ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

REVIEW ARTICLE

AMH and INSL3 in testicular and extragonadal pathophysiology: what do we know?

[1,2]A. Sansone, [1]S. Kliesch, [2]A. M. Isidori and [1]S. Schlatt

[1]Center of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, Institute of Reproductive and Regenerative Biology, Münster, Germany, [2]Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology – Sapienza University of Rome, Rome, Italy

Correspondence:
Andrea Sansone, Centrum für Reproduktionsmedizin und Andrologie, Albert-Schweitzer-Campus 1, Geb. D11, 48149 Münster, Germany and Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy.
E-mails: andrea.sansone@uniroma1.it; andreasansone85@gmail.com

Keywords:
AMH, cryptorchidism, INSL3, Müllerian ducts, testicular development, testis

Received: 6-Dec-2018
Revised: 9-Jan-2019
Accepted: 22-Jan-2019

doi: 10.1111/andr.12597

ABSTRACT

Background: It is commonly accepted that testicular function is prevalently regulated by the hypothalamic–pituitary–gonadal axis: The pulsatile secretion of GnRH by the hypothalamus induces pituitary expression of the two gonadotropins FSH and LH, which then stimulate Sertoli and Leydig cells, respectively, therefore regulating steroidogenesis and spermatogenesis. However, a growing body of evidence has recently suggested that other hormones act on the reproductive tract since the early phases of fetal development. Anti-Müllerian hormone and INSL3 are still largely used only for research purposes despite being increasingly recognized as markers of Sertoli and Leydig cells function, respectively.

Objectives: Provide an up-to-date review of the role of anti-Müllerian hormone and INSL3 in human pathophysiology according to current evidence.

Materials and Methods: A thorough literature review was performed on PubMed, OVID MEDLINE/EMBASE and Google Scholar for papers concerning anti-Müllerian hormone and INSL3 in human males.

Results: INSL3 is not acutely regulated by the hypothalamic–pituitary axis but is constitutively secreted by Leydig cells, therefore representing a valid marker for their number and status. Anti-Müllerian hormone expression, on the other hand, is downregulated by androgens, therefore occurring mostly at the early stages of testicular differentiation and before the onset of puberty. Several conditions affecting testicular development, such as male hypogonadotropic hypogonadism, and their treatment have been associated to specific pattern of INSL3 and anti-Müllerian hormone expression, proving a role for both hormones in the diagnostic and therapeutic management. Recent reports suggest a role for both anti-Müllerian hormone and INSL3 in extra gonadal physiology, such as cardiovascular and bone health.

Conclusion: Anti-Müllerian hormone and INSL3 are markers of Sertoli and Leydig cells maturation, respectively, usually involved in the pathogenesis of disorders of sexual differentiation. However, their role in testicular pathology has only been hinted at in the last decades. Recent evidence supports an involvement of both anti-Müllerian hormone and INSL3 in extragonadal pathophysiology as well.

INTRODUCTION

Testicular functions include spermatogenesis and hormone production– both largely dependent on the integrity of the hypothalamic–pituitary–gonadal (HPG) function and of the testis itself. It is textbook knowledge that GnRH is secreted by hypothalamic GnRH-secreting neurons into the hypophyseal portal circulation; from here, GnRH reaches the anterior pituitary, where it induces release of the two gonadotropins LH and FSH by binding to its receptor on the surface of gonadotrope cells (Stamatiades & Kaiser, 2018). Secretion of GnRH is pulsatile: the pituitary response is affected by frequency and amplitude of each pulse, with low and high pulse frequencies,

respectively, stimulating FSH and LH release (Stamatiades & Kaiser, 2018), and pulsatility changes dramatically during life, decreasing in frequency and amplitude (Bhasin *et al.*, 2000). In males, FSH and LH act on specific receptors predominantly expressed in the testis: receptors for FSH are mostly expressed by Sertoli cells, whereas Leydig cells express LH receptors. The binding of LH to its receptor on Leydig cells induces testosterone synthesis by increased activity of the desmolase enzyme (Dufau *et al.*, 1984); similarly, FSH binds to its receptor on Sertoli cells, activating several pathways ultimately needed for supporting spermatogenesis (Simoni *et al.*, 1997; Walker & Cheng, 2005; Lucas *et al.*, 2014). An intra testicular paracrine role has been clearly identified for testosterone, suggesting that its binding to the androgen receptor is the key stimulus for successful spermatogenesis (Huhtaniemi, 2015). On the contrary, the role of FSH has been somewhat questioned in the last few years: studies in men with polymorphisms in the genes for either the β chain of the FSH molecule (*FSHB*) or the FSH receptor (*FSHR*) have shown that clinical phenotypes may differ, ranging from azoospermia (Huhtaniemi, 2003) to normal sperm counts (Tüttelmann *et al.*, 2012). These polymorphisms could negatively influence serum FSH levels (*FSHB*) or the FSH receptor affinity (*FSHR*), therefore possibly impairing reproductive health (Tüttelmann *et al.*, 2012); however, clinical manifestations largely depend on the combination of the two alleles (Tüttelmann *et al.*, 2012). A different approach to the hormonal regulation of spermatogenesis seems to suggest that in mammals, as a result of evolutionary changes, FSH is acting as an anti-apoptotic factor, rather than a proliferative signal for Sertoli cells (Meachem *et al.*, 2001; Huhtaniemi, 2015). The role of FSH in human spermatogenesis has not been fully elucidated. However, in mature rodent models, germ cells are lost through apoptosis when FSH action is neutralized (Meachem *et al.*, 1999), although pioneering work on the role of FSH in Sertoli cell proliferation has proven differently (Orth, 1986).

