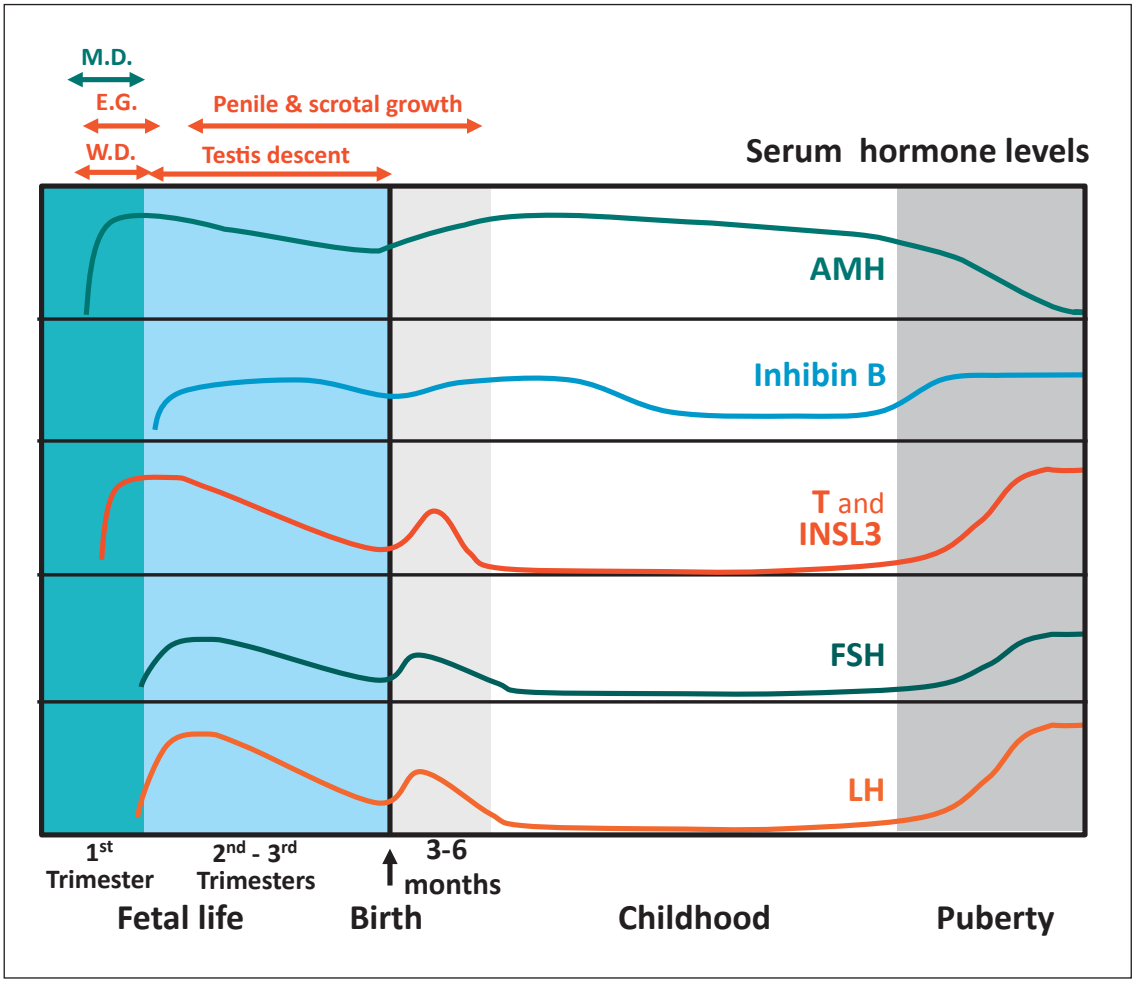

Fig. 1. Serum hormone levels during fetal and postnatal development and events related to fetal sexual differentiation. M.D, müllerian duct regression; E.G., virilization of external genitalia; W.D, wolffian duct differentiation; AMH, anti-müllerian hormone; T, testosterone; INSL3, insulin-like factor 3; FSH, follicle-stimulating hormone; LH, luteinizing hormone. Modified with permission from Grinspon et al. [2014].

ers Bouin and Ancel identified Leydig cells in the interstitial testicular tissue. Further experiments by other French scientists, Courrier and Jost, set the basis for the modern theories of fetal sex differentiation, as they observed that the administration of the steroid 17-ethinyltestosterone masculinized female fetuses without preventing müllerian duct development [reviewed by Josso, 2008]. Jost's ground-breaking experiments of microsurgery in rabbit fetuses provided compelling evidence of the existence of 2 discrete testicular products involved in sex differentiation: testosterone, responsible for the differentiation of wolffian ducts, the urogenital sinus, and external genitalia, and a second testicular secretion responsible for müllerian duct regression [Jost, 1953]. However, the novel concept that this second testicular hormone was not a steroid but a glycoprotein only came to light in the 1980s when Picard and Josso purified the müllerian inhibitor [Picard and Josso, 1984], thereafter called anti-müllerian hormone (AMH) or müllerian inhibiting substance (MIS). DSD are usually associated with disorders in the secretion or action of androgens and/or AMH, as discussed below.

The testes produce protein hormones of 2 other types as well. One of them is also involved in fetal development and was described towards the end of the 20th century: the successively called relaxin-like factor (RLF), Leydig cell insulin-like (Ley-I-L) peptide, and insulin-like factor 3 (INSL3) drives the initial phase of testis descent by

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

Table 1. Serum levels of gonadal hormones during the first month of life

Hormone	Boys		Girls	
	day 2	day 30	day 2	day 30
AMH, pmol/L	371±168	699±245	7±6	18 ± 21
Inhibin B, pg/mL	214±86	361±93	nd	125 ± 41
Testosterone, ng/dL	66±42	210±130	30±10	34 ± 8

Hormone levels are given as mean ± SD. Testosterone levels were measured by RIA using extractive methods, and AMH and inhibin B were measured by ELISA. Modified with permission from Bergadá et al. [2006]. nd, not detectable.

thickening the gubernacular ligaments linking the testes to the inguinal region [reviewed by Ivell et al., 2013]. Defects in the INSL3 axis are rare and only result in cryptorchidism but not in 46,XY DSD.

Finally, the testes secrete peptides of the inhibin/activin family, including α and βB subunits. The dimerization of an α and a βB subunit gives rise to inhibin B, whereas the dimerization of 2 β subunits gives rise to activins [reviewed by Namwanje and Brown, 2016]. Inhibin B is recognized as the major factor involved in the negative feedback of pituitary FSH secretion. Although not involved in fetal sex differentiation, inhibin B has become a useful marker of testicular function in pediatric and adult patients.

