

Measurement of the Anti- Müllerian Hormone (AMH) in Endocrinology: A Mini-Review

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Abstract

In this mini-review the use of the measurement of anti-Müllerian hormone (AMH) as an indicator of the ovarian reserve (OR) is discussed, showing different views in commercial and regular IVF clinics. The automated second generation AMH assay is discussed and the problems with this assay are analyzed. The role of AMH measurements in pediatric endocrinology is reviewed, showing AMH measurement are of proven value in this area. The debate of AMH levels in PCOS is discussed as well as its value in premature ovarian failure and oncology. It is concluded, that now the greatest challenge is collecting new age-specific reference values for a standardized AMH assay.

Introduction

Anti-Müllerian Hormone (AMH), also called Müllerian Inhibiting Substance (MIS), has long been known for its role in the development of the gonads and the urogenital ridge. In the male fetus, AMH, produced by the fetal developing testes, causes regression of the Müllerian ducts [1]. In the female fetus, AMH may play a role in early follicle assembly in the gonad by primordial germ cells, a role distinct from its regulatory role of folliculogenesis postnatally [2,3]. At birth, about 1 million oocytes are present. This number decreases during childhood, resulting in a primordial follicle pool of 300.000-500.000 at menarche [4]. Throughout life, follicles leave the primordial pool to enter the growing follicle pool. Therefore the dormant primordial follicles have to be "awakened". This "awakening process" is still not understood and is one of the mysteries in reproduction. The majority of the growing follicles will be lost as a result of atresia, unless they are rescued by follicle-stimulating hormone (FSH). This starts at puberty, when the hypothalamic-pituitary-gonadal axis is activated. Among the rescued follicles only one follicle will be selected to be the dominant one, that will ovulate under the influence of luteinizing hormone (LH)-[5].

The primordial dormant follicles do not produce AMH. When the primordial follicle has awakened, it can grow to a secondary follicle that starts to produce AMH. This process needs 290 days. The follicle is now in the pre-antral stage and called a pre-antral follicle. These follicles are still too small to detect at an ultrasound. The diameter is then 0,2mm. Another 50 days are needed to reach the antral stage. Antral follicles are visible on ultrasound and range in size from 2-10mm. Counting the number of antral follicles forms the basis of a test of ovarian reserve, the antral follicle count (AFC)-[6].

At a given moment a woman may have only a few antral follicles or a large number, because all primordial follicles are in different stages of development all the time. The size of the AFC rarely exceeds 50-60 follicles and is often less [6]. Antral follicles produce AMH by the granulosa cells. AMH expression ceases in follicles with a diameter between 8 and 10mm [7]. Based on this expression pattern serum AMH was suggested to reflect the number of small-growing follicles and more importantly the number of primordial follicles. Several studies confirmed the strong correlation between serum AMH and AFC [8-10]. Serum AMH reflects the cohort of small growing follicles and therefore constitutes a proxy for the size of the primordial follicle pool. Furthermore it was shown that serum AMH in this way could be used as a marker of diminished ovarian reserve (OR)-[9,10]. Both tools, AFC and OR, reflected by serum AMH levels, were developed to monitor and guide IVF (in vitro fertilization) treatments and not as a fertility test, as age is the ultimate fertility marker. Nevertheless both AFC and OR are marketed widely as fertility tests, urging women desperately freezing eggs, at costs of \$20.000-\$30.000. A recent JAMA article about the subject fueled the public discussion by 2 articles published in the New York Times and in BBC Health [11-13]. In this mini-review AMH assays, AMH in physiology and pathophysiology, and the use of AMH measurements in clinical endocrinology will be discussed.

AMH Assays and AMH Measurements

In an excellent 2014 review Rustamov et al. summarizes the existing problems with the manual ELISA assays used since 1990 and the contemporary automated second generation AMH assays (Gen2; Beckman-Coulter). There appeared to be significant variability in determined AMH concentrations due to a differential

response to pre-analytical, proteolysis, and conformational changes of the AMH dimer. These discrepancies may be explained by differences in sample handling, sampling time (diurnal variation), transport and storage conditions. Besides circadian variability also variability within the menstrual cycle was observed and between menstrual cycles. In addition, significant variability between repeat samples was found. The within-patient variability questions the assumption, that a single AMH measurement is acceptable in guiding individual treatment strategies in fertility treatment. There is a lack of reliable interassay comparability and an urgent need for reporting, how samples are collected, processed and stored. The authors conclude there is a clear need for an international reference standard for AMH and a robust independent evaluation of all commercial assays.