Germ cells do not express receptors for either FSH or androgens, therefore suggesting that the effect on spermatogenesis is mediated through Sertoli cells via production of other hormones acting in a paracrine, autocrine or endocrine fashion. Sertoli cells express the androgen receptor (AR) and it is well-established that adequate levels of intratesticular testosterone are required for successful spermatogenesis. As a member of the TGF-β superfamily (Cate *et al.*, 1986), AMH binds to a heteromeric complex of types I and II single transmembrane serine/threonine kinase receptors (Jamin *et al.*, 2003). Until recently, only the type 2 receptor, AMHR-II, had been identified. This receptor is expressed on mesenchymal cells surrounding the Müllerian ducts and on granulosa cells; AMHR-II is also expressed on Sertoli and Leydig cells, suggesting a more complex interplay between the two cellular populations (Visser, 2003). The type 1 receptor seem to be shared with other members of the TGF-β superfamily: Three type 1 receptors for bone morphogenetic proteins (BMPs), ALK2, ALK3 and ALK6, are possible candidates (Josso & Di Clemente, 2003; Belville *et al.*, 2005).

REGULATION OF AMH EXPRESSION

Anti-Müllerian hormone (AMH), previously described as Müllerian inhibiting substance, is a 140 kD homodimeric disulfide-linked glycoprotein belonging to the TGF-β superfamily (Cate *et al.*, 1986) and secreted in males by Sertoli cells. Expression of AMH is largely dependent on the maturation status of Sertoli cells, as shown by the changes in AMH concentration during fetal life, before puberty and in adults: As expression of androgen receptors increases, AMH production is progressively downregulated (Chemes *et al.*, 2008; Grinspon *et al.*, 2013). Higher AMH concentrations are therefore expected in early stages of testicular development, as a result of reduced androgen receptor expression in Sertoli cells (Chemes *et al.*, 2008; Grinspon *et al.*, 2013). In the fetal male gonad, transcription factor SOX9 acts as a trigger for AMH expression, which is then regulated by different transcription factors including SF1 (steroidogenic factor 1), WT1 (Wilms' tumor 1) GATA4, and FSH (Rey *et al.*, 2003). Evidence from *in vitro* studies hints at the direct interaction between SOX9 and SF1 as a key point for AMH expression (De Santa Barbara *et al.*, 1998), but our knowledge of the exact mechanisms involved in AMH regulation is still largely unknown (Miyamoto *et al.*, 2008). At a later stage of gestation, FSH induces production of AMH by immature Sertoli cells. Following birth, AMH concentrations mirror the delicate balance between the stimulating effects of FSH and the inhibiting effects of testosterone. During infancy and when approaching puberty, Sertoli cells shift their pattern of protein expression and establish tight junctions as a consequence of dramatic changes in both their structure and function: AMH expression is progressively reduced, up to the point of becoming almost undetectable, during transition to adulthood. In puberty, the increasing levels of intra testicular testosterone inhibit AMH secretion, possibly by inducing maturation of Sertoli cells rather than by direct action; however, recent findings suggest a direct effect as well, involving the binding sites for SF1 in the proximal promoter of the AMH gene (Edelsztein *et al.*, 2018). Precocious puberty is associated with a decline in AMH secretion: In a study involving six male patients with central precocious puberty, a significant decrease in serum AMH was observed in subjects older than 1 year which was then restored to pre-pubertal levels by adequate GnRH analogue treatment (Grinspon *et al.*, 2013). The maturation process throughout pubertal development is accompanied by morphological changes of Sertoli cells and reflected by distinct changes in histoskeleton architecture identified by immunohistochemical markers (Franke *et al.*, 2004; Schubert *et al.*, 2011; Pleuger *et al.*, 2016).

In females, AMH is produced by granulosa cells of the preantral and small antral follicles and is therefore detectable in serum only before menopause. Inconsistent findings have emerged from studies investigating the effects of conditions such as vitamin D deficiency, obesity, and smoking on AMH secretion (Shahrokhi *et al.*, 2018). AMH measurement can be used as an adjunct to other metabolic variables in polycystic ovary syndrome (PCOS) diagnosis (Pellatt *et al.*, 2010); although AMH directly correlates with the severity of hyperandrogenism and ovulatory disorders, the lack of a universally accepted threshold and the inter-assay variability both make AMH useful as a surrogate marker, rather than an additional item for the Rotterdam classification (Dewailly *et al.*, 2014). Effects on the progeny of AMH-treated mice have been recently elucidated, suggesting a possible mechanisms of trans-generational transmission (Tata *et al.*, 2018).

AMH IN TESTICULAR PATHOPHYSIOLOGY

The AMH exerts its function on the target tissues by binding to its receptor (AMHR-II), which is expressed on Sertoli and Leydig cells, as well as on the paramesonephric ducts (Matuszczak *et al.*, 2013). In the early stages of fetal life, the interaction between AMH and its receptor induces a change in the morphology of the Müllerian duct mesenchyme, ultimately resulting in apoptosis in the cells of paramesonephric ducts, regression of internal female genitalia, and epitheliomesenchymal transformation (Müllerian ducts, uterus, fallopian tubes, and upper vagina) (Allard *et al.*, 2000; Roberts *et al.*, 2002; Roly *et al.*, 2018). At the same time, testosterone stimulates differentiation of the Wolffian ducts into vas deferens, epididymis, and seminal vesicles. It should be therefore expected that AMH is among the key hormones involved in sex differentiation: Mutations in either AMH or AMHR-II result in a rare condition defined persistent Müllerian duct syndrome (PMDS), in which derivatives of Müllerian ducts are seen in phenotypically normal 46,XY male subjects. This condition is the result of masculinizing effects from endogenous testosterone, which affects Wolffian ducts, and absent pro-apoptotic effects on the Müllerian ducts.

Once Sertoli cells reach their maturation serum concentrations of AMH undergo a rapid decline; however, AMH is preferentially released in the seminiferous tubules, where it reaches far greater concentrations than in the serum (Matuszczak *et al.*, 2013). In newborns, measurement of AMH might be helpful in discerning bilateral cryptorchidism from anorchia. In fact, as Sertoli and granulosa cells are the only source of AMH, pre-pubertal females should have undetectable AMH levels; therefore, measurable concentrations of AMH are strongly suggestive of the presence of testicular tissue. Likewise, serum AMH is a valid and reliable tool for differential diagnosis between congenital hypogonadotropic hypogonadism and constitutional delay in growth and puberty (Condorelli *et al.*, 2018). In the pre-pubertal testis, Sertoli cells are the most prevalent and the most active cell population (Edelsztein *et al.*, 2016), and as such markers of their development are more reliable than those associated with Leydig cell function (Rohayem *et al.*, 2015b). Subjects with delayed puberty show normal AMH levels for their age, while patients with congenital hypogonadotropic hypogonadism have markedly reduced AMH as a result of impaired development of Sertoli cells (Adan *et al.*, 2010).

