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Mini-Review

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Abstract

Context: Anti-Mullerian hormone (AMH) was originally described in the context of sexual differentiation in the male fetus but has gained prominence now as a marker of ovarian reserve and fertility in females. In this mini-review, we offer an updated synopsis on AMH and its clinical utility in pediatric patients.

Design and Results: A systematic search was undertaken for studies related to the physiology of AMH, normative data, and clinical role in pediatrics. In males, AMH, secreted by Sertoli cells, is found at high levels prenatally and throughout childhood and declines with progression through puberty to overlap with levels in females. Thus, serum AMH has clinical utility as a marker of testicular tissue in males with differences in sexual development and cryptorchidism and in the evaluation of persistent Mullerian duct syndrome. In females, serum AMH has been used as a predictive marker of ovarian reserve and fertility, but prepubertal and adolescent AMH assessments need to be interpreted cautiously. AMH is also a marker of tumor burden, progression, and recurrence in germ cell tumors of the ovary.

Conclusions: AMH has widespread clinical diagnostic utility in pediatrics but interpretation is often challenging and should be undertaken in the context of not only age and sex but also developmental and pubertal stage of the child. Nonstandardized assays necessitate the need for assay-specific normative data. The recognition of the role of AMH beyond gonadal development and maturation may usher in novel diagnostic and therapeutic applications that would further expand its utility in pediatric care.

Anti-Mullerian hormone (AMH), also known as Mullerian inhibiting substance (MIS), is a hormone produced exclusively in the gonads. Alfred Jost, a pioneering researcher in the field of fetal endocrinology first proposed the existence of the "hormone inhibitrice" in the 1940s when he demonstrated the regression of the "Mullerian ducts" (paramesonephric ducts), anlagen to the uterus, Fallopian tubes, cervix, and upper third of the vagina, in undifferentiated female rabbit embryos following surgical implants of testicular tissue (1,2). Josso et al demonstrated that the Sertoli cells secreted MIS, a glycoprotein (3) that was eventually purified, and coined the term "AMH" now widely in use today, and Donahoe et al synthesized functional recombinant human MIS (4). In the decades that have followed, the sexually dimorphic functions of AMH have not only played a part in the diagnosis of differences (originally "disorders") in sexual development (DSD) but has found extensive clinical utility in female fertility and reproductive health and its significance continues to evolve with the finding of novel neuroendocrine regulatory actions of AMH (5). In this mini-review, we examine the physiological role of AMH and its clinical utility in pediatric patients.

Search Strategy

We performed a literature review in PubMed limiting to English language articles, with no beginning date and search was last updated in July 2021. The search term was "anti-Mullerian hormone" (MeSH term) comprising all variations of AMH and MIS. We used filters for English language and age (child: birth-18 years), and a total of 599 manuscripts including 41 review articles were identified. We narrowed down the search further with filters for clinical studies and systematic reviews to 70 articles. The reference lists of the original and review articles were then reviewed to ensure completeness.

Physiological Role of AMH

AMH is secreted as a 140kDa dimeric glycoprotein hormone structurally related to transforming growth factor β and inhibin (6). It undergoes proteolytic cleavage and generation of bioactive 25kDa C-terminal dimers that bind to the AMH type 2 receptor (7-13). It is thought that, in a similar manner to other members of the transforming growth factor β family, the ligand bound AMH type 2 receptor phosphorylates the type 1 receptor [also a serine/threonine kinase receptor, that belong to a class of activin-like kinase (ALK2)] inducing signaling through

phosphorylation of intracellular Smad proteins, which then translocate to the nucleus and modulate gene transcription (14) (Fig. 1A and B). The *AMH* gene has been localized to chromosome 19p13.3 (15), and its expression is tightly regulated in Sertoli and granulosa cells specific to sex and developmental stage (fetal, neonatal, pre-and postpubertal) and regulated by SRY with activation of *AMH* by SOX-9 (16) and subsequent increase in *AMH* promoter activity by transcription factors steroidogenic factor 1 (SF-1) (17), GATA-4 (18,19), and WT-1 (20) and downregulation by DAX-1 (20). In the testes, high follicle-stimulating (FSH) levels activate AMH secretion (21), but androgens, specifically intratesticular testosterone, downregulates AMH secretion acting through the androgen receptor (Fig. 1A) but requires SF-1 binding to *AMH* promoter sites (22). In the ovarian granulosa cells, AMH secretion appears to be regulated by transcription factors SF1, FOXL2, FOG2, and GATA-4 and stimulated by FSH and luteinizing hormone (LH) (23-25).

In the fetal Sertoli cells, *AMH* expression was seen starting around 8 weeks of gestation (26) during a short window when Mullerian ducts are responsive to its effect (27), leading to irreversible Mullerian duct regression by the ninth week (28). AMH continues to be produced by the fetal testes throughout gestation despite increase in testosterone since Sertoli cells in the fetus do not express androgen receptors (29). Jost proposed that AMH may play a role in the meiotic and mitotic arrest of germ cells (30), and others have suggested roles for AMH in testicular descent, germ cell maturation, and gonadal morphology, but these remain inconclusive (31). After a transient decline postnatally, AMH levels rise in infancy and continue to remain comparatively higher in males until they decline by stages 2 and 3 of puberty as intratesticular testosterone levels start to rise (32). At this point, AMH levels in postpubertal males are comparable and overlap with AMH levels in females considerably although median AMH remains 2- to 3-fold higher compared with females (33). Immunohistochemistry has shown that the expression of AMH type 2 receptor parallels AMH in serum declining after puberty in males (34). A paracrine effect of AMH was also postulated given findings that AMH pathway activation may directly suppress the expression of steroidogenic enzymes in the Leydig cells of the adult testes (35).