Normal Serum Levels in the Boy and the Adolescent

Like testicular steroids, the protein hormone INSL3 is synthesized by Leydig cells. In the human fetus, Leydig cell expression of INSL3 is maximal already in the first half of gestation. After birth, INSL3 serum levels also mimic those of testosterone (Fig. 1). They are high until the 3rd–6th month and then decline to very low levels until the onset of puberty, when they increase again, following the LH pattern [Bay and Andersson, 2011]. The main difference between testosterone and INSL3 as Leydig cell markers is that INSL3 does not respond acutely to an LH or hCG stimulus. Likewise, INSL3 does not exert any feedback on pituitary LH secretion.

AMH and inhibin B are markers of the seminiferous tubules. Nonetheless, there are differences in their expression patterns (Fig. 1) and regulatory mechanisms, which are responsible for their usefulness and interpretation as functional markers in patients with DSD. AMH is specifically secreted by Sertoli cells. Its expression is initiated by local factors independently of FSH in the 7th week of gestation, but then FSH regulates AMH testicular output by modulating Sertoli cell proliferation and AMH expression in each Sertoli cell [Lasala et al., 2004, 2011]. Like all testicular hormones (Table 1), AMH levels decrease perinatally [Bergadá et al., 2006] but then increase again gradually until the second year of life [Aksglæde et al., 2010; Grinspon et al., 2011]. During childhood, serum AMH remains stable and decreases dramatically during the first stages of puberty, when its expression is inhibited by the increasing intratesticular androgen concentration [reviewed by Rey et al., 2009].

Inhibin α and βB subunits are mainly synthesized by Sertoli cells, but expression in other testicular cells has been described [Andersson et al., 1998; Anderson et al., 2002]. Like AMH, inhibin B is also low at birth (Table 1) and increases during the first month of life [Bergadá et al., 2006]. Serum inhibin B peaks in the first 2 years in boys [Kelsey et al., 2016], then declines but remains clearly above female levels, and increases again from the onset of puberty to reach adult levels [Bergadá et al., 1999; Lahlou and Roger, 2004; Kelsey et al., 2016]. Inhibin B reflects Sertoli cell number and its regulation by FSH in the prepubertal boy [Raivio et al., 2007], whereas it also reflects spermatogenic development after pubertal onset [Andersson and Skakkebæk, 2001].

Pathophysiological Classification of DSD

A better interpretation of circulating levels of testicular protein hormones can be achieved in the assessment of patients with DSD when the underlying pathophysiology is considered. A major division can be made between those conditions where there is an abnormal secretion or action of the sex hormones and those that are not endocrine-related.

Endocrine-related disorders represent the vast majority of DSD. They include conditions associated with fetal-onset male primary hypogonadism and with end-organ

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

Table 2. Serum hormone levels in DSD

	DSD	AMH	Inhibin B	INSL3	Testosterone
Non-endocrine (malformative) DSD					
Defective morphogenesis of the gonadal ducts					
Müllerian duct aplasia					
Absence of the vasa deferentia					
Defective morphogenesis of the cloaca and urogenital sinus					
Cloacal malformations	46,XY DSD or 46,XX DSD	normal for sex	normal for sex	normal for sex	normal for sex
Exstrophy of the bladder					
Defective morphogenesis of the primordia of the external genitalia					
Aphallia, bifid phallus					
Isolated hypospadias					
Endocrine DSD					
Dysgenetic DSD (abnormal gonadal differentiation)					
Complete gonadal dysgenesis	46,XY DSD or chromosomal DSD	undetectable	undetectable	undetectable	undetectable
Partial testicular dysgenesis					
Asymmetric gonadal differentiation	46,XY DSD or chromosomal DSD	between M & F	between M & F	between M & F	between M & F
Ovotesticular DSD					
Non-dysgenetic DSD 46,XY					
Leydig cell aplasia	46,XY DSD	M	M	undetectable	undetectable
Leydig cell hypoplasia	46,XY DSD	M	M	between M & F	between M & F
Steroidogenic defects	46,XY DSD	M	M	M	between M & F
Androgen insensitivity	46,XY DSD	M	M	M	M
Defects of AMH production	46,XY DSD	undetectable	M	M	M
AMH resistance	46,XY DSD	M	M	M	M

Levels of INSL3 are those theoretically expected. F, female range; M, male range.

insensitivity to testicular hormones in patients carrying a Y chromosome or those related to an excessive androgen production in XX patients [reviewed by Rey and Grinspon, 2011]. Fetal-onset primary hypogonadism may result from an abnormal differentiation of the gonads, i.e., dysgenetic DSD with its wide range from pure gonadal dysgenesis to the formation of ovotestes, characterized by an insufficient production of all testicular hormones. Alternatively, a dissociated fetal-onset primary hypogonadism may exist, characterized by an isolated defect in hormone production by either Leydig cells (androgens/INSL3) or Sertoli cells (AMH/inhibin B). Finally, ambiguous genitalia may result in eugonadal states owing to defects in the androgen or the AMH receptor in XY patients or to excessive androgen production, e.g., congenital adrenal hyperplasia or aromatase deficiency in XX patients.

Non-endocrine related DSD include all malformations of the external and internal genitalia due to morphogenetic defects in their primordia occurring in early embryogenesis, i.e., during the undifferentiated stage, not associated with gonadal dysfunction or hormone insensitivity [Grinspon and Rey, 2014].

The scope of this review is to address the usefulness of testicular protein hormones in the differential diagnosis of DSD. Therefore, we will not include Turner and Klinefelter syndromes in our discussion, since these patients usually seek medical attention for other reasons than sexual ambiguity.