In Klinefelter patients, normal levels of serum AMH, inhibin B, and FSH are observed until late puberty (Bastida *et al.*, 2007; Aksglaede *et al.*, 2011), with a subsequent decline possibly from hyalinization of seminiferous tubules. In these subjects, chances of sperm retrieval via mTESE (microdissection testicular sperm extraction) are largely dependent on spermatogenetic maturity —therefore suggesting that markers of Leydig cell development, as well as age, should be considered more reliable predictors than AMH and Inhibin B (Rohayem *et al.*, 2015a).

Exogenous FSH administration, as currently used in the treatment of infertile males, is associated with an increase in serum AMH (Young *et al.*, 2005; Colacurci *et al.*, 2018), possibly as a result of enhanced gonadal function. However, hCG administration inhibits AMH secretion from Sertoli cells, whether alone or in combination with FSH (Young *et al.*, 2005; Sinisi *et al.*, 2008). This effect is possibly the result of the increased intra testicular concentration of testosterone due to hCG and fits nicely with evidence suggesting that priming with FSH improves testicular function (Raivio *et al.*, 2007; Isidori *et al.*, 2017; Rohayem & Nieschlag, 2017).

Serum AMH is positively correlated with testicular volume and negatively correlated with serum FSH, but only in men with history of testicular maldescent (Tüttelmann *et al.*, 2009), possibly proving the presence of persisting damage and functional de-differentiation of Sertoli cells (Sharpe *et al.*, 2003). Several reports suggest lower levels of AMH in children with bilateral or unilateral cryptorchidism (Condorelli *et al.*, 2018; Grinspon *et al.*, 2018), providing further confirmation of this theory; similarly, testicular dysgenesis syndrome has been associated with disruption in Sertoli cell maturation, a phenomenon which might contribute to the functional impairment of the Sertoli cell and therefore to reduced AMH secretion (Nistal *et al.*, 2013).

Few studies have assessed the relationship between varicocele and AMH. A single study (Trigo *et al.*, 2004) found increased serum AMH levels in adolescent males with varicocele compared to healthy controls, whereas another study on adult men found no significant difference compared to controls in regard to serum AMH, but found lower concentration of AMH in the spermatic veins of varicocele patients (Goulis *et al.*, 2011). While more solid evidence concerning decreased inhibin B in patients with varicocele suggests a negative effect on Sertoli cell development, the paucity of studies on the association between AMH and varicocele does not allow drawing conclusions in these regards.

Modifications of Sertoli cell structure and patterns of protein expression in most forms of cancer also provide an explanation in regard to the changes in serum AMH detectable in patients with testicular tumors (Rey *et al.*, 2000; Edelsztein *et al.*, 2016). So far, however, the role of AMH as a testicular tumor marker is debated: In females, AMH is most commonly recognized as a marker for granulosa cell tumors of the ovary (Rey *et al.*, 1996; Färkkilä *et al.*, 2015), whereas reports concerning its validity in the diagnosis of Sertoli cell tumors mostly come from animal models (Holst & Dreimanis, 2015; Claes & Ball, 2016). New findings also suggest a possible role for serum AMH and the ratio of AMH to total testosterone as independent predictor biomarkers for successful sperm retrieval at microTESE (Alfano *et al.*, 2017).

REGULATION OF INSL3 EXPRESSION

INSL3 is a 'neohormone'—that is, an adaptation of the endocrine system, stemming from the increasingly complex regulations of reproduction resulting from evolution (Anand-Ivell *et al.*, 2013). Production of INSL3 occurs exclusively in mammalian Leydig cells: In fact, INSL3 is undetectable in anorchid men (Foresta *et al.*, 2004; Bay & Andersson, 2011). INSL3 is constitutively secreted by Leydig cells without direct acute regulation by the HPG axis, therefore being a marker of Leydig cell function and differentiation status (Ivell *et al.*, 2014). It is therefore unsurprising that INSL3 expression closely mirrors the patterns of Leydig cell activity and population size, with a transient increase during fetal life, a second peak occurring roughly 3 months after birth ('mini-puberty'), and a third increase during puberty (Ferlin *et al.*, 2006), which ultimately leads to persisting high concentrations during adult life (Bay & Andersson, 2011) with a slight reduction in older age (Anand-Ivell *et al.*, 2006). Although acute regulation of the HPG axis is not

involved in INSL3 expression, it should be noted that LH stimulation is needed for Leydig cells differentiation and measurable levels of INSL3 after pubertal development: Further proof of this comes from treatment of congenital hypogonadotropic hypogonadism, as men undergoing testosterone treatment show undetectable INSL3 levels whereas a significant increase in INSL3 is observed in patients treated with hCG (Bay *et al.*, 2005). As the differentiating effect of LH on the Leydig cell is conserved during adult life as well, reduced INSL3 levels are observed following suppression of the HPG axis (Bay *et al.*, 2006). Autocrine and paracrine factors have also been considered in the regulation of INSL3 expression: Testosterone and estradiol possibly regulate transcription of the *INSL3* gene by binding to their receptors, respectively, stimulating and inhibiting the transcription factors SF1 and NUR77 (Bay & Andersson, 2011; Lee *et al.*, 2012). A testosterone-responsive element acting as a binding site for NUR77 and SF1 has been identified in the INSL3 promoter (Laguë & Tremblay, 2008); several hypotheses have been postulated concerning the role of estrogens, such as estradiol-mediated disruption of NUR77 and SF1 acetylation status and antagonism between androgen and estrogen receptors, but the exact mechanisms are still largely unknown (Laguë & Tremblay, 2009).