In contrast, *AMH* expression in fetal ovaries was detected around 23 weeks of gestation, with serum AMH detectable postnatally by 26 weeks of gestation (36). AMH expression was highest in the granulosa cells of secondary, preantral, and small antral cells (less than 4 mm in diameter)

and significantly declines in larger antral follicles (6-8 mm) (37). Data from rodent studies (38) and immunostaining patterns in human ovaries (37) suggest that AMH may suppress initial recruitment of primordial follicles and also modulate follicular sensitivity to FSH (39), regulating the growth of follicles and the cyclic recruitment of the larger antral follicles. AMH also downregulates the aromatase activity in granulosa cells decreasing estradiol production
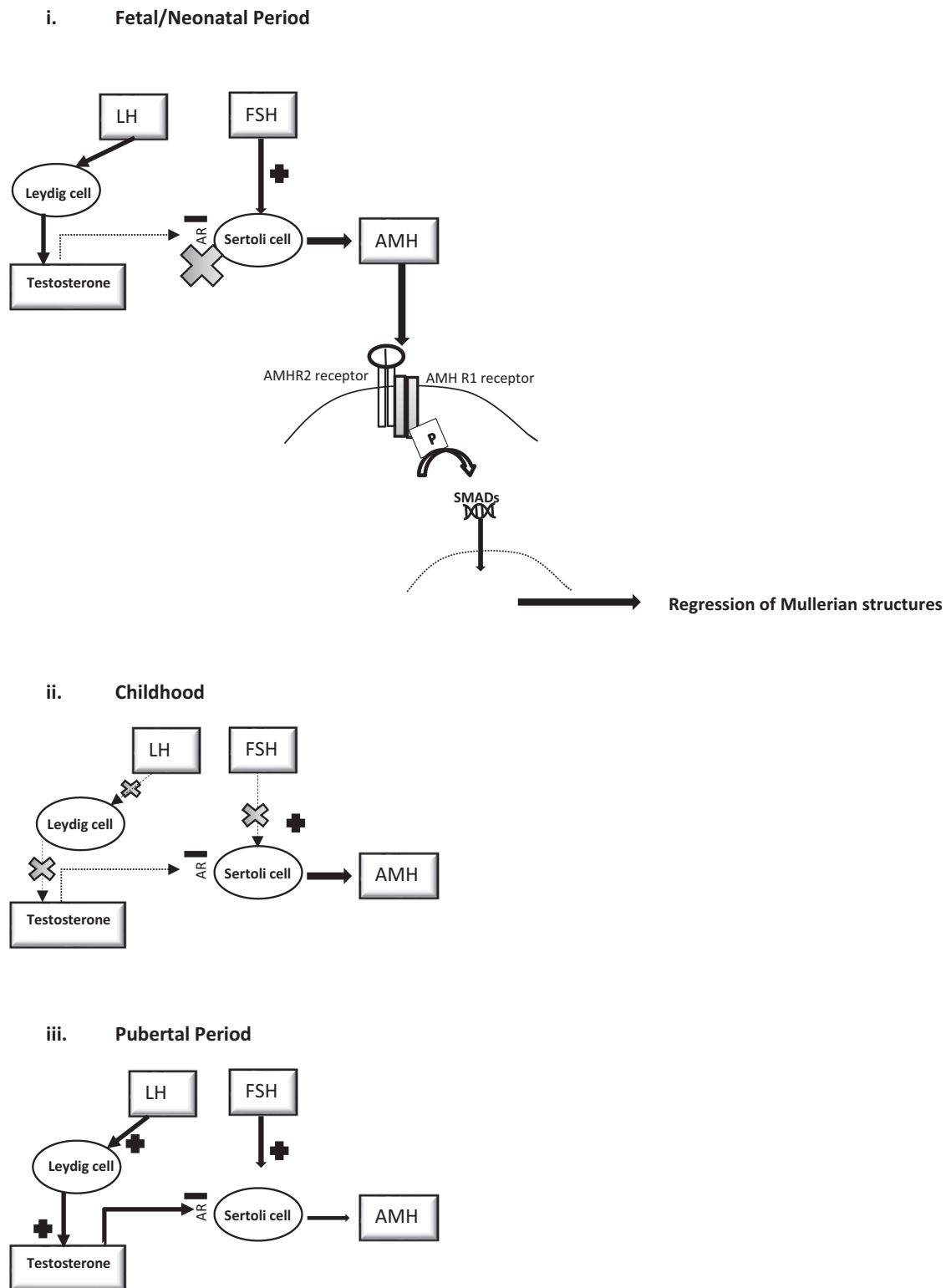