Serum Levels of Testicular Protein Hormones in DSD

Non-Endocrine Related DSD: Malformative DSD

Because the genital abnormalities are not due to defective secretion or action of gonadal hormones, serum levels of testicular protein hormones are expected to be normal in malformative DSD (Table 2), for which only very few data exist in the literature. In a large series of patients with isolated hypospadias and no other signs of undervirilization, AMH and inhibin, as well as androgens, were within the normal range in more than 85% of the cases, suggesting that the condition was malformative [Rey et al., 2005]. Rarely, mutations have been identified in genes related to sexual development in patients with distal hypospadias and apparently normal testicular hormone lev-

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

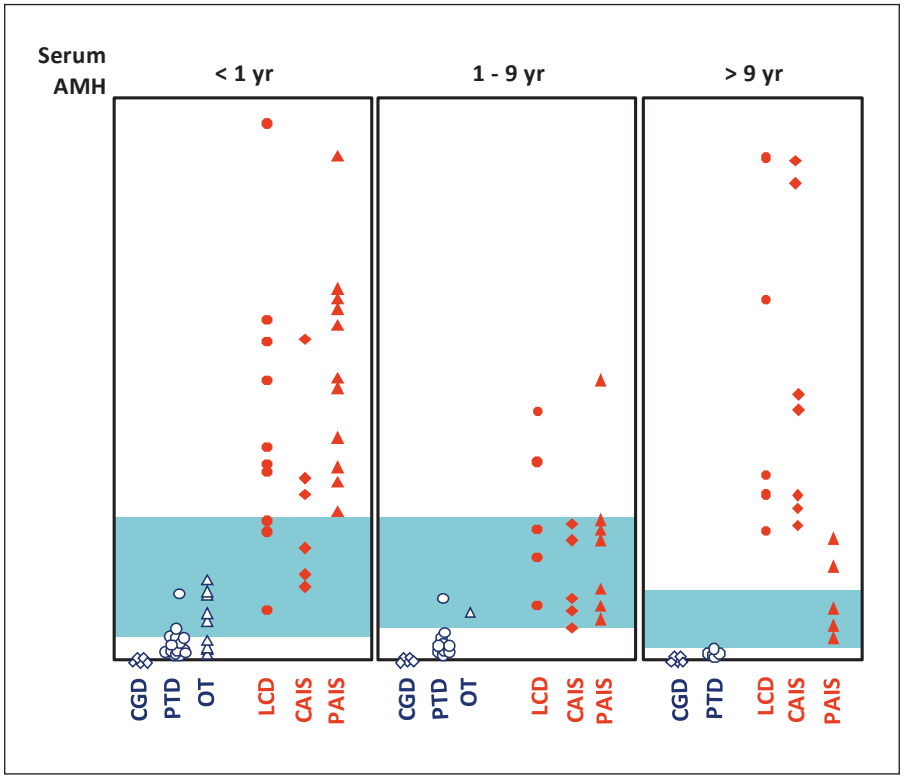

Fig. 2. AMH levels in patients with DSD per age. The shaded area represents the normal male levels. CGD, complete gonadal dysgenesis; PTD, partial testicular dysgenesis; OT, ovotesticular dysgenesis; LCD, Leydig cell disorders (aplasia, hypoplasia, steroid enzyme defects); CAIS, complete androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome. Modified with permission from Josso et al. [2012].

els [Kalfa et al., 2013; Ahmed et al., 2016]. In patients with proximal (penoscrotal, scrotal, or perineal) hypospadias, even if isolated, an endocrine etiology is likely in up to 30% of the cases [Boehmer et al., 2001], and a genetic cause may be identified in approximately 40% [Ahmed et al., 2013].

From a practical point of view, the diagnosis of malformative DSD should be considered when there is inconsistency in the development of the different components of the reproductive tract and gonadal hormones are within the normal range. Other situations where the determination of testicular hormones is useful are those where the external malformation precludes the anatomic assessment of the genitalia in the newborn, e.g., bladder exstrophy or cloacal malformations. In these cases, serum hormone levels of testicular protein and steroid hormones can easily define if the newborn has testes or ovaries [reviewed in Grinspon and Rey, 2014].

Dysgenetic DSD

The undifferentiated gonadal ridges are bipotential until the 6th week in the human embryo. The prevalence of one of the antagonistic signaling networks operating at that stage leads the gonadal ridges to engage in the testicular or the ovarian pathway, irrespective of the XY or XX karyotype of the embryo [Svingen and Koopman, 2013; Lin and Capel, 2015]. Disruption of these signaling networks results in a disordered gonadal differentiation which may range from complete (pure) gonadal dysgenesis, characterized by the existence of streak gonads, to milder forms of testicular or ovarian dysgenesis, and even to the occurrence of bisexual gonads containing both testicular and ovarian tissue, i.e., ovotestes [reviewed by Rey and Grinspon, 2011].

Testicular, but not ovarian, dysgenesis affects genital differentiation during fetal life. In this category, we have included gonadal dysgenesis seen in patients with different karyotypes (Table 2): 46,XY, 45,X/46,XY, and other mosaicisms containing a Y chromosome, and also 46,XX patients with testicular differentiation (i.e., 46,XX testicular DSD or XX males, and 46,XX ovotesticular DSD). Testicular dysgenesis results in insufficient production of all male hormones, i.e., congenital primary hypogonadism affecting all cell lineages, which impairs the virilization of the genital primordia. The resulting phenotype may vary from completely female to mild undervirilization.

AMH. In patients with dysgenetic DSD, serum AMH is low or undetectable (Fig. 2), reflecting the number of

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

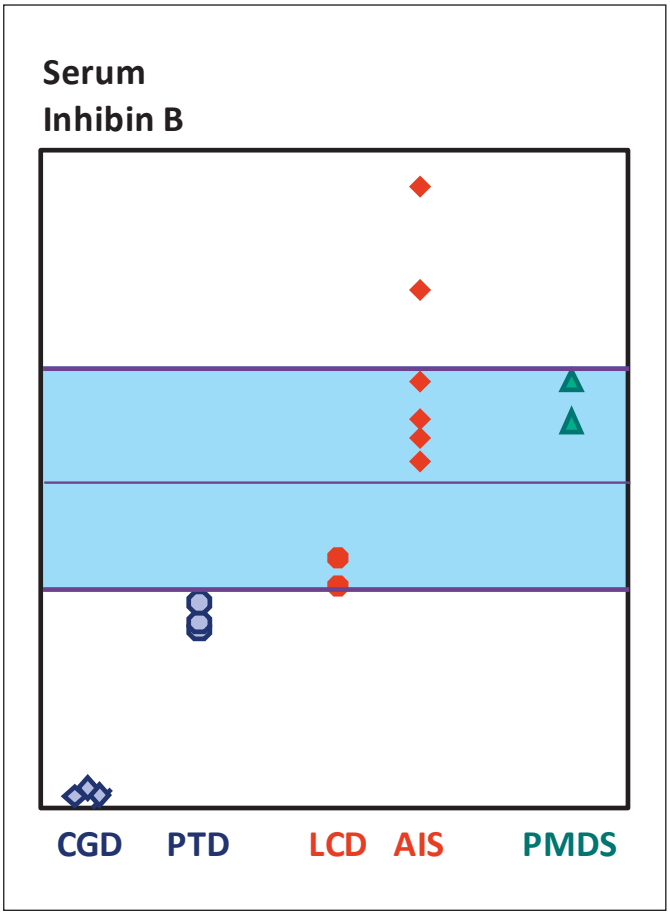

Fig. 3. Inhibin B levels in patients with DSD. The shaded area represents the normal male level; thin line = 50th centile, thick lines = 5th and 95th centiles. CGD, complete gonadal dysgenesis; PTD, partial testicular dysgenesis; LCD, Leydig cell disorders (aplasia, hypoplasia); AIS, androgen insensitivity syndrome; PMDS, persistent müllerian duct syndrome. Data obtained from Kubini et al. [2000].