INSL3 IN TESTICULAR PATHOPHYSIOLOGY

INSL3 binds to its receptor RXFP2, mainly expressed on germ cells and Leydig cells. There is solid evidence supporting a role for INSL3 in the transabdominal phase of testicular descent (Bay & Andersson, 2011), as cells of the gubernacular bulb express RXFP2; when INSL3 binds to RXFP2, the following cascade of events in the cell causes thickening of the bulb, effectively 'anchoring' the testis in the inguinal region, next to the abdominal wall (Bay & Andersson, 2011). The following

Figure 1 Testicular endocrine function before and after testicular maturity.

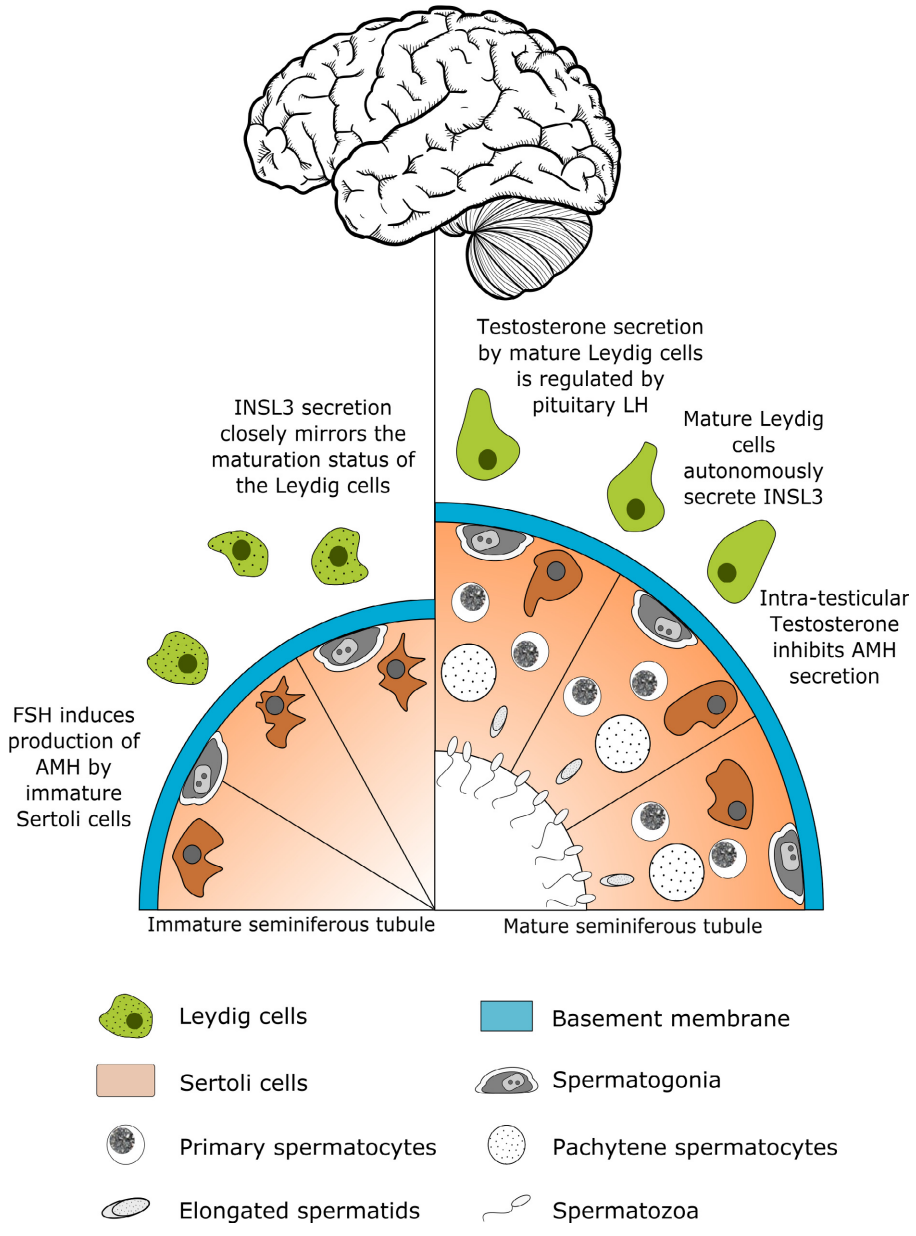

Table 1 Reference values for AMH levels by age. Source (Edelsztein et al., 2016; Ferlin et al., 2006)

Age	Serum AMH pmol/l	ng/ml	Serum INSL3 pg/ml
<14 days	250–1000	35–140	-
15 days – 6 months	400–1500	55–210	-
6 months – 2 years	600–2300	85–320	-
2–9 years	400–1800	55–250	-
9–18 years:			
Tanner 1	250–1400	35–200	10.3–19.8
Tanner 2	70–1000	10–140	30.6–43.7
Tanner 3	30–400	4–55	74.3–92.8
Tanner 4	30–160	4–22	85.5–150.0
Tanner 5	30–150	4–21	100.1–145.8
Adults	25–130	3–18	493.1–643.5

phases of testicular descent likely require both INSL3/RXFP2 and testosterone/AR interaction (Yuan *et al.*, 2010). Mutations in the *INSL3* or *RXFP2* genes are associated with cryptorchidism, but they only account for a small percentage of cases: Frequency of these mutations is 1.8% and 2.9% for INSL3 and RXFP2, respectively, but clinical phenotypes vary, and spontaneous descent during the first years of life has also been reported (Foresta *et al.*, 2008).

INSL3 has proven useful as a surrogate marker of endocrine disruption. Studies in rodent models have proven downregulation of *Insl3* gene expression in the fetal testis following administration of diethylstilbestrol (DES) to pregnant mice (Emmen *et al.*, 2000). This finding corroborates the clinical finding that associated cryptorchidism with administration of DES to pregnant mothers ('blacklisted' by the FDA in the early 1970s, but widely used before that).