Figure 1. Regulation of anti-Mullerian hormone (AMH) secretion and signaling. (A) Regulation of AMH secretion in males by developmental stage. (B) Regulation of AMH secretion in females and its role in ovarian follicle maturation. Abbreviation: AR, Androgen receptor.
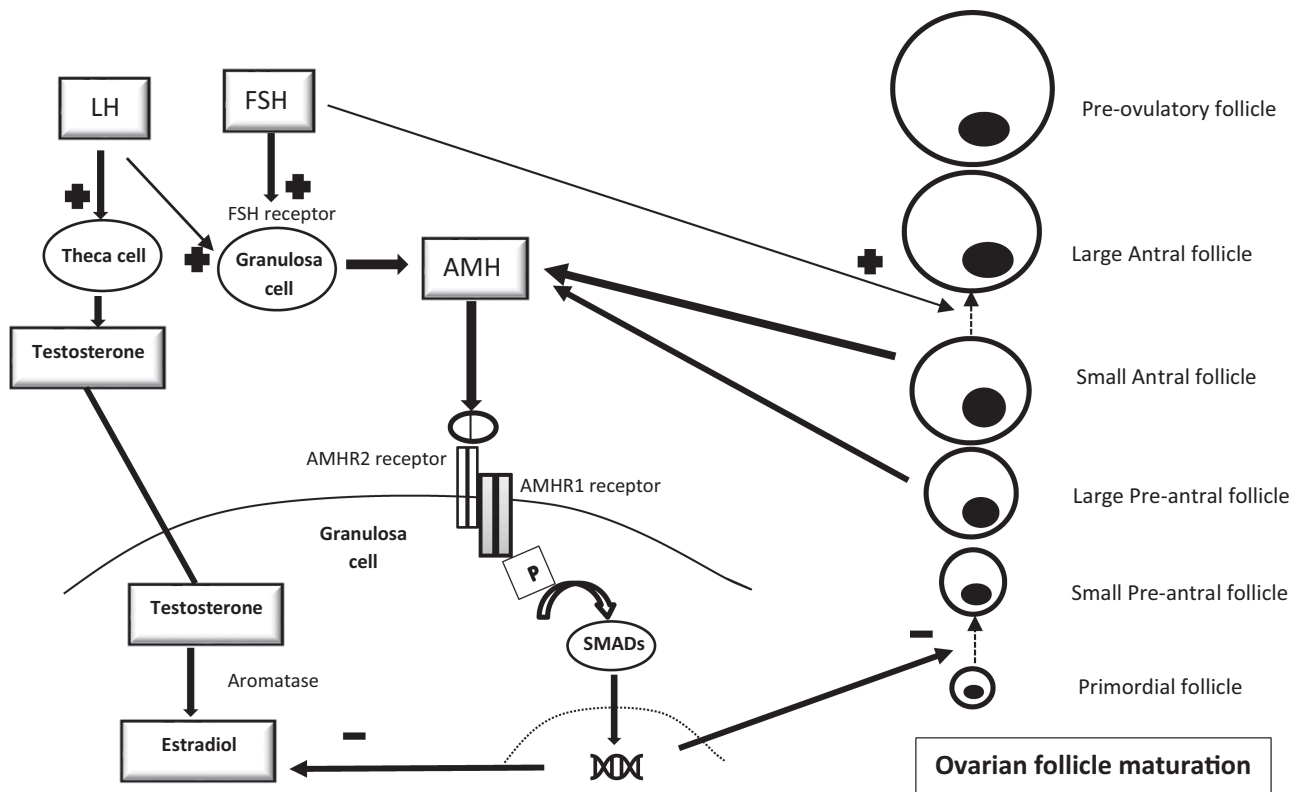
Figure 1. Continued.

until follicular selection (40,41). AMH tends to be 35-fold lower in females compared with males in infancy and remains stable through childhood and adolescence (42). Recent longitudinal data suggest a slight rise in AMH by 7 to 9 years with a dip around the ages of 10 to 14 years correlating with the transition through puberty, rising again by age 16 years to peak in young adulthood (43). AMH then declines over the reproductive age proportionate to the decline in the antral follicle pool and decreases rapidly by menopause and, as such, is suggested as a marker of ovarian aging (44).

Serum AMH Measurements

Enzyme-linked immunosorbent assays were first developed to measure AMH in the 1990s (45) but were not adequately sensitive to measure levels especially in females. In 2000, an ultrasensitive assay was developed (46) with a detection limit of 2 ng/mL [Immunotech (IOT), Marseille, France] followed by a more sensitive assay with a detection limit of 6.3 pg/mL [Diagnostic Systems Laboratory (DSL), Webster, TX, USA] (41). Since the calibrators and antibodies were different, the DSL assay results for serum AMH in women was 4.6-fold lower than the IOT assay (47), invalidating comparisons between studies using these 2 different nonstandardized assays. A Gen II assay of AMH

(Beckman Coulter, Chaska, MN, USA) has been in use since 2010 (48), and normative data on serum AMH are depicted in Figure 2 (49). Although calibrated to the IOT assay, several studies have raised concerns with regard to the Gen II AMH assay, especially related to sample characteristics and differences in calibration compared with newer immunoassays in the past few years (50). Hence, there is still a need to establish assay-specific normative data in the absence of an international reference standard.