functional Sertoli cells and indirectly the amount of testicular tissue [Rey et al., 1999; Josso et al., 2012; Lindhardt Johansen et al., 2013; Hafez et al., 2014]. Because of absent or insufficient AMH action during fetal life, these patients present with müllerian remnants. Serum AMH determination is not helpful to distinguish between bilateral testicular dysgenesis and asymmetric gonadal differentiation, also called mixed testicular dysgenesis, usually associated with a 45,X/46,XY karyotype [Rey et al., 1999]. Because AMH acts locally during fetal differentiation of the sex organs, a hemi-uterus and one fallopian tube is generally present on the side of the streak gonad. However, it should be noted that 45,X/46,XY may present with bilaterally dysgenetic testes or even with normal gonads.

The spectrum of testicular differentiation in 46,XX DSD fetuses is wide. Typical XX males do not present genital abnormalities, because the testes secrete normal levels of testicular hormones until the age of puberty; this is usually the case in *SRY*-positive patients [reviewed by Grinspon and Rey, 2016]. When the testes are clearly dysgenetic thus resulting in the development of ambiguous genitalia, as frequently observed in *SRY*-negative patients, serum AMH is low (Fig. 2). Finally, in patients with ovotesticular DSD, testicular tissue is usually dysgenetic, especially in *SRY*-negative cases [Grinspon et al., 2016]. However, the circulating levels of AMH are clearly higher than in patients with exclusively ovarian tissue. Therefore, an AMH value higher than that expected for a female suggests the existence of ovotestes and rules out other causes of virilization in XX newborns, like aromatase deficiency, androgen-secreting tumors, or the exposure to androgenic compounds [reviewed by Rey and Grinspon, 2011; Lindhardt Johansen et al., 2013].

Inhibin B. Like AMH, inhibin B is a useful serum marker for the existence of functional testicular tissue. A recent study in DSD patients that assessed cut-off levels for inhibin B to discriminate between the absence and presence of testicular tissue reported a positive predictive value of 86% and a negative predictive value of 90% for inhibin B levels <41.9 pg/mL [Hafez et al., 2014]. Serum inhibin B is low in patients with partial testicular dysgenesis (Fig. 3) [Kubini et al., 2000] with certified mutations disrupting genes of the testicular differentiating pathway like *SF1* [Köhler et al., 2009; Allali et al., 2011; Warman et al., 2011] and in patients with asymmetric gonadal differentiation [Martinerie et al., 2012]. Lower expression of the inhibin α subunit has been observed by immunohistochemistry in dysgenetic testicular samples [Lepais et al., 2016].

Serum inhibin B is particularly useful for the assessment of seminiferous tubule function at the age of puberty in DSD patients assigned as males. The occurrence of a low inhibin B level is usually associated with impaired pubertal development and infertility in 45,X/46,XY patients [Martinerie et al., 2012], whereas normal inhibin B levels may predict the occurrence of spontaneous pubertal onset, although a subsequent fall of inhibin B and an elevation of FSH may indicate primary gonadal failure in adulthood [Lindhardt Johansen et al., 2012]. In XX males, serum inhibin B may be normal/low before puberty [Kubini et al., 2000; Jain et al., 2013; Haines et al., 2015] but decreases to undetectable levels later on, reflecting germ cell loss like in Klinefelter patients [Aksglæde et al., 2008].

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

INSL3. Insufficiency of INSL3 might be partially responsible for testicular maldescent in DSD patients. However, except for Klinefelter syndrome [Rohayem et al., 2015], no reports exist on serum INSL3 in genetically certified DSD. A recent report showed the existence of low INSL3 levels in males with hypospadias and cryptorchidism, associated with the exposure to an environmental disruptor [Toft et al., 2016].

Non-Dysgenetic DSD

In these cases, either the androgen- or the AMH-dependent fetal sex differentiation pathway is affected, but not both (Table 2). In the former, the newborn presents with undervirilized or female external genitalia but does not have fully developed müllerian derivatives. Conversely, in the latter, a uterus and fallopian tubes are present in the externally virilized patient [for a detailed review, see Rey and Grinspon, 2011].

Defects of Androgen Synthesis

Congenital isolated Leydig cell disorders occur in 46,XY fetuses with testicular differentiation and normal Sertoli cell development due to inactivating mutations in the luteinizing hormone/chorionic gonadotropin receptor (LHCG-R) leading to Leydig cell aplasia/hypoplasia, or in the proteins or enzymes involved in testosterone or DHT synthesis, i.e., StAR, P450scc, P450c17, 3β-HSD, 17β-HSD, and 5α-reductase.

AMH. Normal to high serum levels of AMH are a distinctive characteristic of DSD owing to primary Leydig cell-specific congenital hypogonadism [Rey et al., 1999]. This feature is helpful in patients with ambiguous or female genitalia to rule out gonadal dysgenesis [Grinspon et al., 2014]. The elevation of AMH testicular output, especially in the first months after birth and at the age of puberty in non-gonadectomized patients (Fig. 2), is probably due to an elevation of FSH, which induces Sertoli cell proliferation and AMH expression [Lasala et al., 2004, 2011], and the lack of the inhibition normally exerted by androgens on AMH production [Edelsztein et al., 2016].

In the few patients with deficiency of 5α-reductase in whom it was assessed, serum AMH was in the lowest part or below the normal male range. Because intratesticular androgen concentration and androgen receptor expression are normal and FSH is not elevated, serum AMH is not increased in these patients [Stuchi-Perez et al., 2005; Vija et al., 2014].