BEYOND REPRODUCTION: EXTRA GONADAL PATHOPHYSIOLOGY OF AMH AND INSL3

AMH has been largely considered in recent times as a valid marker for ovarian reserve (van Rooij *et al.*, 2002), whereas no function has been attributed to it in adult males despite detectable serum levels. Some reports have suggested a possible role for AMH in cardiovascular prevention in elderly men (Chong *et al.*, 2013; Dennis *et al.*, 2013), as well as in Klinefelter pre-pubertal boys (Davis *et al.*, 2016) and in premenopausal women (Appt *et al.*, 2012): in fact, AMH inversely correlated with the ultrasonographic diameters of the distal- and mid-infrarenal aorta, independently of other markers of Sertoli cell function such as inhibin B (Dennis *et al.*, 2013). These results require further confirmation, but are plausible given that AMH has been identified as a potent regulator of TGF-β/BMP signaling (Beck *et al.*, 2016), which is in turn involved in vascular development (Lowery & de Caestecker, 2010; Cai *et al.*, 2012).

The most known function of INSL3, as previously stated, involves testicular descent. However, the expression of INSL3 occurs throughout all adult life—therefore suggesting that INSL3 might actually be involved in other conditions. Further proof of an endocrine role for INSL comes from identification of RXFP2 in several other organs, including thyroid, seminal vesicles, kidney, brain, and bone marrow.

It has been hypothesized that some of the defining features of male hypogonadism might actually be the result of reduced INSL3 levels, or at least that low INSL3 might contribute to signs and symptoms of male hypogonadism, such as muscle wasting (Ferlin *et al.*, 2017, 2018). Impaired bone mineral density was the first clinical finding described in association with inactivating mutations in the humans (Ferlin *et al.*, 2008). This hypothesis seemed valid, considering the common origin of both testosterone and INSL3—the Leydig cell—and the identification of RXFP2 on osteoblasts: Following studies confirmed a role for INSL3 in bone remodeling, as receptor activation stimulates osteoblast proliferation and bone anabolic activity while at the same time influencing osteoclastogenesis (Ferlin *et al.*, 2017). Furthermore, lower levels of INSL3 have been described in Klinefelter patients compared to healthy controls (Overvad *et al.*, 2014; Di Nisio *et al.*, 2018) and negatively correlated with sclerostin, an osteocyte-specific protein with anti-anabolic effects on bone formation.

CONCLUSIONS

It is currently clear that Leydig and Sertoli cells are able to influence the endocrine milieu of the testes by several pathways (Fig. 1) and also affecting exocrine testicular function. In these regards, functions of AMH and INSL3 are only beginning to emerge. It is accepted that both hormones are involved in sexual differentiation: Mutations of AMH or its receptor lead to persistence of the Müllerian duct, whereas a decrease in INSL3 levels accounts for a small, but relevant, percentage of cases of cryptorchidism. So far, INSL3 and AMH have prevalently been considered for differential diagnosis as reliable markers of maturation of Leydig and Sertoli cells (Table 1). The role of both INSL3 and AMH in several conditions, such as varicocele, has only marginally been hinted at. Furthermore, effects of AMH and INSL3 extend beyond gonadal range: Symptoms of male hypogonadism have been often associated with decline in serum INSL3 levels, and reduced AMH concentrations have been correlated with worse cardiovascular conditions.

ACKNOWLEDGMENTS

We would like to thank Dr. Joachim Wistuba for his assistance in preparing Fig. 1.

FUNDING INFORMATION

No funding was requested for this work.

DISCLOSURES

The authors have no competing interests to disclose.

AUTHORS' CONTRIBUTIONS

AS and StS designed the study. AS drafted the early manuscript which was later critically appraised and improved by all authors.

REFERENCES

Adan L, Lechevalier P, Couto-Silva A-C, Boissan M, Trivin C, Brailly-Tabard S & Brauner R. (2010) Plasma inhibin B and antimüllerian hormone concentrations in boys: discriminating between congenital hypogonadotropic hypogonadism and constitutional pubertal delay. *Med Sci Monit*, 16, CR511–R517.

Aksglaede L, Christiansen P, Sørensen K, Boas M, Linneberg A, Main KM, Andersson AM, Skakkebæk NE & Juul A. (2011) Serum concentrations

of Anti-Müllerian Hormone (AMH) in 95 patients with Klinefelter syndrome with or without cryptorchidism. *Acta Paediatr Int J Paediatr* 100, 839–845.

Alfano M, Ventimiglia E, Locatelli I, Capogrosso P, Cazzaniga W, Pederzoli F, Frego N, Matloob R, Saccà A, Pagliardini L, Viganò P, Zerbi P, Nebuloni M, Pontillo M, Montorsi F & Salonia A. (2017) Anti-mullerian hormone-to-testosterone ratio is predictive of positive sperm retrieval in men with idiopathic non-obstructive azoospermia. *Sci Rep* 7, 1–9.

Allard S, Adin P, Gouédard L, di Clemente N, Josso N, Orgebin-Crist MC, Picard JY & Xavier F. (2000) Molecular mechanisms of hormone-mediated Müllerian duct regression: involvement of beta-catenin. *Development* 127, 3349–3360.

Anand-Ivell R, Wohlgemuth J, Haren MT, Hope PJ, Hatzinikolas G, Wittert G & Ivell R. (2006) Peripheral INSL3 concentrations decline with age in a large population of Australian men. *Int J Androl* 29, 618–626.

Anand-Ivell R, Dai Y & Ivell R. (2013) Neohormones as biomarkers of reproductive health. *Fertil Steril* 99, 1153–1160.

Appt SE, Chen H, Clarkson TB & Kaplan JR. (2012) Premenopausal antimüllerian hormone concentration is associated with subsequent atherosclerosis. *Menopause J North Am Menopause Soc* 19, 1353–1359.

Bastida MG, Rey RA, Bergadá I, Bedecarrás P, Andreone L, Del Rey G, Boywitt A, Ropelato MG, Cassinelli H, Arcari A, Campo S & Gottlieb S. (2007) Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. *Clin Endocrinol (Oxf)* 67, 863–870.

