**ORIGINAL ARTICLE**

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**Anti-Mullerian Hormone (AMH) as a Novel Marker for Ovarian Function: A Review**

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**ABSTRACT**

**Background:** Anti-Mullerian Hormone (AMH) is a homodimeric glycoprotein linked by disulfide bonds which belongs to the Transforming Growth Factor (TGF) beta superfamily. AMH is produced by gonadal tissue namely testicular Sertoli cells and ovarian granulosa cells especially pre-antral and antral follicles. AMH involved in embryonic sexual differentiation in male, while it has an inhibitory effect on primordial follicle recruitment and responsiveness of the growing follicles to FSH, in female. Current studies show promising clinical utilities of AMH measurement in predicting ovarian function.

**Objective:** This review aims to explore further about the physiology of AMH and its role as a novel marker for ovarian function.

**Method:** A review of relevant literature was performed to elaborate AMH involvement in ovarian function. A total of 14 qualified published literature of all years until 2018 were collected from several electronic database and manual search and included in this review.

**Results:** AMH level reflected ovarian follicular reserve, an important indicator in infertility treatment (assisted reproduction technique) and a sensitive marker for ovarian aging. AMH is also a valuable tool in diagnosis and recognition of recurrence granulosa cell tumors, and a marker of ovarian dysfunction particularly polycystic ovary syndrome. A low circulating AMH level is observed in obesity and male with fertility problems.

**Conclusion:** AMH level can be used as primary or supplementary markers to aid in the diagnosis and treatment of several reproductive related conditions in males and females. A set of guidelines about sample storage and handling and age-specific standardized reference values are needed to optimize clinical application of AMH.

**Keyword:** Anti-Mullerian hormone, marker, ovarian function


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**INTRODUCTION**

At early stages of development in mammals, there are two pairs of ducts in fetuses of both sexes: the Wolfian and Mullerian ducts. Regression of Mullerian duct later in male responsible for normal sexual organ development. In 1940, Alfred Jost showed that there is a testicular product beside testosterone that accountable for the regression of Mullerian duct in the male fetus called “hormone inhibitrice/Anti-Mullerian Hormone (AMH)”.

The human gene for AMH have already been isolated and sequenced. AMH has molecular weight 140 kDa, and the human gene is located on short arm of chromosome 19, band 19p.13.3. AMH gene is 2.750 bp long and divided into five exons. The 3’ part of the fifth exon codes for the bioactive part of the molecule and extremely GC rich.1

AMH or Mullerian Inhibiting Substance (MIS) is a homodimeric glycoprotein linked by disulfide bonds and belongs to Transforming Growth Factor (TGF) beta family, which acts on tissue growth and differentiation. AMH induces regression of Mullerian duct, allowing Wolfian ducts to develop into male reproductive tract under the influence of testosterone. AMH plays a vital role in folliculogenesis, inhibits recruitment of primordial follicles and diminishes response of selectable follicle to FSH, thus impairing selection of dominant follicle.2-6 AMH acts through two receptors: type I receptor (AMHR1) and type II receptor (AMHRII) which are present on AMH target organs (gonad and Mullerian ducts).2-4,6

This article will review the physiology of AMH and clinical utility of AMH measurement.

**PHYSIOLOGY OF AMH**

AMH plays a vital role in sexual differentiation during embryonic development. In the male fetus, AMH produced by fetal Sertoli cells induces regression of Mullerian duct, and there is normal male reproductive tract development. The absence of AMH in female fetus allows the Mullerian duct to differentiate into the upper vagina, uterus, and oviduct.7

At birth, about 1 million oocytes are present, and decreases during childhood, resulting in a primordial follicle pool of 300.000 – 500.000 at menarche. Throughout life, follicles leave the primordial pool to enter the growing follicle pool. Majority of growing follicles will be lost as a result of atresia unless the follicles rescued by Follicle Stimulating Hormone (FSH). 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).5,7

The dormant primordial follicles do not produce AMH, and after it grows to pre-antral follicles, it starts to produce AMH. This process needs 290 days. Pre-antral follicles are small follicles with diameter 0, 2 mm and still too small to detect at an ultrasound. Another 50 days are required to reach the antral stage. Antral follicles are visible on ultrasound and range size from 2 – 10 mm. Counting the number of antral follicles forms the basis ovarian reserve test, the antral follicle count (AFC). A woman may have a variable number of antral follicles because all primordial follicles are in different stages of development all the time. Size of AFC rarely exceeds 50 – 60 follicles.9

Figure 1 shows that AMH is produced in early stages of follicle development, characterized by gonadotrophin independent growth, as opposed to Inhibin B and estradiol produced by follicles at later stages of development where growth is FSH dependent.9