Inhibin B. Inhibin B levels have been studied in very few patients with impaired testicular androgen production (Fig. 3). Like AMH, it was found within the lower part of the normal range [Kubini et al., 2000; Sánchez-Garvín et al., 2013], indicating a fairly normal functional status of prepubertal Sertoli cells.

INSL3. Although serum INSL3 is a proven marker of Leydig cell function before and after birth [Mitsui et al., 2015], it has not been assessed in DSD patients with purportedly isolated Leydig cell impairment.

Defects of Androgen Action

Androgen receptor mutations lead to complete (CAIS) or partial (PAIS) androgen insensitivity syndrome (AIS). Usually, partial forms seek medical attention at birth owing to genital ambiguity, whereas complete forms are diagnosed at pubertal age owing to primary amenorrhea in an otherwise normal girl unless a karyotype is performed before. Testicular hormone production is not impaired before puberty, and these patients can therefore be considered eugonadal during fetal life and childhood.

AMH. Because Sertoli cells differentiate normally in AIS fetuses, serum AMH is normal or elevated [Rey et al., 1994, 1999; Morel et al., 2002]. Interestingly, the pituitary-gonadal axis shows different features during the first months of life in CAIS and PAIS. In a newborn with CAIS, FSH remains low, which probably explains why serum AMH is not as high as expected. Conversely, in PAIS, FSH is higher and induces AMH elevation [Bouvattier et al., 2002]. During childhood, gonadotropin levels drop and serum AMH remains within the normal male range [Rey et al., 1994, 1999]. At the age of puberty, if gonadectomy has not been performed, serum AMH increases to very high levels in CAIS (Fig. 2), reflecting the lack of the inhibitory effect normally exerted by androgens [Rajpert-De Meyts et al., 1999; Vija et al., 2014]. In PAIS, the elevation of intratesticular testosterone concentration induces a partial inhibition of AMH expression. Nonetheless, AMH levels remain inadequately high for the concomitant circulating testosterone [Rey et al., 1999; Bouvattier et al., 2006].

Inhibin B. There are few studies reporting inhibin B levels in pediatric patients with AIS (Fig. 3). In prepubertal subjects with AIS, inhibin B levels are in the normal male range or slightly elevated [Kubini et al., 2000; Hellmann et al., 2012]. After pubertal onset and through adulthood, inhibin B accurately reflects spermatogenic function in PAIS males: it is low in patients with impaired sexual function [Bouvattier et al., 2006] but normal in patients with milder forms with preserved fertility [Giwercman et al., 2000].

INSL3. No reports exist on INSL3 levels in patients with AIS.

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

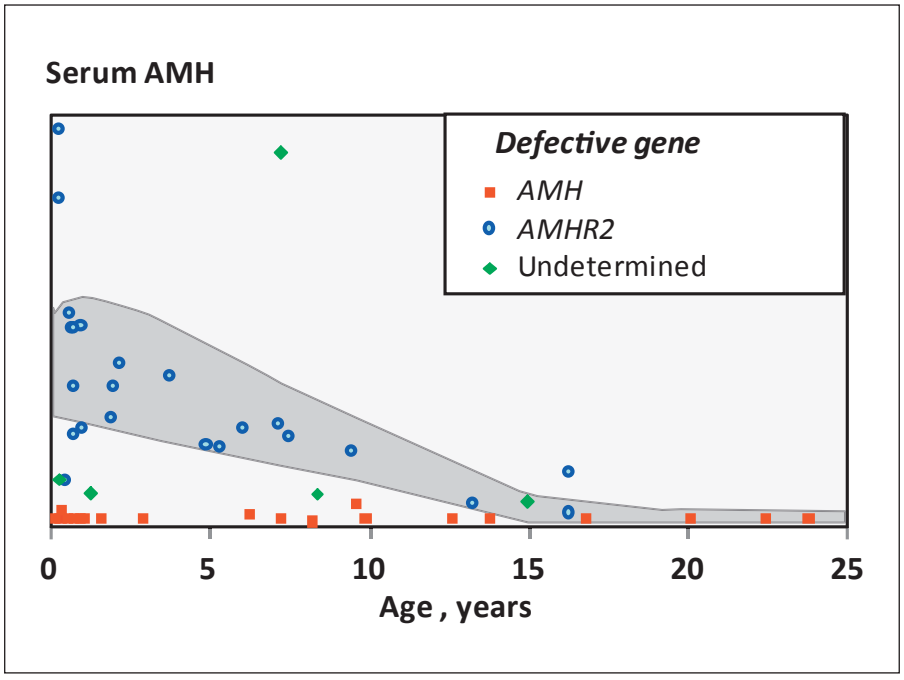

Fig. 4. AMH levels in patients with persistent müllerian duct syndrome (PMDS). The shaded area represents the normal male level. *AMHR2*, AMH receptor type 2. Modified with permission from Imbeaud et al. [1996]

Defects of AMH Synthesis or Action

Isolated failure of AMH secretion or action on müllerian ducts in the 46,XY fetus results in the persistent müllerian duct syndrome (PMDS), characterized by the presence of a uterus and fallopian tubes in an otherwise normally virilized patient who is usually seeking medical attention for cryptorchidism. Approximately 85% of cases are due to mutations in the genes coding for AMH or its type 2 receptor in roughly equal proportions [reviewed by Josso et al., 2012].

AMH. In patients with mutations in the *AMH* gene, serum AMH levels are undetectable or extremely low due to a lack of AMH expression or to instability of the mutant protein (Fig. 4). Since these patients are evaluated for cryptorchidism, anorchia may be suspected. However, normal serum inhibin B (see below) and testosterone response to hCG rules out this possibility and leads to the diagnosis of PMDS. In rare cases, normal serum AMH levels have been described where a mutation disturbs the AMH receptor interaction but not its detection by antibodies used in ELISA tests [Belville et al., 2004]. In patients with insensitivity to AMH due to mutations in the *AMHR2* gene, serum AMH is readily detectable within a variable range.

Inhibin B. The association of normal inhibin B and FSH levels is clearly indicative for the presence of functional testicular tissue in PMDS boys with bilaterally non-palpable gonads and undetectable serum AMH [Kubini et al., 2000; van der Zwan et al., 2012].

INSL3. Like testosterone, INSL3 levels are expected to be within the male range, yet no studies have been published reporting serum INSL3 determination in PMDS.

