Review Article

Anti-Mullerian hormone – Promises and pitfalls

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Abstract Anti-Mullerian hormone (AMH) measurement is a routine practice that precedes every assisted reproductive techniques (ART) cycle in the current day. However, there is much more to AMH than just prediction of the cycle response. Many more applications have emerged in keeping with its role in understanding ovarian physiology and pathology. Many more contentious aspects need exploration on its measurements adding dimensions for future research.

Keywords: AMH, infertility, ovarian reserve

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INTRODUCTION

AMH has emerged as an important biomarker in reproductive medicine with varied clinical applications. Secreted by the antral and preantral follicles in the ovary, it plays an important role in ovarian folliculogenesis and steroidogenesis. AMH inhibits early follicular recruitment and preserves the primordial follicles and, thus, prevents premature exhaustion of follicles. It is not only being utilized as a marker of ovarian reserve helping in individualizing therapeutic strategies and stimulation protocols in infertility treatment but has also found a potential role in monitoring chemotherapeutic and radiation-induced ovarian toxicity, surgery-induced ovarian damage, in predicting menopause, as a surrogate marker in the diagnosis of patients of polycystic ovarian syndrome (PCOS) and certain ovarian tumors. However, due to the lack of standardization of lab assay and cutoffs for various ethnic populations, the reliability of AMH especially in the infertile population remains controversial. Further research into the follicular levels of AMH may help in quantifying this marker for the prediction of quantity as well as quality of oocytes.

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PHYSIOLOGY OF ANTI-MULLERIAN HORMONE

AMH, also known as Mullerian inhibiting substance is a dimeric glycoprotein belonging to the transforming growth factor β superfamily.[1] It was originally identified because of its role in male sexual differentiation. When expressed in the Sertoli cells of fetal testis, it leads to regression of Mullerian ducts. The absence of AMH in the female fetus allows the development of the Mullerian duct into uterus, tubes, and upper part of vagina.[2] In females, AMH is produced by the ovarian granulosa cells from the preantral stage to the antral follicle and acts as an inhibitor of primordial follicle recruitment. It also has a role in selection of the dominant follicle in late follicular phase by regulating the threshold of follicle stimulating hormone (FSH) sensitivity.[3] AMH levels are undetectable at birth and rise with the onset of puberty and remain stable in the reproductive period and again decline with advancing age and become almost indiscernible with imminent menopause. The serum levels are proportional to the number of developing follicles, and so AMH reflects the process of ovarian aging.[4] The many promising areas,

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where the utility of AMH measurements have been assessed and proven, are discussed.

Anti-Mullerian hormone as a marker of ovarian reserve

The reproductive potential of a female is determined by the biological age (ovarian reserve) rather than the chronological age. Ovarian reserve is constituted by the quantity and quality of the primordial follicles in the ovary. Since AMH is produced exclusively in the pre-antral and antral ovarian follicles, it serves as a good marker of the functional ovarian reserve and can help in predicting the reproductive life span guiding women on planning over delaying conception.[4]

There is a non-significant inter-cycle and intra-cycle variability of AMH during the menstrual cycle. Also the levels are not significantly altered by short-term exogenous hormone intake including oral contraceptive pills.[5]

AMH as a predictor of natural fertility has been evaluated in a few studies. In a study by Steiner,[6] women in their 30s with low AMH had a significantly reduced fecundability.[6] Contrastingly, in healthy young women with no prior knowledge of their fecundity, even if very low AMH levels were present, there was no compromise in the fecundability. In women with high AMH the fecundability was again reduced reflecting probable anovulation.[6]

Anti-Mullerian hormone as a marker of ovarian response in assisted reproductive techniques

ART have become a standard treatment of care in indicated cases of male and female infertility. Age and ovarian reserve help in determining the success of ART. AMH especially along with antral follicle counts (AFCs) has been shown to correlate with the number of oocytes retrieved as well as cycle cancelations. AMH helps in identifying both poor responders as well as the hyper-responders prior to initiating *in vitro* fertilization cycle.[7-9] Thus, it aids the clinicians in counseling patients and providing a realistic prognostication before embarking on an expensive and physically and emotionally taxing procedure of ART. It also helps in individualizing stimulation protocols and dosage of gonadotropins to optimize results with minimum cycle cancelations and complications, most dreaded being ovarian hyperstimulation syndrome.[7,8] However, AMH does not correlate well with pregnancy rates probably because it is an indicator of the quantity rather than the quality of oocytes.[10]