AMH concentrations are age and sex-specific (49), rise in early infancy starting 1 month after birth peaking around 6 months of age in male infants while they remain low in female infants (Fig. 2). Throughout childhood, AMH concentrations are distinctly higher in males (almost 35-fold) (51), so despite individual variability and a broad normal range, it is easily discriminated from female norms (33) (Fig. 2). In adolescence with progression through puberty, the mean AMH levels decline in males and increase in females with considerable overlap (33,52).

Clinical Utility of AMH

AMH and Differences in Sexual Development

The process of sexual differentiation is a carefully orchestrated process with multiple regulatory elements impacting
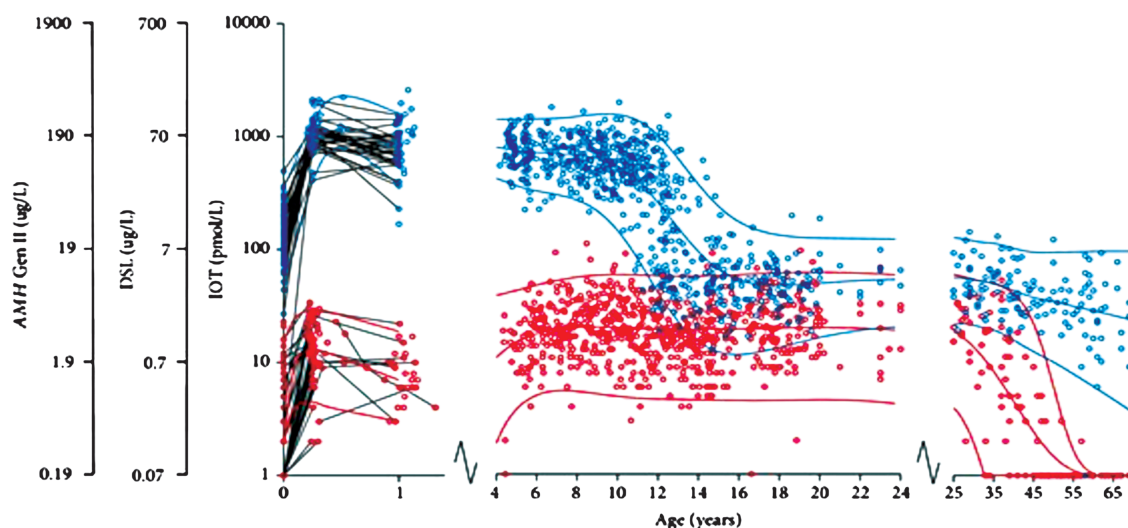
Figure 2. Reference ranges for serum anti-Mullerian hormone (AMH). Serum AMH in 1027 males (blue circles) and 926 females (red circles) is depicted with blue and red lines depicting (median, +/−2 SD) reference ranges for males and females, respectively. Connecting grey lines represent longitudinal values in infancy. Age in years on the *x*-axis and serum AMH (pmol/L) measured on Immunotech (IOT) on the logarithmic *y*-axis. Comparative data for Diagnostic Systems Laboratory (DSL) and Gen II assays were calculated as follows: AMH (IOT) pmol/L = 2.0 × AMH (DSL) µg/L × 7.14 pmol/µg and AMH (IOT) pmol/L = 0.74 × AMH (Gen II) µg/L × 7.14 pmol/µg. This figure is reproduced from Johansen ML, et al (49). Copyright © 2013 Marie Lindhardt Johansen et al. This is an open access article distributed under the Creative Commons Attribution License http://creativecommons.org/licenses/by/3.0/ which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

steps of differentiation. AMH was first described in the context of dysregulated sexual differentiation, and the first clinical utility was in the diagnosis of differences of sexual development. While the presence of Mullerian duct derivatives reflects the lack of AMH secretion during the sixth to tenth weeks of fetal life, the serum AMH measurements after birth reflect not only Sertoli cell function in patients with DSD but also serve as a biomarker of the relative influence of FSH and androgens (53). FSH increases Sertoli cell mass and AMH secretion, but testosterone acting via the androgen receptors in Sertoli cells postnatally suppresses AMH (22,54). So, AMH concentrations are directly correlated to FSH but inversely to testosterone levels in childhood and adolescence, in contrast to prenatal values. AMH concentrations vary by pathology, genetic defect, and developmental stage and are summarized in Table 1. In 46,XY gonadal dysgenesis, the AMH concentrations are proportionate to the presence of testicular tissue, typically being low or undetectable in conditions affecting testicular development (55). In Leydig cell hypoplasia, and androgen synthetic defects, the AMH concentrations are typically normal and may even be high during the minipuberty of infancy due to a lack of the suppressive effect of androgens (55,56). In 5-alpha reductase deficiency, characterized by a high testosterone:dihydrotestosterone ratio, AMH was in the lower range of normal (<−1 SD) since dihydrotestosterone is not required for suppression of AMH secretion (57). In androgen insensitivity syndrome, however, the relatively high gonadotropins and lack of a functional

androgen receptor cause AMH levels to be inappropriately high for the degree of testosterone in the serum (56). Recent studies indicate that the hyperestrogenic state in complete AIS may also play a role in AMH elevation (58). A study of the testosterone response to human chorionic gonadotropin (hCG) stimulation in prepubertal patients with 46,XY DSD found that a normal serum AMH had a positive predictive value of 84% for a normal post-hCG testosterone level, but a low AMH was not useful in predicting a suboptimal testosterone response (59). Gonadal dysgenesis and ovotestes were associated with mean AMH concentrations in between levels seen in anorchia and undescended testes (60). In summary, AMH can be a useful tool for assessment of Sertoli cell function in 46,XY DSD and can help distinguish testicular dysgenesis from biosynthetic defects.