Usefulness of Serum Testicular Protein Hormone Assessment in the Diagnosis of DSD

The results of the endocrine assessment of DSD patients need to be analyzed according to their age (Table 1; Fig. 1). Basal AMH and inhibin B are extremely useful to assess the existence and functional state of testicular tissue during childhood, when testosterone or INSL3 determination can only be used after an hCG test. When a patient of pubertal age needs to be evaluated, AMH may be less informative since it normally declines at puberty, whereas inhibin B and INSL3 are very useful, like testosterone and gonadotropins.

In the following sections, the diagnostic value of testicular protein hormones will be addressed according to the widely accepted clinical classification of DSD, proposed after the recently updated Chicago Consensus Conference [Hughes et al., 2006; Lee et al., 2016].

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

46,XY DSD

Ambiguous Genitalia

As discussed above, the occurrence of ambiguous genitalia in 46,XY newborns indicates either an early nonendocrine dysmorphogenesis of the genital primordia or a later insufficient androgen secretion or action.

Serum AMH, inhibin B, and INSL3 in the normal male range indicate the existence of functionally normal testes in a newborn with multiple malformations of the abdominal wall or the pelvic and anogenital region, thus leading to the diagnosis of malformative DSD (Table 2).

When AMH, inhibin B, and INSL3 are low, testicular dysgenesis is the most likely diagnosis (Fig. 2, 3). The interpretation of the results may prove difficult in the first week, owing to the normal transient decrease occurring at that moment, and may require a new evaluation in the 2nd or 3rd week of life.

Serum AMH and inhibin B levels in the male range with low INSL3 – in association with low testosterone – should prompt the diagnosis of Leydig cell hypoplasia, whereas steroidogenic defects should be suspected when AMH, inhibin B, and INSL3 are normal or elevated but testosterone is low. Finally, if all testicular hormones are in the male range, AIS and 5α-reductase deficiency are the main differential diagnoses.

In older infants or children, basal INSL3 (and testosterone) are no longer informative. However, determination of basal AMH and inhibin B remains useful to distinguish between dysgenetic DSD and defects of androgen production (including Leydig cell aplasia, steroidogenic defects, and 5α-reductase deficiency) or action (AIS).

At the age of puberty, if the patient has not undergone gonadectomy, persistence of relatively high levels of AMH is indicative of PAIS, whereas a decline is more compatible with 5α-reductase deficiency or partial forms of steroidogenic defects allowing some testosterone production. The functional prognosis is better asserted by measuring inhibin B levels; a lack of normal pubertal increase is a sign of poor prognosis for spermatogenic development and fertility.

Female Genitalia

In a 46,XY newborn/infant with completely feminized external genitalia, pure gonadal dysgenesis, Leydig cell aplasia, extremely severe steroidogenic defects, and CAIS are the possible diagnoses. Undetectable levels of AMH and inhibin B are indicative of pure (complete) gonadal dysgenesis (Fig. 2, 3). Normal/high levels of AMH and inhibin B are seen in the remaining conditions, with undetectable INSL3 expected in Leydig cell aplasia and normal INSL3 expected in steroidogenic defects and CAIS. Basal INSL3 is not supposed to be informative during childhood. If the patient has not been gonadectomized at the age of puberty, results are similar to those observed during the postnatal activation period.

46,XX DSD

Ambiguous Genitalia

In a 46,XX patient of any age with ambiguous external genitalia, serum AMH and inhibin B levels that are above the normal female range raise the suspicion of ovotesticular or testicular DSD (Fig. 2, 3). The interpretations already made for 46,XY dysgenetic DSD apply. Other forms of 46,XX DSD (aromatase deficiency, congenital adrenal hyperplasia) can be ruled out.

Male Genitalia

If gonads are not palpable, the finding of AMH and inhibin B in the male range confirm the presence of intraabdominal testes in an XX male. Female-range levels are found in patients with congenital adrenal hyperplasia with complete virilization (Prader V score).

In patients with scrotal testes, AMH and inhibin B are in the normal male range until puberty, and then they decline like in patients with Klinefelter syndrome, reflecting the deterioration of seminiferous tubules.

Chromosomal DSD

In these patients, serum levels of AMH and inhibin B are commensurate with the amount of functional testicular tissue that has developed. The interpretation of hormone levels is similar to that described in 46,XY dysgenetic DSD patients.

Concluding Remarks

The assessment of testicular protein hormone levels in serum can be very useful and complementary with testosterone and gonadotropin determination in patients with DSD. Reasonable amount of information is available for AMH and somewhat less for inhibin B. Both markers are extremely useful particularly during childhood, when the other reproductive hormones are normally low, thus not informative unless dynamic tests are performed. Serum AMH and inhibin B above the female range are indicative of the presence of testicular tissue, and their levels reflect the amount of functional Sertoli cells. If they attain normal male levels, the diagnosis of

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

gonadal dysgenesis is unlikely, and those related to isolated androgen secretion deficiency or AIS may be favored. At pubertal age, inhibin B levels serve to predict spermatogenic development.

Biochemical investigations are a helpful tool for the achievement of an accurate diagnosis in patients with DSD. However, lack of sufficient sensitivity and overlapping results may represent drawbacks in partial forms of DSD. As the availability of high-throughput genetic technologies increases and their cost decreases, the use of comparative genomic hybridization microarray and exome- or even whole-genome sequencing is likely to become a routine diagnostic tool for DSD patients in a near future.

References

Ahmed SF, Bashamboo A, Lucas-Herald A, McElreavey K: Understanding the genetic aetiology in patients with XY DSD. Br Med Bull 106:67–89 (2013).

Ahmed SF, Achermann JC, Arlt W, Balen A, Conway G, et al: Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clin Endocrinol (Oxf) 84:771–788 (2016).

Aksglæde L, Jensen RB, Carlsen E, Kok P, Keenan DM, et al: Increased basal and pulsatile secretion of FSH and LH in young men with 47,XXY or 46,XX karyotypes. Eur J Endocrinol 158:803–810 (2008).

Aksglæde L, Sorensen K, Boas M, Mouritsen A, Hagen CP, et al: Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. J Clin Endocrinol Metab 95:5357–5364 (2010).

Allali S, Muller JB, Brauner R, Lourenco D, Boudjenah R, et al: Mutation analysis of *NR5A1* encoding steroidogenic factor 1 in 77 patients with 46,XY disorders of sex development (DSD) including hypospadias. PLoS One 6:e24117 (2011).

Anderson RA, Cambray N, Hartley PS, McNeilly AS: Expression and localization of inhibin alpha, inhibin/activin βA and βB and the activin type II and inhibin β-glycan receptors in the developing human testis. Reproduction 123:779–788 (2002).