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

Bay K, Hartung S, Ivell R, Schumacher M, Jürgensen D, Jorgensen N, Holm M, Skakkebaek NE & Andersson AM. (2005) Insulin-like factor 3 serum levels in 135 normal men and 85 men with testicular disorders: relationship to the luteinizing hormone-testosterone axis. *J Clin Endocrinol Metab* 90, 3410–3418.

Bay K, Matthiesson KL, McLachlan RI & Andersson A-M. (2006) The effects of gonadotropin suppression and selective replacement on insulin-like factor 3 secretion in normal adult men. *J Clin Endocrinol Metab* 91, 1108–1111.

Beck TN, Korobeynikov VA, Kudinov AE, Georgopoulos R, Solanki NR, Andrews-Hoke M, Kistner TM, Pépin D, Donahoe PK, Nicolas E, Einarson MB, Zhou Y, Boumber Y, Proia DA, Serebriiskii IG & Golemis EA. (2016) Anti-Müllerian hormone signaling regulates epithelial plasticity and chemoresistance in lung cancer. *Cell Rep* 16, 657–671.

Belville C, Jamin SP, Picard JY, Josso N & Di Clemente N. (2005) Role of type I receptors for anti-Müllerian hormone in the SMAT-1 Sertoli cell line. *Oncogene* 24, 4984–4992.

Bhasin S, Huang G, Travison TG & Basaria S. (2000) Age-related changes in the male reproductive axis. In: *Endotext [Internet]* (eds. KR Feingold, B Anawalt, A Boyce, G Chrousos, K Dungan, A Grossman, JM Hershman, G Kaltsas, C Koch, P Kopp, M Korbonits, R McLachlan, JE Morley, M New, L Perreault, J Purnell, R Rebar, F Singer, DL Trence, A Vinik & DP Wilson). MDText.com, Inc., South Dartmouth (MA).

Cai J, Pardali E, Sanchez-Duffhues G & ten Dijke P. (2012) BMP signaling in vascular diseases. *FEBS Lett* 586, 1993–2002.

Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A & Donahoe PK. (1986) Isolation of the bovine and human genes for müllerian inhibiting substance and expression of the human gene in animal cells. *Cell* 45, 685–698.

Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, González-Peramato P & Serrano Á. (2008) Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in sertoli cells. *J Clin Endocrinol Metab* 93, 4408–4412.

Chong YH, Dennis NA, Connolly MJ, Teh R, Jones GT, van Rij AM, Farrand S, Campbell AJ & McLennan IS. (2013) Elderly men have low levels of anti-müllerian hormone and inhibin B, but with high interpersonal variation: a cross-sectional study of the sertoli cell hormones in 615 community-dwelling men. *PLoS ONE* 8, e70967.

Claes ANJ & Ball BA. (2016) Biological functions and clinical applications of anti-müllerian hormone in stallions and mares. *Vet Clin North Am – Equine Pract* 32, 451–464.

Colacurci N, De Leo V, Ruvolo G, Piomboni P, Caprio F, Pivonello R, Papaleo E, La Verde E, Depalo R, Lispi M, Longobardi S, Paoli D, Pallotti F & Lombardo F. (2018) Recombinant FSH improves sperm DNA damage in male infertility: a phase II clinical trial. *Front Endocrinol (Lausanne)* 9, 383.

Condorelli RA, Cannarella R, Calogero AE & La Vignera S. (2018) Evaluation of testicular function in prepubertal children. *Endocrine* 62, 274–280.

Davis S, Lahlou N, Bardsley M, Temple M-C, Kowal K, Pyle L, Zeitler P & Ross J. (2016) Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. *Andrology* 4, 1169–1177.

De Santa Barbara P, Bonneaud N, Boizet B, Desclozeaux M, Moniot B, Sudbeck P, Scherer G, Poulat F & Berta P. (1998) Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. *Mol Cell Biol* 18, 6653–6665.

Dennis NA, Jones GT, Chong YH, van Rij AM & McLennan IS. (2013) Serum anti-Müllerian hormone (AMH) levels correlate with infrarenal aortic diameter in healthy older men: is AMH a cardiovascular hormone? *J Endocrinol* 219, 13–20.

Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH & Anderson RA (2014) The physiology and clinical utility of anti-Müllerian hormone in women. *Hum Reprod Update* 20, 370–385.

Di Nisio A, De Toni L, Rocca MS, Ghezzi M, Selice R, Taglialavoro G, Ferlin A & Foresta C. (2018) Negative association between sclerostin and INSL3 in isolated human osteocytes and in klinefelter syndrome: new hints for testis-bone crosstalk. *J Clin Endocrinol Metab* 103, 2033–2041.

Dufau ML, Winters CA, Hattori M, Aquilano D, Barañao JLS, Nozu K, Baukal A & Catt KJ. (1984) Hormonal regulation of androgen production by the Leydig cell. *J Steroid Biochem* 20, 161–173.

Edelsztein NY, Grinspon RP, Schteingart HF & Rey R. (2016) 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.

Edelsztein NY, Racine C, di Clemente N, Schteingart HF & Rey RA. (2018) Androgens downregulate anti-Müllerian hormone promoter activity in the Sertoli cell through the androgen receptor and intact steroidogenic factor 1 sites. *Biol Reprod* 99, 1303–1312.

Emmen JMA, McLuskey A, Adham IM, Engel W, Verhoef-Post M, Themmen APN, Grootegoed JA & Brinkmann AO. (2000) Involvement of insulin-like factor 3 (Insl3) in diethylstilbestrol-induced cryptorchidism. *Endocrinology* 141, 846–849.

Färkkilä A, Koskela S, Bryk S, Alfthan H, Bützow R, Leminen A, Puistola U, Tapanainen JS, Heikinheimo M, Anttonen M & Unkila-Kallio L. (2015) The clinical utility of serum anti-Müllerian hormone in the follow-up of ovarian adult-type granulosa cell tumors – A comparative study with inhibin B. *Int J Cancer* 137, 1661–1671.

Ferlin A, Garolla A, Rigon F, Rasi Caldogno L, Lenzi A & Foresta C. (2006) Changes in serum insulin-like factor 3 during normal male puberty. *J Clin Endocrinol Metab* 91, 3426–3431.