These critics were accepted by Beckman-Coulter and they released a field safety notice stating, [14,15] that all kit lots were affected by complement interference, which causes a time-dependent 70% reduction in AMH values. They revised their assay methodology (i.e. premixing the sample with a buffer before plating). This technical issue is of critical importance and it may have hampered the clinical use of AMH measurements. So new age-specific values for the revised Gen-2 AMH assay are urgently needed [16]. Reference values for serum AMH concentrations were established mostly in fertility clinic populations [16]. Few studies established reference values for normal, healthy, female populations and regarding the revision of the used assay, their value is in doubt now [17,18]. The relationship between serum AMH and other clinical markers as body mass index, AFCs and other sex related hormones have not been studied well in normal populations [17].

Several investigators reported disrupting influences on the AMH serum concentrations of vitamin D deficiency, use of oral contraceptives (OACs), smoking, obesity, and race or ethnicity [19-28]. Circulating 25.(OH)VitD3 levels correlated with serum AMH levels in late reproductive-aged women. Vitamin D deficiency was associated with lower ovarian reserve (OR), as measured by serum AMH concentrations [19]. Vitamin D supplementation normalized serum AMH levels in these women [20]. Vitamin D is believed to change AMH production pattern in ovarian follicle granulosa cells by steroidogenic factor 1 and altering FSH sensitivity. It is recommended both partners should receive vitamin D supplementation therapy in fertility treatment [21].

AMH levels are some 20% lower during oral contraceptive (OAC) use Kullio et al. measured AMH, inhibin B, FSH, LH and E2 at baseline, 5 and 9 weeks later after starting OACs via different routes in 42 healthy women. After 9 weeks AMH levels were significantly lower vs baseline, independent of the route of administration. This is not unexpected as OACs reduce the effect of FSH and inhibit the growth of follicles [22]. Obesity affects serum AMH levels adversely, at least in Caucasian women. The mechanism behind the negative correlation between BMI and AMH levels is largely unknown [23]. Some believe, that elevated inflammatory markers and oxidative stress markers found in follicular fluid of obese women are

involved [24,25]. Others believe the leptin-AMH2 receptor pathway is involved, as leptin treatment significantly suppressed AMH levels and AMHR-2 mRNA levels [26].

Smoking is associated with increased FSH levels and early menopause. Active smoking but not former smoking is associated with decreased AMH values in late reproductive-age and perimenopausal women. The authors found no association between passive smoking and AMH levels. Former smokers had the same AMH levels as never smokers. These results suggest a direct effect of smoking on ovarian follicle depletion, and on pre-antral and antral follicles in particular. The direct impact of active smoking on AMH levels in young women require further investigation [27]. There is accumulating evidence for significant racial differences in OR and reproductive aging. Multiple genetic and environmental may underlie these racial differences to determine the relative contributions of these factors, genome wide association studies are required [28].

The AMH Receptor Complex

The actions of AMH are mediated by the AMH receptor complex. AMH is a member of the transforming growth factor-beta (TGF-beta) family of growth and differentiation factors. AMH is a 140kD homodimeric glycoprotein. Human AMH is synthesized as a 560 amino acid precursor, with a 24-25 amino acid leader, containing a 16-18 amino acid signal sequence and a putative 7-8 residue pro-sequence. The carboxy-terminal fragment of AMH shows homology with other members of the TGF-beta family. Most members of these families require proteolytic cleavage at a site 110 amino acids from the carboxyl terminus to be active. The cleaved N-terminal domain interacts and enhances the activity of the bio-active C-terminus [29,30].

AMH has two receptors. These are serine-threonine-kinase receptors. The AMH1 receptor (AMHR1) is a non-specific kinase 2 receptor. The AMH2 receptor (AMHR2) is specific by the AMHR2 gene. These genes are located on chromosome 19. Binding of AMH to AMHR2 suppresses follicle maturation by inhibiting recruitment of primordial follicles into the pool of growing follicles. Once AMH binds to AMHR2 the type 1 receptor, AMHR1, is recruited for forming a receptor complex. This causes downstream signalling of receptor-related cytoplasmic effectors, known as receptor-related Smad proteins (R-Smads 1,5,8) and a common Smad. After forming the AMHR2-AMHR1 complex the AMHR1 becomes activated causing phosphorylation of R-Smads. These proteins bind not the common Smad4 protein, resulting in translocation into the nucleus and binding directly to DNA to regulate gene expression or interacting with other DNA binding proteins [31].

AMH in Physiology and Pathophysiology

In the male the changes of AMH expression follow the development of the hypothalamic-pituitary-gonadal (HPG) axis. AMH is synthesized in Sertoli cells already in an early stage of development. In this fetal stage there is already LH and FSH production. By the action of LH on the Leydig cells receptors



relatively high amounts of testosterone are formed, detectable in the newborn's circulation. Testosterone is responsible for the differentiation of the Wolffian ducts. Testosterone can not inhibit AMH, because insufficient androgen receptors are present at this time. On the other hand FSH, through its receptors on the membranes of Sertoli cells, stimulates AMH expression [32]. In this fetal, early postnatal stage Inhibin B is also formed. Sertoli cells constitute then half of the testicular volume and serum AMH levels are relatively high in this stage [33].