In 46,XX DSD, serum AMH above the female reference standards suggest the presence of testicular tissue (as seen in ovotesticular or testicular DSD), which distinguishes these disorders from virilized female infants with high extratesticular androgens (such as congenital adrenal hyperplasia) (55) who have typical female AMH levels.

AMH in Klinefelter syndrome was found to be within the normal reference range during minipuberty of infancy and throughout childhood (61). The decline in AMH with puberty was delayed in adolescents with Klinefelter syndrome although pubertal onset is still on time (62). This has been attributed to a downregulation of *AMH* in these affected individuals (63), as well as relatively low testosterone concentrations coupled with Sertoli cell destruction

Table 1. AMH and differences in sexual development

Pathology	Karyotype	Phenotype	Serum AMH	Other markers
46,XY DSD				
Complete gonadal dysgenesis (Swyer syndrome)	46,XY	Female external genitalia and presence of Mullerian structures. Streak/dysgenetic gonads	Undetectable	Low testosterone, inhibin B and elevated LH/FSH at puberty
Partial gonadal dysgenesis	46,XY	Genital ambiguity +/− presence of Mullerian structures. Dysgenetic testes	Low and reflective of testicular Sertoli cell mass	Low testosterone, inhibin B and elevated LH/FSH at puberty
Persistent Mullerian duct syndrome	46,XY	Male external genitalia and Persistence of Mullerian structures. Transverse testicular ectopia common	Low (AMH gene mutations) Normal (AMH receptor mutations) male	Normal age appropriate FSH/LH and testosterone.
Testicular synthetic defects (including steroidogenic defects and Leydig cell hypoplasia)	46,XY	Undervirilized male/ambiguous genitalia and absence of Mullerian structures with bilateral testes	Normal in childhood/high for male standard in neonates/puberty;	Low testosterone and normal to high LH based on cause
5-alpha reductase deficiency	46,XY	Undervirilized male/ambiguous genitalia and absence of Mullerian structures. Bilateral testes	Lower range of normal male	Increased testosterone: dihydrotestosterone ratio
Androgen insensitivity	46,XY	Absence of Mullerian structures generally with Ambiguous genitalia (partial androgen insensitivity). Female external genitalia (complete androgen insensitivity). Bilateral testes	High (in first year of life and during puberty with FSH stimulation); normal in childhood	Normal to high testosterone
Anorchia and testicular regression syndrome	46,XY	Absence of Mullerian structures and normal male external genitalia. Absent testes	Undetectable	Low testosterone and elevated FSH/LH during puberty
46,XX DSD				
46,XX males (SRY translocation, etc)	46,XX	Male external genitalia or ambiguous genitalia. Dysgenetic testes	Above female reference ranges in childhood and declines at puberty	Low testosterone and elevated FSH/LH at puberty
Congenital adrenal hyperplasia, aromatase deficiency etc	46,XX	Virilized female or ambiguous genitalia. Bilateral ovaries and presence of Mullerian structures.	Normal female range	High testosterone and/or other androgens based on cause
Sex chromosome DSD				
Turner syndrome	45,X and variable mosaic karyotype	Female external genitalia with presence of Mullerian structures. Streak or dysgenetic gonads typically	Typically, low for female reference range but may be normal and correlates with karyotype/age	Variable but typically low estradiol and elevated LH/FSH at puberty
Klinefelter syndrome	47 XXY	Male external genitalia and absent Mullerian structures. Dysgenetic testes	Normal male reference range but declines after puberty to low levels	Low or low-normal testosterone and elevated FSH/LH at puberty

Table 1. Continued

Pathology	Karyotype	Phenotype	Serum AMH	Other markers
Ovotesticular DSD	46,XX or 46,XX/46,XY typically	Ambiguous genitalia with +/− persistent unilateral/bilateral Mullerian structures; Ovotestes or unilateral dysgenetic testes/contralateral ovarian tissue	AMH above female reference standards after birth but decline in childhood	Variable but typically low testosterone/estradiol and elevated FSH/LH at puberty
Mixed gonadal dysgenesis	45,X/46XY typically	Ambiguous or male external genitalia; Asymmetric gonadal dysgenesis, with dysgenetic testes/streak gonads; Mullerian structures variable and often absent on testicular side	AMH above female reference standards after birth typically, but variable and may decline in childhood	Variable, but typically low testosterone for reference male for age

Abbreviations: AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

and lack of meiotic germ cells. AMH levels in adults with Klinefelter syndrome were lower than healthy males and attributed to the testicular tissue and Sertoli cell destruction seen in these individuals (62).