Ferlin A, Pepe A, Gianesello L, Garolla A, Feng S, Giannini S, Zaccolo M, Facciolli A, Morello R, Agoulnik AI & Foresta C. (2008) Mutations in

the insulin-like factor 3 receptor are associated with osteoporosis. *J Bone Miner Res* 23, 683–693.
Ferlin A, De Toni L, Sandri M & Foresta C. (2017) Relaxin and insulin-like peptide 3 in the musculoskeletal system: from bench to bedside. *Br J Pharmacol* 174, 1015–1024.
Ferlin A, De Toni L, Agoulnik AI, Lunardon G, Armani A, Bortolanza S, Blaauw B, Sandri M & Foresta C. (2018) Protective role of testicular hormone INSL3 from atrophy and weakness in skeletal muscle. *Front Endocrinol (Lausanne)* 9, 1–15.
Foresta C, Bettella A, Vinanzi C, Dabrilli P, Meriggiola MC, Garolla A & Ferlin A. (2004) A novel circulating hormone of testis origin in humans. *J Clin Endocrinol Metab* 89, 5952–5958.
Foresta C, Zuccarello D, Garolla A & Ferlin A. (2008) Role of hormones, genes, and environment in human cryptorchidism. *Endocr Rev* 29, 560–580.
Franke FE, Pauls K, Rey R, Marks A, Bergmann M & Steger K. (2004) Differentiation markers of Sertoli cells and germ cells in fetal and early postnatal human testis. *Anat Embryol (Berl)* 209, 169–177.
Goulis DC, Mintziori G, Koliakos N, Hatzichristou D, Papadimas I, Hatzimouratidis K & Goulis DG. (2011) Inhibin B and anti-Müllerian hormone in spermatic vein of subfertile men with varicocele. *Reprod Sci* 18, 551–555.
Grinspon RP, Andreone L, Bedecarrás P, Ropelato MG, Rey RA, Campo SM & Bergadá I. (2013) Male central precocious puberty: serum profile of anti-müllerian hormone and inhibin B before, during, and after treatment with GnRH analogue. *Int J Endocrinol* 2013, 2–7.
Grinspon RP, Gottlieb S, Bedecarrás P & Rey R. (2018) Anti-Müllerian hormone and testicular function in prepubertal boys with cryptorchidism. *Front Endocrinol (Lausanne)* 9, 182.
Holst BS & Dreimanis U. (2015) Anti-Müllerian hormone: a potentially useful biomarker for the diagnosis of canine Sertoli cell tumours. *BMC Vet Res* 11, 1–7.
Huhtaniemi I. (2003) Mutations affecting gonadotropin secretion and action. *Horm Res* 60(Suppl 3), 21–30.
Huhtaniemi I. (2015) A short evolutionary history of FSH-stimulated spermatogenesis. *Hormones* 14, 468–478.
Isidori AM, Sansone A & Gianfrilli D. (2017) Hormonal treatment of male infertility: gonadotropins and beyond. In: *Endocrinology of the testis and male reproduction*(eds. M Simoni & I Huhtaniemi), pp. 1–20. Springer International Publishing, Cham.
Ivell R, Heng K & Anand-Ivell R. (2014) Insulin-like factor 3 and the HPG axis in the male. *Front Endocrinol (Lausanne)* 5, 6.
Jamin SP, Arango NA, Mishina Y, Hanks MC & Behringer RR. (2003) Genetic studies of the AMH/MIS signaling pathway for Müllerian duct regression. *Mol Cell Endocrinol* 211, 15–19.
Josso N & Di Clemente N. (2003) Transduction pathway of anti-Müllerian hormone, a sex-specific member of the TGF-β family. *Trends Endocrinol Metab* 14, 91–97.
Laguë É & Tremblay JJ. (2008) Antagonistic effects of testosterone and the endocrine disruptor mono-(2-ethylhexyl) phthalate on INSL3 transcription in Leydig cells. *Endocrinology* 149, 4688–4694.
Laguë É & Tremblay JJ. (2009) Estradiol represses Insulin-like 3 expression and promoter activity in MA-10 Leydig cells. *Toxicology* 258, 101–105.
Lee SY, Park E, Kim SC, Ahn RS, Ko CM & Lee K. (2012) ERα/E2 signaling suppresses the expression of steroidogenic enzyme genes via cross-talk with orphan nuclear receptor Nur77 in the testes. *Mol Cell Endocrinol* 362, 91–103.
Lowery JW & de Caestecker MP. (2010) BMP signaling in vascular development and disease. *Cytokine Growth Factor Rev* 21, 287–298.
Lucas TF, Nascimento AR, Pisolato R, Pimenta MT, Lazari MFM & Porto CS. (2014) Receptors and signaling pathways involved in proliferation and differentiation of Sertoli cells. *Spermatogenesis* 4, e28138.
Matuszczak E, Hermanowicz A, Komarowska M & Debek W. (2013) Serum AMH in physiology and pathology of male gonads. *Int J Endocrinol* 2013, 1–6.
Meachem SJ, Mclachlan RI, Stanton PG, Robertson DM & Wreford NG (1999) FSH immunoneutralization acutely impairs spermatogonial development in normal adult rats. *J Androl* 20, 756–762; discussion 755.
Meachem SJ, von Schönfeldt V & Schlatt S. (2001) Spermatogonia: stem cells with a great perspective. *Reproduction* 121, 825–834.
Miyamoto Y, Taniguchi H, Hamel F, Silversides DW & Viger RS. (2008) A GATA4/WT1 cooperation regulates transcription of genes required for mammalian sex determination and differentiation. *BMC Mol Biol* 9, 1–18.
Nistal M, Gonzalez-Peramato P & De Miguel MP. (2013) Sertoli cell dedifferentiation in human cryptorchidism and gender reassignment shows similarities between fetal environmental and adult medical treatment estrogen and antiandrogen exposure. *Reprod Toxicol* 42, 172–179.
Orth JM. (1986) FSH-induced sertoli cell proliferation in the developing rat is modified by β-endorphin produced in the testis. *Endocrinology* 119, 1876–1878.
Overvad S, Bay K, Bojesen A & Gravholt CH. (2014) Low INSL3 in Klinefelter syndrome is related to osteocalcin, testosterone treatment and body composition, as well as measures of the hypothalamic-pituitary-gonadal axis. *Andrology* 2, 421–427.
Pellatt L, Rice S & Mason HD. (2010) Anti-Müllerian hormone and polycystic ovary syndrome: a mountain too high? *Reproduction* 139, 825–833.
Pleuger C, Fietz D, Hartmann K, Weidner W, Kliesch S, O'Bryan MK, Dorresteijn A & Bergmann M. (2016) Expression of katanin p80 in human spermatogenesis. *Fertil Steril* 106, 1683–1690.e1.
Raivio T, Wikström AM & Dunkel L. (2007) Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol* 156, 105–111.
Rey R, Lhomme C, Marcillac I, Lahlou N, Duvillard P, Jesse N & Bidart JM. (1996) Antimullerian hormone as a serum marker of granulosa cell tumors of the ovary: comparative study with serum α-inhibin and estradiol. *Am J Obstet Gynecol* 174, 958–965.
Rey R, Sabourin JC, Venara M, Long WQ, Jaubert F, Zeller WP, Duvillard P, Chemes H & Bidart JM. (2000) Anti-mullerian hormone is a specific marker of sertoli- and granulosa-cell origin in gonadal tumors. *Hum Pathol* 31, 1202–1208.
Rey R, Lukas-Croisier C, Lasala C & Bedecarrás P. (2003) AMH/MIS: what we know already about the gene, the protein and its regulation. *Mol Cell Endocrinol* 211, 21–31.
Roberts LM, Visser JA & Ingraham HA. (2002) Involvement of a matrix metalloproteinase in MIS-induced cell death during urogenital development. *Development* 129, 1487–1496.
Rohayem J & Nieschlag E. (2017) Stimulation of spermatogenesis in hypogonadotropic men. In: *Male hypogonadism*(eds. SJ Winters & IT Huhtaniemi), pp. 423–436. Springer International Publishing, Cham.
Rohayem J, Fricke R, Czeloth K, Mallidis C, Wistuba J, Krallmann C, Zitzmann M & Kliesch S. (2015a) 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.
Rohayem J, Nieschlag E, Kliesch S & Zitzmann M. (2015b) Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. *Andrology* 3, 882–887.
Roly ZY, Backhouse B, Cutting A, Tan TY, Sinclair AH, Ayers KL, Major AT & Smith CA. (2018) The cell biology and molecular genetics of Müllerian duct development. *Wiley Interdiscip Rev Dev Biol* 7, e310.