AMH is specifically expressed in granulosa cells of small growing follicles (pre-antral and small antral follicles). AMH is no longer expressed by granulosa cells during FSH dependent stages of follicular growth and atretic follicles. The intrafollicular concentration of AMH in normal human antral follicles show a gradual reduction as the diameter of the follicle increases and sharply decline as follicles diameter reach 8 mm. The rapid reduction in AMH expression corresponds with the selection of follicles for dominance, which is characterized by a transition from low estrogen producing state into rapidly increasing estrogen production. AMH is the biological regulator of folliculogenesis and primordial follicular rupture. It reduces the rate of follicle conversion from primordial to the growing stage and regulates follicle growth by inhibiting FSH-induced conversion from early to late stage.5,7

Figure 2 shows that AMH produced by granulosa cells of small growing follicles inhibits initial follicle recruitment and FSH dependent growth and selection on pre-antral and small antral follicles. AMH remains highly expressed in cumulus cells of mature follicles. The inset shows the inhibitory effect of AMH on FSH-induced CYP19a1 expression leading to reduced estradiol levels, and the inhibitory effect of estradiol on AMH expression.8

The expression of AMH by Sertoli cells in testis is started at eight weeks and remains high until puberty. Sertoli cell maturation is characterized by decreasing of AMH production. During pubertal, AMH expression falls, coinciding with the increase in androgen secretion by Leydig cells. The reduction in AMH levels at puberty is considered a marker of elevation of intratesticular androgen concentration which inhibits Sertoli cell AMH production at puberty. The primary physiological role of AMH in adult male seems to be limited to the paracrine control of the testicular function. AMH inhibits aromatase activity in Sertoli cells and testosterone production by Leydig cells. As AMH is a specific marker of Sertoli cell function and is secreted in serum and seminal fluid, its measurement is useful in obtaining information on spermatogenesis, particularly in infertile men.7

**CLINICAL UTILITY OF AMH MEASUREMENT**

**AMH as a marker of ovarian reserve**

The increase in FSH and decrease in Inhibin B and antral follicle count (AFC) are laboratory parameters which are often used for investigating ovarian reserve. However, recent studies suggest AMH as a novel parameter for ovarian reserve. AMH is secreted by the ovaries to the circulation so that AMH can be measured in serum samples. AMH can be used as a marker of functional ovarian reserves, both in the general population and subfertile women before induction of ovulation. AMH is an ideal marker to measure the functional ovarian reserve because AMH is only formed by pre-antral and antral follicles that are proportional to the primordial follicle pool. Several studies have suggested that there is a good correlation between serum AMH levels and the number of growing follicles and functional ovarian reserve.4,5,7

The number of primordial follicles decrease with age and virtually depleted at menopause. AMH level shown significant fall with age and correlated with loss of ovarian reserve. AMH levels also decreased with increasing age, which corresponds with the loss of functional ovarian reserve, where significant reductions can be detected earlier than FSH. Thus, AMH could serve as the marker of ovarian aging as its levels reflect ovary’s age-dependent falls in follicular potential. AMH level have higher sensitivity than Inhibin B, FSH and estradiol values in predicting ovarian follicular reserve.4

**AMH in Assisted Reproductive Technology (ART)**

In women over 35 years old, AMH may be used as a screening test to assess fertility status. Measurement of AMH levels is an excellent prognostic marker for assessing ovarian response to ovarian stimulation during the in vitro fertilization (IVF) cycle. AMH also has an advantage over Inhibin B in determining the rate of response to ovarian stimulation.
It was reported that AMH levels could recognize women prone to express ovarian hyperstimulation syndrome (OHSS) during multiple ovulation induction with human gonadotropins. In patients with Ovarian Hyperstimulation Syndrome (OHSS) during stimulation therapy, AMH levels are six times higher than normal controls, so AMH levels should be measured prior the action of IVF / ICSI.1

Several studies have suggested that serum AMH levels on the third day of the cycle may provide better predictor of pregnancy in IVF cycles than age parameter, serum FSH levels, inhibin B and estradiol. Basal serum AMH levels <1.1 ng / ml are associated with the risk of IVF failure. Other studies stated that a cut off AMH levels of 1.13 ng/ml was able to predict ovarian reserve with sensitivity and specificity of 80% and 85%, respectively. Using a 0.1 ng/ml cut off, serum AMH levels gave 87.5% sensitivity and 72.2% specificity in predicting IVF cycle cancellation.1

AMH as tumor marker
AMH expressed by granulosa cells of primary follicles could be used as ovarian tumor marker of granulosa cells origin. AMH increased in 76 – 93% of women with granulosa cells tumors (GCTs). Elevation of AMH levels precedes clinical tumor recurrence by up to 16 months providing the relevancy of AMH uses as early diagnostic and GCTs recurrence markers.4 Monitoring GCTs using AMH is better than alpha-inhibin and estradiol, where AMH only increases in GCTs while inhibin may increase in various types of cancer.1