Andersson A, Skakkebæk NE: Serum inhibin B levels during male childhood and puberty. Mol Cell Endocrinol 180:103–107 (2001).

Andersson AM, Müller J, Skakkebæk NE: Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. J Clin Endocrinol Metab 83:4451–4458 (1998).

Bay K, Andersson AM: Human testicular insulin-like factor 3 in relation to development, reproductive hormones and andrological disorders. Int J Androl 34:97–109 (2011).

Belville C, Van Vlijmen H, Ehrenfels C, Pepinsky B, Rezaie AR, et al: Mutations of the anti-Mullerian hormone gene in patients with persistent Mullerian duct syndrome: biosynthesis, secretion, and processing of the abnormal proteins and analysis using a three-dimensional model. Mol Endocrinol 18:708–721 (2004).

Bergadá I, Rojas G, Ropelato MG, Ayuso S, Bergadá C, Campo SM: Sexual dimorphism in circulating monomeric and dimeric inhibins in normal boys and girls from birth to puberty. Clin Endocrinol (Oxf) 51:455–460 (1999).

Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, et al: Time course of the serum gonadotropin surge, inhibins, and anti-Mullerian hormone in normal newborn males during the first month of life. J Clin Endocrinol Metab 91:4092–4098 (2006).

Boehmer AL, Nijman RJ, Lammers BA, de Coninck SJ, Van Hemel JO, et al: Etiological studies of severe or familial hypospadias. J Urol 165:1246–1254 (2001).

Bouvattier C, Carel JC, Lecointre C, David A, Sultan C, et al: Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the *AR* gene. J Clin Endocrinol Metab 87:29–32 (2002).

Bouvattier C, Mignot B, Lefevre H, Morel Y, Bougneres P: Impaired sexual activity in male adults with partial androgen insensitivity. J Clin Endocrinol Metab 91:3310–3315 (2006).

Edelsztein NY, Grinspon RP, Schteingart HF, Rey RA: Anti-Müllerian hormone as a marker of steroid and gonadotropin action in the testis of children and adolescents with disorders of the gonadal axis. Int J Pediatr Endocrinol 2016:20 (2016).

Giwercman A, Kledal T, Schwartz M, Giwercman YL, Leffers H, et al: Preserved male fertility despite decreased androgen sensitivity caused by a mutation in the ligand-binding domain of the androgen receptor gene. J Clin Endocrinol Metab 85:2253–2259 (2000).

Grinspon RP, Rey RA: When hormone defects cannot explain it: malformative disorders of sex development. Birth Defects Res C Embryo Today 102:359–373 (2014).

Grinspon RP, Rey RA: Disorders of sex development with testicular differentiation in *SRY*-negative 46,XX individuals: clinical and genetic aspects. Sex Dev 10:1–11 (2016).

Grinspon RP, Bedecarrás P, Ballerini MG, Iñíguez G, Rocha A, et al: Early onset of primary hypogonadism revealed by serum anti-Müllerian hormone determination during infancy and childhood in trisomy 21. Int J Androl 34:e487–e498 (2011).

Grinspon RP, Loreti N, Braslavsky D, Valeri C, Schteingart H, et al: Spreading the clinical window for diagnosing fetal-onset hypogonadism in boys. Front Endocrinol (Lausanne) 5:51 (2014).

Grinspon RP, Nevado J, Mori Alvarez ML, del Rey G, Castera R, et al: 46,XX ovotesticular DSD associated with a *SOX3* gene duplication in a *SRY*-negative boy. Clin Endocrinol (Oxf) 85:669–675 (2016).

Hafez M, El Dayem SM, El Mougy F, Atef A, Kandil M, et al: The role of anti-Mullerian and inhibin B hormones in the evaluation of 46,XY disorders of sex development. J Pediatr Endocrinol Metab 27:891–899 (2014).

Haines B, Hughes J, Corbett M, Shaw M, Innes J, et al: Interchromosomal insertional translocation at Xq26.3 alters *SOX3* expression in an individual with XX male sex reversal. J Clin Endocrinol Metab 100:E815–E820 (2015).

Hellmann P, Christiansen P, Johannsen TH, Main KM, Duno M, Juul A: Male patients with partial androgen insensitivity syndrome: a longitudinal follow-up of growth, reproductive hormones and the development of gynaecomastia. Arch Dis Child 97:403–409 (2012).

Hughes IA, Houk C, Ahmed SF, Lee PA, LWPES/ESPE Consensus Group: Consensus statement on management of intersex disorders. Arch Dis Child 91:554–563 (2006).

Imbeaud S, Belville C, Messika-Zeitoun L, Rey R, et al: A 27 base-pair deletion of the anti-Müllerian type II receptor gene is the most common cause of the persistent Müllerian duct syndrome. Hum Mol Genet 5:1269–1279 (1996).

Ivell R, Wade JD, Anand-Ivell R: INSL3 as a biomarker of Leydig cell functionality. Biol Reprod 88:147 (2013).

Jain M, Veerapandian V, Chaudhary I, Halder A: The Sertoli cell only syndrome and glaucoma in a sex determining region Y (SRY) positive XX infertile male. J Clin Diagn Res 7:1457–1459 (2013).

Josso N: Professor Alfred Jost: the builder of modern sex differentiation. Sex Dev 2:55–63 (2008).

Josso N, Rey R, Picard JY: Testicular anti-Mullerian hormone: clinical applications in DSD. Semin Reprod Med 30:364–373 (2012).

Jost A: Problems of fetal endocrinology: the gonadal and hypophyseal hormones. Recent Prog Horm Res 8:379–418 (1953).

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM

Kalfa N, Philibert P, Werner R, Audran F, Bashamboo A, et al: Minor hypospadias: the 'tip of the iceberg' of the partial androgen insensitivity syndrome. PLoS One 8:e61824 (2013).

Kelsey TW, Miles A, Mitchell RT, Anderson RA, Wallace WH: A normative model of serum inhibin B in young males. PLoS One 11:e0153843 (2016).

Köhler B, Lin L, Mazen I, Cetindag C, Biebermann H, et al: The spectrum of phenotypes associated with mutations in steroidogenic factor 1 (*SF-1, NR5A1, Ad4BP*) includes severe penoscrotal hypospadias in 46,XY males without adrenal insufficiency. Eur J Endocrinol 161:237–242 (2009).

Kubini K, Zachmann M, Albers N, Hiort O, Bettendorf M, et al: Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. J Clin Endocrinol Metab 85:134–138 (2000).