Anti-Mullerian hormone and polycystic ovarian syndrome

PCOS is the most common cause of chronic anovulation and hyperandrogenism affecting around 5–10% of women. It is characterized by an increase in the number of ovarian follicles at all growing stages, particularly the preantral and antral follicles which are accepted to produce AMH. The levels of AMH in PCOS women are 2–4 times higher than that in the healthy women, being 75-fold higher in anovulatory PCOS and 20-fold higher in normo-ovulatory PCOS suggesting an intrinsic granulose cell dysfunction.[11] Different studies have displayed that AMH levels highly correspond with the number of follicles in polycystic ovaries and further suggested a strong positive correlation of severity of symptoms including hyperandrogenism and oligo-anovulation with the levels.[12] Thus AMH has been proposed as a surrogate marker for AFC in the diagnosis of PCOS. But this diagnostic transition has not materialized or been accepted in defining disease, as there is no uniformity and international standards in AMH lab assay. Further, the threshold of AMH across various populations worldwide has not yet been determined. One study has proposed a cutoff of 35 pmol/L (4.9 ng/mL) using the enzyme immunoassay technique by Beckmann Coulter, which needs to be validated by similar studies.[13]

AMH levels, therefore, are of utmost help in deciding the treatment protocols and in defining the best strategy for ovulation induction in such patients. It can also help in assessing the response to treatment such as ovarian drilling and predict hyperstimulation. More studies are needed to clearly define these thresholds that predict response.

Anti-Mullerian hormone beyond infertility

Anti-Mullerian hormone and assessment of iatrogenic ovarian damage

The measurement of AMH levels have found utilization in assessing damage to the ovarian reserves caused by iatrogenic sources such as gonadotoxic chemotherapy, pelvic irradiation, uterine artery embolization, and ovarian surgery (cystectomy, stripping, fulguration) and may, therefore, guide in devising strategies to preserve fertility.[5] The option of cryopreservation of oocytes or embryos before chemotherapy or radiotherapy should be considered based on the AMH levels. Studies have shown declining AMH in young adults exposed to chemotherapy in childhood cancers and breast cancers.[14] In addition to reflecting post treatment ovarian damage AMH can also predict ongoing ovarian activity useful to counsel women as regards menstruation besides fertility issues. The impact of ovarian surgery (particularly for endometrioma) has been elucidated in two systematic reviews; both of which have demonstrated a decline in AMH levels post procedure indicating a significant attenuation of

ovarian reserve despite surgical expertise.[15,16] All these should be considered when planning surgery in a female desirous of future pregnancy.

Anti-Mullerian hormone as a tumor marker and tumor inhibitor

Since AMH is synthesized by the granulosa cells, it has been evaluated as a tumor marker for the diagnosis of granulosa cell tumor of the ovary. AMH levels are elevated in 76–93% of women with granulosa cell tumors.[17] Moreover, a rise in AMH levels precedes the clinical recurrence of tumor by up to 16 months.[17] Therefore, AMH could be used as an early diagnostic marker of as well as to follow up for the detection of recurrence in cases of granulosa cell tumor. A recent theory has been proposed that most epithelial ovarian tumors originate from fimbriated ends of fallopian tubes or the secondary Mullerian system which can be potentially inhibited by AMH just because it induces the regression of Mullerian duct in fetal life. Thus, AMH is being explored as a therapeutic and diagnostic agent in epithelial ovarian tumors.[17]

Anti-Mullerian hormone in the prediction of menopause and premature ovarian insufficiency

The number of primordial follicles diminishes with advancing age and is virtually depleted at menopause. Data have emerged from studies suggesting a relation between AMH level at a certain age and the timing of menopause.[18]

This can help in individualized prediction of the reproductive lifespan, and time of onset of menopause and in diagnosis of premature ovarian insufficiency. This can provide a significant clinical tool in assisting women to plan child bearing, timely referral for ART and potentially prevent infertility based on early ovarian aging.

Anti-Mullerian hormone in pediatric disorders of sex development

AMH is a marker of gonadal development and plays an important role in sex differentiation and development of testes. Lower concentration or absence in males is indicative of dysfunctional testis while higher levels in females suggest existence of testicular tissue. AMH is currently used to assess the presence of testicular tissue in ambiguous genitalia, anorchia, and cryptorchidism with potential follow-up post treatment for ovotestis.[19] It can also aid in differentiating between various causes of virilization in girls.