van Rooij IAJ, Broekmans FJM, te Velde ER, Fauser BCJM, Bancsi LFJMM, de Jong FH & Themmen APN. (2002) Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 17, 3065–3071.

Schubert K, Polte T, Bönisch U, Schader S, Holtappels R, Hildebrandt G, Lehmann J, Simon JC, Anderegg U & Saalbach A. (2011) Thy-1 (CD90) regulates the extravasation of leukocytes during inflammation. *Eur J Immunol* 41, 645–656.

Shahrokhi SZ, Kazerouni F & Ghaffari F. (2018) Anti-Müllerian Hormone: genetic and environmental effects. *Clin Chim Acta* 476, 123–129.

Sharpe RM, McKinnell C, Kivlin C & Fisher JS. (2003) Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction* 125, 769–784.

Simoni M, Gromoll J & Nieschlag E. (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr Rev* 18, 739–773.

Sinisi AA, Esposito D, Maione L, Quinto MC, Visconti D, De Bellis A, Bellastella A, Conzo G & Bellastella G. (2008) Seminal anti-Müllerian hormone level is a marker of spermatogenic response during long-term gonadotropin therapy in male hypogonadotropic hypogonadism. *Hum Reprod* 23, 1029–1034.

Stamatiades GA & Kaiser UB. (2018) Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. *Mol Cell Endocrinol* 463, 131–141.

Tata B, Mimouni NEH, Barbotin A-L, Malone SA, Loyens A, Pigny P, Dewailly Di, Catteau-Jonard S, Sundström-Poromaa I, Piltonen TT, Dal Bello F, Medana C, Prevot V, Clasadonte J & Giacobini P. (2018) Elevated prenatal anti-Müllerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. *Nat Med* 24, 834–846.

Trigo RV, Bergadá I, Rey R, Ballerini MG, Bedecarrás P, Bergadá C, Gottlieb S & Campo S. (2004) Altered serum profile of inhibin B, Pro-αC and anti-Müllerian hormone in prepubertal and pubertal boys with varicocele. *Clin Endocrinol (Oxf)* 60, 758–764.

Tüttelmann F, Dykstra N, Themmen APN, Visser JA, Nieschlag E & Simoni M. (2009) Anti-Müllerian hormone in men with normal and reduced sperm concentration and men with maldescended testes. *Fertil Steril* 91, 1812–1819.

Tüttelmann F, Laan M, Grigorova M, Punab M, Sõber S & Gromoll J. (2012) Combined effects of the variants FSHB -211G>T and FSHR 2039A>G on male reproductive parameters. *J Clin Endocrinol Metab* 97, 3639–3647.

Visser JA. (2003) AMH signaling: from receptor to target gene. *Mol Cell Endocrinol* 211, 65–73.

Walker WH & Cheng J. (2005) FSH and testosterone signaling in Sertoli cells. *Reproduction* 130, 15–28.

Young J, Chanson P, Salenave S, Noël M, Brailly S, O'Flaherty M, Schaison G & Rey R. (2005) Testicular anti-Müllerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 90, 724–728.

Yuan FP, Li X, Lin J, Schwabe C, Büllesbach EE, Rao CV & Lei ZM. (2010) The role of RXFP2 in mediating androgen-induced inguinoscrotal testis descent in LH receptor knockout mice. *Reproduction* 139, 759–769.