Lahlou N, Roger M: Inhibin B in pubertal development and pubertal disorders. Semin Reprod Med 22:165–175 (2004).

Lasala C, Carré-Eusèbe D, Picard JY, Rey R: Subcellular and molecular mechanisms regulating anti-Mullerian hormone gene expression in mammalian and nonmammalian species. DNA Cell Biol 23:572–585 (2004).

Lasala C, Schteingart HF, Arouche N, Bedecarrás P, Grinspon RP, et al: SOX9 and SF1 are involved in cyclic AMP-mediated upregulation of anti-Mullerian gene expression in the testicular prepubertal Sertoli cell line SMAT1. Am J Physiol Endocrinol Metab 301:539–547 (2011).

Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, et al: Global disorders of sex development update since 2006: perceptions, approach and care. Horm Res Paediatr 85:158–180 (2016).

Lepais L, Morel Y, Mouriquand P, Gorduza D, Plotton I, et al: A novel morphological approach to gonads in disorders of sex development. Mod Pathol 29:1399–1414 (2016).

Lin YT, Capel B: Cell fate commitment during mammalian sex determination. Curr Opin Genet Dev 32:144–152 (2015).

Lindhardt Johansen M, Hagen CP, Rajpert-De Meyts E, Kjærgaard S, Petersen BL, et al: 45,X/46,XY mosaicism: phenotypic characteristics, growth, and reproductive function: a retrospective longitudinal study. J Clin Endocrinol Metab 97:E1540–E1549 (2012).

Lindhardt Johansen M, Hagen CP, Johannsen TH, Main KM, Picard JY, et al: Anti-mullerian hormone and its clinical use in pediatrics with special emphasis on disorders of sex development. Int J Endocrinol 2013:198698 (2013).

Martinerie L, Morel Y, Gay CL, Pienkowski C, de Kerdanet M, et al: Impaired puberty, fertility, and final stature in 45,X/46,XY mixed gonadal dysgenetic patients raised as boys. Eur J Endocrinol 166:687–694 (2012).

Mitsui T, Araki A, Imai A, Sato S, Miyashita C, et al: Effects of prenatal Leydig cell function on the ratio of the second to fourth digit lengths in school-aged children. PLoS One 10: e0120636 (2015).

Morel Y, Rey R, Teinturier C, Nicolino M, Michel-Calemard L, et al: Aetiological diagnosis of male sex ambiguity: a collaborative study. Eur J Pediatr 161:49–59 (2002).

Namwanje M, Brown CW: Activins and inhibins: roles in development, physiology, and disease. Cold Spring Harb Perspect Biol 8: pii:a021881 (2016).

Picard JY, Josso N: Purification of testicular anti-Müllerian hormone allowing direct visualization of the pure glycoprotein and determination of yield and purification factor. Mol Cell Endocrinol 34:23–29 (1984).

Raivio T, Wikström AM, Dunkel L: Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. Eur J Endocrinol 156:105–111 (2007).

Rajpert-De Meyts E, Jørgensen N, Græm N, Müller J, Cate RL, Skakkebæk NE: Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 84:3836–3844 (1999).

Rey RA, Grinspon RP: Normal male sexual differentiation and aetiology of disorders of sex development. Best Pract Res Clin Endocrinol Metab 25:221–238 (2011).

Rey RA, Mebarki F, Forest MG, Mowszowicz I, Cate RL, et al: Anti-müllerian hormone in children with androgen insensitivity. J Clin Endocrinol Metab 79:960–964 (1994).

Rey RA, Belville C, Nihoul-Fékété C, Michel-Calemard L, Forest MG, et al: Evaluation of gonadal function in 107 intersex patients by means of serum antimüllerian hormone measurement. J Clin Endocrinol Metab 84:627–631 (1999).

Rey RA, Codner E, Iñíguez G, Bedecarrás P, Trigo R, et al: Low risk of impaired testicular Sertoli and Leydig cell functions in boys with isolated hypospadias. J Clin Endocrinol Metab 90:6035–6040 (2005).

Rey RA, Musse M, Venara M, Chemes HE: Ontogeny of the androgen receptor expression in the fetal and postnatal testis: its relevance on Sertoli cell maturation and the onset of adult spermatogenesis. Microsc Res Tech 72:787–795 (2009).

Rohayem J, Fricke R, Czeloth K, Mallidis C, Wistuba J, et al: Age and markers of Leydig cell function, but not of Sertoli cell function predict the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome. Andrology 3:868–875 (2015).

Sánchez-Garvín D, Albaladejo S, Ezquieta B, Corripio R: Disorder of sex development as a diagnostic clue in the first Spanish known newborn with P450 oxidoreductase deficiency. BMJ Case Rep pii:bcr2013010251 (2013).

Stuchi-Perez EG, Hackel C, Oliveira LE, Ferraz LF, Oliveira LC, et al: Diagnosis of 5alpha-reductase type 2 deficiency: contribution of anti-Mullerian hormone evaluation. J Pediatr Endocrinol Metab 18:1383–1389 (2005).

Svingen T, Koopman P: Building the mammalian testis: origins, differentiation, and assembly of the component cell populations. Genes Dev 27:2409–2426 (2013).

Toft G, Jonsson BA, Bonde JP, Norgaard-Pedersen B, Hougaard DM, et al: Perfluorooctane sulfonate concentrations in amniotic fluid, biomarkers of fetal Leydig cell function, and cryptorchidism and hypospadias in Danish boys (1980–1996). Environ Health Perspect 124:151–156 (2016).

van der Zwan YG, Bruggenwirth HT, Drop SL, Wolffenbuttel KP, Madern GC, et al: A novel *AMH* missense mutation in a patient with persistent Mullerian duct syndrome. Sex Dev 6:279–283 (2012).

Vija L, Ferlicot S, Paun D, Bry-Gauillard H, Berdan G, et al: Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5 alpha-reductase type 2 deficiency: case report and review of the literature in a perspective of evaluation of potential fertility of these patients. BMC Endocr Disord 14:43 (2014).

Warman DM, Costanzo M, Marino R, Berensztein E, Galeano J, et al: Three new *SF-1* (*NR5A1*) gene mutations in two unrelated families with multiple affected members: within-family variability in 46,XY subjects and low ovarian reserve in fertile 46,XX subjects. Horm Res Paediatr 75:70–77 (2011).

Downloaded by:
190.195.103.168 - 8/30/2017 10:19:11 PM