Childhood, until prepuberty is a stage of relative rest of the HPG axis. Leydig cells produce only very low amounts of testosterone then. Half of testosterone production originates of the adrenals at that time. Sertoli cells are still immature and spermiogenesis is arrested in a premeiotic stage. They still produce AMH in amounts comparable with the prenatal period thanks to FSH stimulation [32,33]. With the onset of puberty the HPG axis is activated, and Leydig cells increase testosterone production. The effect of LH on testosterone production is more pronounced, due to the inhibitory effect of Inhibin B on FSH secretion. The inhibitory effect of testosterone prevails over FSH stimulation, resulting in down regulation of AMH expression, the levels of which sink rapidly. Germinal cells undergo meiosis and spermatogenesis starts. The secretion of AMH reaches adult values and is maintained almost constantly during the rest of life.

AMH as a Marker of Male Hypogonadism

The term hypogonadism is applied in the adult male to describe testicular failure, associated with androgen deficiency and/or disorders in sperm production. Male hypogonadism in the prepubertal boy can only be discovered, if Sertoli cell function is assessed, at that time the only origin of testicular testosterone production. The Sertoli cell function can be tested by serum AMH levels, without dynamic stimulation tests. Male hypogonadism can be classified, according to the level of the HPG axis, that is primarily affected. In central hypogonadism gonadal failure results from a malfunction of the hypothalamic GnRH pulse or the pituitary to generate FSH and LH. Low serum AMH levels have been reported in infants with congenital central hypogonadism. Treatment with recombinant human FSH (rhFSH) results in an increase in testicular size by FSH-induced Sertoli cell proliferation, and an elevation of serum AMH levels [34]. In untreated patients of pubertal age with congenital hypogonadotropic hypogonadism. AMH levels are too high for age, as at the start of puberty AMH levels sink rapidly, as discussed above [35].

When primary hypogonadism is caused by early-onset complete gonadal dysgenesis, the absence of testicular tissue results in a DSD (disorder of sexual differentiation) with female external genitalia, whereas vanishing or regression of testicular tissue is associated with male genitalia, hypoplastic scrotum and micropenis. In all cases the absence of gonadal tissues results in undetectable AMH levels [36,37]. Early-onset partial testicular dysgenesis in XY patients usually result in DSD with ambiguous genitalia. Both tubular and interstitial compartments of the testes are affected, producing insufficient AMH and testosterone [36]. In

most boys with isolated hypospadias, AMH and testosterone are normal [38]. Patients with the Klinefelter syndrome have typically late-onset testicular dysgenesis. Before puberty there are no overt signs of hypogonadism. AMH levels are normal and in correlation with FSH and Inhibin B levels. In these patients Sertoli cell function deteriorates rapidly from mid-puberty, resulting in extremely low or undetectable AMH levels, in coincidence with very high FSH levels and undetectable Inhibin B levels and small testis volume [39].

Cryptorchidism may have many possible origins. It may present as "testicular dysgenesis syndrome", a primary hypogonadal disorder, with whole testicular dysfunction, with hypospadias, reduced semen quality and increased testicular cancer risk [40]. Alternatively, cryptorchidism may result from anatomical defects in the inguinal region or abdominal wall (not associated with hypogonadism), from hypogonadotropic hypogonadism or from rare mutations of INSL3 or its receptor [41]. So cryptorchidism may be associated with normal or impaired Sertoli cell function, depending on its etiology. AMH is normal in patients with inguinal or abdominal wall defects, unless longstanding abnormal testis position occurs. In boys with bilateral cryptorchidism AMH is low in 75% of those with non-palpable gonads and in 35% of those with inguinal gonads, indicating Sertoli cell dysfunction [42]. AMH is low in patients with cryptorchidism, due to central hypogonadotropic hypogonadism, as discussed before.

Furthermore AMH measurements are of help in the evaluation of patients with mutations of the FSH receptor provoking Sertoli cell dysfunction, mutations of the AMH gene, the androgen insensitivity syndrome (AIS), and in prepubertal patients with the Prader-Willi syndrome, where central and peripheral hypogonadism are combined [43-45]. The physiology of AMH in the female is already discussed above. Besides the use as a marker of the ovarian reserve, AMH serves as a biomarker in several other conditions.

Polycystic Ovarian Syndrome (PCOS)

PCOS causes anovulatory infertility and hyperandrogenism along with elevated AMH levels in premenopausal women [46-49]. AMH has not, however proven as a sensitive and specific diagnostic marker of neither PCOS nor of ovarian morphology [50]. Clear cutoffs do not exist in adolescence [49] and several thresholds have been suggested in adults, with varying degrees of sensitivity and specificity [51-53].