Isolated absence of AMH effect with normal testicular Leydig cell function is seen in persistent Mullerian duct syndrome (PMDS), a rare autosomal recessive disorder caused by loss of function mutations in the *AMH* gene (AMH deficiency) or the AMH type 2 receptor gene, *AMHR2* (AMH receptor insensitivity) detectable in nearly 88% of affected individuals (64). PMDS is characterized by completely virilized male external genitalia in 46,XY males and variable persistence of Mullerian structures, presenting with the following phenotypes: (1) bilateral cryptorchidism and testes in the pelvis attached to Fallopian tubes/uterus, (2) unilateral cryptorchidism associated with a testis in the inguinal canal attached to the herniated uterus/Fallopian tube (hernia uteri inguinalis), or (3) transverse testicular ectopia (unique to PMDS, with bilateral testes and Mullerian structures herniated into a single processus vaginalis) (64). *AMH* gene mutations with a few exceptions, are associated with an unstable protein and, hence, very low or undetectable serum AMH in prepubertal male patients compared to a normal-for-age AMH in AMH receptor defects (65). The AMH concentrations are not elevated in prepubertal males with AMH receptor defects causing PMDS since the testosterone and FSH concentrations are normal in these patients. There is no reported clinical phenotype in females with *AMH* gene or *AMHR2* receptor mutations although there is some speculation for possible early menopause in these individuals based on animal studies (64).

AMH and Cryptorchidism

In infants with male external genitalia and cryptorchidism, the detection of AMH secreted from the Sertoli cells can help differentiate between absent gonads (anorchia with undetectable AMH) and undescended testicles with intact Sertoli cell function. In patients with cryptorchidism without microphallus or genital ambiguity, AMH demonstrated 98% sensitivity and 91% specificity for the identification of testicular tissue (60,66). AMH was low or absent in nearly half of the patients with isolated cryptorchidism, and the number increased to 61% in the presence of cryptorchidism and microphallus (67). In addition, a low AMH was seen in 74% with bilateral nonpalpable gonads and 35% of those with gonads palpable in the inguinal region reflective of diminished Sertoli cell function (67). Additional studies have also shown lower AMH (and inhibin B values) compared to normative data in 2-year-old male children with cryptorchidism, suggesting a functional defect of Sertoli cells in this condition (68). In a large

cohort of males with cryptorchidism, AMH was shown to be significantly lower in those with bilateral cryptorchidism compared with unilateral cryptorchidism and controls; abnormally low AMH (<3%) was seen in 36.5% of patients with bilateral cryptorchidism between the ages of 6 months and 2 years (69). In pooled data of the studies by Lee et al (60), normal AMH was 100% predictive of the presence of testes while a measurable but low value was predictive 86% of the time, with an unmeasurable AMH being predictive of absent testicular tissue (anorchia) 94% of the time. One exception to this would be patients with AMH-negative PMDS due to *AMH* gene mutations, who also have undetectable AMH but nondescent of testes due to the presence of Mullerian-derived structures affecting descent. A pelvic ultrasound to detect Mullerian structures can distinguish this condition from anorchia due to testicular regression syndrome where Mullerian derivatives are absent (53).

AMH and Pubertal Disorders

Low AMH is also characteristic of prepubertal males with hypogonadotropic (central) hypogonadism who have diminished Sertoli cell number and function due to FSH deficiency and has been shown to be low in hypogonadotropic hypogonadism due to multiple pituitary hormone deficiency in infants (70). Inhibin B is often a useful adjunct that parallels AMH secretion as well, and basal inhibin B had a higher discriminatory value for distinguishing hypogonadotropic hypogonadism from constitutional delay of growth and puberty (71). During puberty, AMH is low for Tanner stage (lack of FSH effect) and high for age (lack of testosterone effect) (72). Further, treatment with combined recombinant FSH and hCG lowers AMH during pubertal induction in hypogonadotropic hypogonadism, but testosterone therapy for pubertal induction does not appear to lower AMH (73). This has been attributed to the LH-driven rise in intratesticular testosterone, which suppresses AMH and overshadows the stimulating effect of FSH on AMH during combination therapy, whereas intratesticular testosterone levels are not adequately raised by exogenous testosterone therapy alone (73,74).

In isolated Leydig cell disorders, AMH was normal or high (increased FSH effect but lack of testosterone effect) (75). Precocious puberty in males including central precocious puberty and gonadotropin-independent forms such as familial male limited precocious puberty were associated with low AMH secretion for age due to rise in intratesticular testosterone (76). Gonadotropin-releasing hormone (GnRH) analogue therapy normalizes AMH to prepubertal values, suggesting that the Sertoli cell maturation in early puberty may be reversible and AMH could potentially serve as an additional tool for diagnosis of precocious puberty and treatment efficacy (77), although not of routine clinical utility compared to LH/testosterone.

In females, AMH was normal in most patients with acquired multiple pituitary hormone deficiency but low in severe congenital hypopituitarism reflective of gonadotropin deficiency (78). AMH has been shown to be lower in premature thelarche in females ages 1 to 3 years and negatively correlated with FSH compared with age-matched controls (79). In girls with precocious puberty, AMH levels did not differ from healthy age-matched controls at baseline but were suppressed compared to the pretreatment levels with GnRH analogue therapy and returned to pretreatment levels 6 months after discontinuation of treatment (80). The authors speculated that the suppression of AMH was consistent with gonadotropin-dependence of AMH secretion, but the normal AMH levels and negative correlation with FSH at baseline were due to an individual set point for the pituitary-gonadal feedback loop (80). Regardless, there does not appear to be any clinical utility to routine monitoring of AMH in the management of precocious puberty in female children.