Anti-Mullerian hormone in male infertility

AMH is a specific marker of Sertoli cell function secreted in both serum and seminal fluid. It is secreted from the testis from around the 8th week of pregnancy and remains high till puberty when androgen secretion by Leydig cells increases and AMH levels fall having an indirect bearing on spermatogenesis. Recent studies have shown lower levels of serum AMH in men with obstructive azoospermia compared to non-obstructive azoospermia.[20] Also as discussed above, AMH indicates the existence of functional testicular tissue and helps in differentiating gonadal dysgenesis from tubulo-interstitial dysfunction in men with infertility or sexual dysfunction.

PITFALLS OF ANTI-MULLERIAN HORMONE

Though AMH has emerged as a promising marker with a wide array of clinical applications, it is still shrouded with shortcomings. There is still confusion as regards the most ideal and acceptable AMH assay.[21] In the last 20 years, there has been a constant evolution of the assay from single laboratory versions, through to the more recent commercially available Diagnostic Systems Lab (DSL) and Immunotech (IOT) (also branded as the Immunotech Beckman Coulter) assays. However, these assays utilized two different primary antibodies against AMH and different standards and consequently the crude values reported by authors and between papers can differ substantially, with the IOT assay giving values for AMH that are higher than those obtained with the DSL assay. But with the recent consolidation of these two companies by Beckman Coulter, there is finally a single commercially available assay − the AMH Generation II assay (AMH Gen II assay). Enzyme linked immunosorbent assay initially developed has evolved from IOT to the present Gen II assay. It is not recommended to adapt clinical cut-off values from the IOT assay to the Gen II assay, because a different antibody pair is used.

It is important to develop an international standard for AMH to allow harmonization of current and potential new AMH assays, thereby eliminating the need to establish assay-specific normative and cut-off values. To resolve these issues on AMH assay, currently the fully automated assay, Elecsys® AMH assay is indicated for the quantification of AMH, which (in conjunction with other clinical and laboratory findings) can help to determine ovarian reserve.[22] The assay is highly sensitive and precise, with a broad linear range, and correlates well with manual AMH assays and transvaginal sonographic

assessment of AFC, but has the benefit of being less variable. The Elecsys® AMH assay also has a shorter testing time than some other AMH assays and was shown to provide a reproducible measure of ovarian reserve in the women of reproductive age in a large prospective cohort study, demonstrating its usefulness in this setting.

There is also high inter-individual variability of AMH. Ethnic variation has been shown in few studies with African-American and Hispanic women having lower levels of AMH as compared to Caucasians. In one study, it was concluded that Asians have faster ovarian aging as compared to their European counterparts.[23] Also body mass index (BMI) may affect the ovarian reserve and subsequently the AMH levels, and a negative correlation between the two has been proposed.[24] Intra-individual variability with different AMH levels at different times has also been studied. Majority of the studies indicate that AMH levels remain relatively stable throughout the menstrual cycle, yet a recent small study found a reduction in AMH levels in the luteal phase.[25] Also the use of prolonged oral contraceptive pills or gonadotropin releasing hormone agonists leads to a reduction in AMH. In relation to pregnancy, it has been seen that AMH levels fall significantly in the second and third trimesters as compared to the first trimester.

Therefore, it is important to establish valid nomograms of AMH levels in diverse populations across the world. La Marca *et al.*[26] and Nelson *et al.*[27] have created nomograms for the European and the American population, but a uniform international standard is the need of the hour. Currently, in the reproductive aged women (25–40 years), studies in terms of fertility recommend, levels of 1.0–3.0 ng/mL AMH as "normal," 0.7–0.9 ng/mL as "low normal," and 0.3–0.6 ng/mL as "low" and <0.3 ng/mL to be a "very low" range, but whether it can be extrapolated to all ethnic groups is debatable.

Thus, fluctuations in serum AMH levels should be considered while interpreting values in clinical practice and should not be the sole criteria of refusing treatment especially in ART.

CONCLUSION

AMH is an important biomarker in both male and female reproductive physiology. It reflects the acyclic ovarian activity, thus, providing a window to the submerged part of the tip of the iceberg of follicle growth. An international standard for AMH and improved assay validity are urgently needed to maximize the clinical utility of this very valuable marker of ovarian function in a large array of clinical situations, right from childhood to adolescence to adult females.

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