Premature Ovarian Failure (POF)

Primary ovarian failure or sometimes called primary ovarian insufficiency is a disorder of fertility of women younger than 40 years. It is the result of premature exhaustion of the follicle pool, due to follicular dysfunction caused by mutations in the FSH receptor or steroidogenic cell autoimmunity. Many cancer patients develop POF, due to chemotherapy and/or radiotherapy. AMH could be a marker for women at risk for POF. In cancer survivors serum AMH levels correlate with the extent of gonadal damage [31]. Alipour et al., compared the sensitivity and specificity of AMH and FSH in the diagnosis of POF. In this study (n=96), AMH had more sensitivity

than FSH (80% vs 28,6%) and almost equal specificity (78,9% vs 78,6%). The negative predictive value for AMH was 98,6% vs 87,5% for FSH, but the positive predictive value was the same (17,4%). Early diagnosis of POF can prevent expensive ways of infertility treatment. AMH is a slightly better marker than FSH of POF, having more sensitivity. In cancer patients decisions about freezing eggs, ovarian tissue and embryo freezing are made on other grounds, than serum AMH and FSH. Nevertheless combined together they can be of some help.

AMH in Turner syndrome

Gonadal dysgenesis in Turner syndrome (TS) result in pubertal delay and premature ovarian failure and infertility in most patients. However up to 30% of girls with TS have spontaneous pubertal development and 2-5% have regular menstrual cycles. Serum AMH levels can serve as a useful marker of the follicle pool. AMH levels in these patients are related to karyotype, pubertal development and growth hormone treatment [55]. The majority of women with mosaic karyotype 45, X/46, XX have ongoing ovarian function in early adulthood. An AMH level <2 SD predicts failure to enter puberty in young TS patients and imminent premature ovarian failure [56].

AMH in Female Virilization

AMH levels can indicate if the virilization of a girl originates from testicular tissue or of a granulosa cell tumor, where AMH concentrations are in the male reference range or from adrenal androgens, as in the case of congenital adrenal hyperplasia (AMH levels in the female reference range). Values within the normal female reference range do not exclude the presence of abnormal gonadal tissue, but undetectable levels exclude the presence of testicular tissue in mildly virilized females [42,57].

AMH Levels in Precocious Puberty

CPP is defined as the full activation of the HPG axis before 8 years of age in girls and before 9 years in boys. Whereas boys with CPP have low AMH values, as discussed before, the AMH levels in girls with CPP are in the normal range. AMH levels decrease at the early stages of GnRH agonist treatment in these girls, but return to pretreatment levels after discontinuation of the treatment, indicating GnRH treatment does not affect fertility [58].

AMH as a Tumor Marker

AMH is a reliable tumor marker for ovarian tumors of granulosa cell origin in diagnosis work-up and monitoring during therapy. Raised levels are found in 76-93% of women with granulosa cell tumors [59,60]. AMH levels were evaluated also in breast cancer patients as the AMHR2 is also expressed in both normal breast tissue as well as in breast cancer tissue. However, studies yielded conflicting results [61,62]. In the largest study to date, the "Nurses' Health Studies" a positive correlation between premenopausal AMH levels and the risk of breast cancer was observed [63].

Conclusion

Anti-Müllerian hormone (AMH) is largely known to the public and physicians for estimating ovarian reserve (OR). In combination with the antral follicle count of the ovary (AFC), it is a useful tool for guiding and monitoring IVF treatment. However, AMH measurements are not calibrated for egg quality and clinical pregnancy outcome [10,13]. Nevertheless, AMH measurements are marketed commercially widely as a fertility predictor [11,12]. The measurement of AMH by a new automated second generation ELISA revealed substantial problems with the assay methodology, urging the supplier (Beckman-Coulter) in 2013 to revise the assay, and releasing a field safety notice [14,15]. Comparability of different assays is poor and it is questionable, if a single AMH measurement is yet acceptable in guiding individual IVF treatment strategies. There is an urgent need for a new international reference standard for the AMH assay. Vitamin D deficiency, smoking, obesity, use of oral contraceptives (OACs) and race have their influence on serum AMH concentrations [19-28]. The AMH measurement is of proven value in the evaluation of gonadal differentiation and function in pediatric endocrinology [32-45]. The use of AMH as a marker in the polycystic ovary syndrome (PCOS) is still in debate [46-53]. In oncology AMH measurements are a reliable tumor marker of ovarian granulosa cell tumors. AMH measurements to predict premature ovarian failure in chemotherapy and/or radiotherapy of cancer are promising [59-63]. The greatest challenge, is to collect new age-specific standardized reference values for the AMH assay, however.

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