AMH as a Marker of Granulosa Cell Tumors

Granulosa cell tumors (GCTs), the most common subtype of ovarian sex cord-stromal tumors, represent 2% to 5% of all ovarian cancers (81). GCTs are divided histologically into juvenile GCTs, occurring primarily in children and young adults, and adult GCTs, occurring typically in adult women. Signs of excessive estrogen secretion such as precocious puberty in a prepubertal child or menstrual irregularities including hypermenorrhea in a postmenarchal adolescent in the presence of an adnexal mass can lead to the diagnosis of GCT. However, making an accurate preoperative diagnosis remains difficult. These tumors secrete estrogen, inhibin B, and AMH. An elevation in AMH level has been reported in both juvenile and adult GCTs (82-84). AMH can be used as a marker of treatment efficacy and tumor progression and recurrence and correlates with tumor mass as determined by radiology or pathology (84-86). In a recent meta-analysis evaluating the performance of AMH in the diagnosis of GCT, the pooled sensitivity was reported as 89% with a pooled specificity of 93% (87). However, negative testing does not rule out GCTs, and there are reported cases of discrepancy between inhibin B and AMH levels in patients with progressive GCT (88). Given the possibility of false negatives, measuring both AMH and inhibin may improve the detection of GCT.

AMH and Polycystic Ovary Syndrome

Polycystic ovarian syndrome (PCOS) is the most common cause of chronic anovulation and hyperandrogenism in

young women, with a prevalence of 8% to 13% (89,90). The International Guideline for the Assessment and Management of PCOS endorses the Rotterdam PCOS diagnostic criteria in adults (2 of oligo- or anovulation, clinical and/or biochemical hyperandrogenism, or polycystic ovaries on ultrasound), after exclusion of related disorders. In adolescents, both oligoanovulation and hyperandrogenism are required, with ultrasound not recommended for diagnosis (91). Serum AMH levels are consistently higher in women with PCOS (92,93). Abnormally slow growth of primary follicles results in an elevated number of AMH-producing cells. There also appears to be an increased production of AMH per follicle as evidenced by a mean AMH level 4× higher in granulosa cells from ovulatory PCOS and 75× higher in granulosa cells of anovulatory PCOS (94). Adolescent daughters of women affected by PCOS have higher AMH levels compared to girls with obesity (95,96). Higher AMH levels in adolescent daughters of women affected by PCOS is associated with menstrual cycle irregularities and higher ovarian volumes, suggesting differences in ovarian folliculogenesis in adolescents at risk of developing PCOS (97). Currently, there is no consensus on an AMH threshold for the diagnosis of PCOS. AMH levels have been reported to be a more reliable marker of polycystic ovarian morphology (PCOM) than follicle number with an AMH threshold of 35 pmol/L (5ng/mL) suggested as a possible inclusion in the diagnostic criteria of PCOS (98). Some authors have proposed increased AMH levels as an adjunct in the diagnosis of PCOS in adolescents and reported that an AMH level > 3.4ng/mL best distinguishes adolescents with PCOS from controls (99). The International Guideline for the Assessment and Management of PCOS does not recommend the use of serum AMH as an alternative to the detection of PCOM or as a single test for the diagnosis of PCOS. However, there is mention that with improved standardization of assays and established cutoff levels or thresholds based on large-scale validation in populations of different ages and ethnicities, AMH assays would be more accurate in the detection of PCOM (91).

AMH as a Marker of Ovarian Reserve/Fertility

Ovarian reserve, as defined by American Society for Reproductive Medicine, represents the number of oocytes remaining in the ovary or oocyte quantity (oocyte number) (100). Ovarian reserve tests include both ultrasound imaging and biochemical tests. Antral follicle count (AFC), a marker of ovarian reserve, is traditionally performed in the early follicular phase by transvaginal ultrasound, which precludes its use in prepubertal children as well as postmenarchal adolescents who do not tolerate transvaginal imaging. Although transabdominal

AFC measurements have been performed in both prepubertal and pubertal children, AMH was only moderately correlated to AFC in premenarchal girls (101). AMH is a sensitive biochemical marker of ovarian reserve and may be more sensitive when compared to early follicular phase FSH and estradiol levels (102). The ideal timing of AMH measurement depends on whether serum AMH levels vary throughout the follicular and luteal phases of the menstrual cycle. Numerous studies have investigated AMH variations during the menstrual cycle, and some have reported only mild intra- and intercycle fluctuations of AMH secretion (103-105), while others have reported a peak AMH level in the mid-follicular phase with a subsequent decrease in the luteal phase (106,107). However, these fluctuations are not large enough to warrant a recommendation of timed AMH measurement on specific menstrual cycle days or phases (107,108). Although AMH has the ability to predict ovarian responsiveness to gonadotropin stimulation and oocyte yield in assisted reproductive technologies, it is a poor predictor of pregnancy and live birth rates (100).

AMH is expressed by granulosa cells of growing follicles after recruitment of primordial follicles from the resting pool and expression increases until the large preantral and small antral follicular stage (109,110) (Fig. 1B). AMH expression is lost in the atretic follicle as well as corpus lutea. With age, AMH decreases, and serum AMH levels correlate strongly with the decrease in size of the antral follicle pool (111,112). There is no marker that can directly measure the number of primordial follicles. However, the number of growing follicles recruited from the primordial follicle pool reflect the number of primordial follicles. As a marker for the number of growing follicles, AMH is used as a proxy for the number of primordial follicles and the quantitative aspect of the ovarian reserve in adults (113). In prepubertal children, however, ovarian follicles remain in a quiescent state after the minipuberty of infancy. AMH is not expressed by primordial or small preantral follicles and, prior to the onset of puberty, may correlate poorly with ovarian reserve in children. In vitro evaluation of ovarian tissue in children undergoing ovarian tissue cryopreservation (OTC) prior to gonadotoxic therapies due to malignant diseases that do not affect ovarian reserve parameter revealed high follicle density despite low AMH levels (114). In addition, studies reporting intervals for AMH in children demonstrated wide variations in AMH in healthy girls ages 1 to 12 years (42,115). Despite these limitations, AMH has been proposed as a potential biomarker of ovarian reserve in childhood to determine possible candidates for fertility preservation and the timing of such interventions in children and adolescents at risk of primary ovarian insufficiency (POI).

Turner syndrome (TS), caused by X-chromosome anomalies with or without mosaicism, is characterized by an increased risk of POI due to accelerated ovarian follicular apoptosis before and/or after puberty (116). In adolescents and young adults with TS, higher AMH levels are associated with spontaneous puberty and ongoing ovarian function (42,115) and negatively correlated with FSH, LH and 45,X karyotype (117). In addition, AMH < 4 pmol/L has been reported as predictive of absent puberty in prepubertal girls and POI in adolescents and adult patients (118). Care should be taken in the generalization of these results, however, as the evaluation of AMH as a predictor of spontaneous puberty was based only on 15 patients before pubertal onset (5 with spontaneous puberty and 10 with puberty induced by hormone replacement therapy). Some investigators have proposed guidelines for performing OTC based on serum AMH levels in prepubertal girls with TS (119). However, the available limited data on OTC in girls <12 years with TS do not allow for meaningful clinical predictions of the feasibility of OTC and ovarian follicular density based on AMH levels alone (120-125).

As the number of childhood cancer survivors increase, attention to long-term adverse health effects outcomes including future fertility has been identified as a major concern of patients and their families (126). Risk factors for POI include age at diagnosis, abdominal/pelvic radiation therapy, and exposure to alkylating agents (127). In a small study of 16 postmenarchal adolescents undergoing chemotherapy for oncology diagnoses (leukemia, lymphoma, and sarcoma), 94% showed a decline in mean AMH levels at 6 months postdiagnosis, but 80% showed at least some recovery of AMH by 18 to 24 months (128). Longitudinal follow-up in female childhood cancer survivors showed that although AMH levels were significantly low compared to age-matched controls, in women with sustained ovarian function (AMH > 1.0µg/L), the decline in AMH is similar to that in the normal population (129). In adolescents requiring treatment with chemotherapy, the predictive value of AMH as it relates to spontaneous pregnancy requires further longitudinal studies.

Future Directions

The clinical utility of AMH has continued to expand with the understanding of sexual differentiation and the elucidation of the molecular basis of AMH action. Originally, *AMH* expression was thought to be limited to the gonads, but recent studies not only demonstrate *AMH* and *AMHR2* expression in different neurons but also confirm AMH actions at different levels of the hypothalamic-pituitary-gonadal axis to increase the activity of GnRH neurons and the sensitivity of gonadotropes to GnRH signaling, LH pulsatility (130), modulating GnRH/LH/FSH secretion through possibly endocrine (circulating gonadal AMH), or even autocrine effects (5). Loss-of-function heterozygous mutations in *AMH* and *AMHR2* genes were identified in congenital hypogonadotropic hypogonadism, suggesting a possible role of AMH signaling in the development, migration, and function of GnRH neurons (131). Paracrine actions of AMH in adult ovaries regulating follicular recruitment and in adult testes regulating steroidogenesis and Leydig cell/germ cell maturation, as well as a postulated role for AMH in the prenatal reprogramming of neuroendocrine pathways for pathogenesis of PCOS in the offspring, suggest that our understanding of this multifaceted hormone and its clinical utility continues to evolve (5,132). Therapeutic applications of AMH for treating ovarian and endometrial cancer and PCOS, preserving fertility, and delaying ovarian aging (133) hold promise but will require standardization of current assays and development of AMH analogues (134).

Conclusions

AMH is a valuable tool in the care of pediatric patients with diverse conditions affecting gonadal development and function. The interpretation of serum AMH should be informed by assay-specific normative data accounting for age/sex and pubertal stage. In conjunction with gonadotropins and testosterone, AMH can be a useful diagnostic marker of Sertoli cell mass and function in males. Although widely used as a biochemical marker of ovarian reserve in females, the predictive value of AMH in prepubertal females for fertility outcomes needs further study. Assay standardization and widespread availability will further enhance its utility in clinical practice.

Acknowledgments

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