AMH as a marker of ovarian dysfunction
AMH levels can aid in differentiating hypergonadotropic amenorrhoea differentiation (premature ovarian failure/POF) from hypogonadotropic amenorrhea (functional hypothalamic amenorrhoea). AMH levels were within normal limits in women with hypogonadotropic amenorrhoea, and 83% of AMH levels were undetectable in women with hypergonadotropic amenorrhoea.1

AMH reflects the number of growing follicles, so its measurement may be used as a marker of ovarian follicle impairment in Polycystic Ovary Syndrome (PCOS). AMH level in serum samples and follicular fluid samples in women with PCOS were higher than controls. AMH measurements in PCOS had a specificity of 92% and a sensitivity of 67%. It indicates that elevated AMH levels are associated with an increased number of small follicles of 2-5 mm diameter in women with PCOS. High AMH levels are associated with follicular arrest, during the dominant follicle selection process.4

AMH as tumor inhibitor
A new theory showed that ovarian epithelial tumors originate from tissues that embryologically derive from Mullerian ducts, although a long time ago it was thought to originate from coelomic epithelium.
that covers the ovarian surface. Recent data indicate that a great number of tumors arise from fimbriated end of fallopian tube. Some researchers hypothesized that AMH could be used in the treatment of ovarian epithelial tumors and several studies showed that AMH inhibited epithelial ovarian cancer cells in vitro. Further studies are required to establish whether AMH has clinical use in the treatment of these tumors.4

AMH in obesity
A study by Freeman et al. suggested that obese women at late reproductive age (35-49 years) have significantly lower AMH levels than women of normal weight at the appropriate age. The inverse correlation between body mass index and AMH levels is considered due to several reasons including the following:

1. Obesity affects the catabolism of AMH,
2. Obesity may decrease functional ability of the ovaries,
3. Obesity possibly related to ovarian dysfunction.

More studies are still needed to prove the effect of obesity on ovarian function.10

One study by Panidis et al. demonstrated a positive correlation between AMH and LH levels. Low LH levels as seen in obese women is associated with increased androgen to estrogen aromatization occurring in peripheral lipid tissue leading to LH suppression. Additionally, obese women also showed increased in inflammatory markers and oxidative stress markers in follicular fluid. There is also leptin-AMH2 receptor pathway involvement, whereas leptin treatment significantly reduces levels of AMH and AMHR2 mRNA levels.11

AMH in male fertility
AMH can be used as a marker of hypogonadism in men. Hypogonadism in adult males reflects testicular failure associated with androgen deficiency and/or disorders in sperm production. In prepubertal children, hypogonadism can be detected if Sertoli cell function can be assessed. In central hypogonadism, gonadal failure results from malfunctions of the hypothalamic Gonadotropin-releasing Hormone (GnRH) pulse or pituitary to generate FSH and LH. AMH levels are very low in infants with congenital central hypogonadism. Treatment with recombinant human FSH (rhFSH) results in increased testicular size and elevated serum AMH levels.8

In primary hypogonadism caused by early-onset complete gonadal dysgenesis, the absence of testicular tissue results in disorder of sexual differentiation with female external whereas regression of testicular tissue is associated with male genitalia, hypoplastic scrotum dan micropenis. In all cases, the absence of gonadal tissues results in undetectable AMH level. Patients with Klinefelter syndrome...
typically have late-onset testicular dysgenesis with no overt signs of hypogonadism before puberty and AMH levels are normal. However, Sertoli cell function deteriorates rapidly from mid-puberty, resulting in extremely low or undetectable AMH, very high FSH, and undetectable inhibin B levels, and small testis volume. Cryptorchidism may have many possible origins. One of the is testicular dysgenesis syndrome, a primary hypogonadal disorder with whole testicular dysfunction and hypospadias, reduced semen quality and increased testicular cancer risk. Alternatively, cryptorchidism may result from anatomical defects in the inguinal region or abdominal wall. Hence, cryptorchidism may be associated with normal or impaired Sertoli cell function depends on its etiology. AMH is normal in patients with inguinal or abdominal wall defects and is low in 75% of patients with non-palpable gonad and 35% of those with inguinal gonads indicating Sertoli cell dysfunction. 

**Factor Influencing AMH Level**

AMH is produced and secreted by gonads into the circulation, and AMH is measurable in serum from both men and women. AMH levels in women are lower than in men throughout life. In women, AMH serum levels almost undetectable at birth, with a subtle increase within the first 2 – 4 years of age, then stable until adulthood. It eventually decreases as a sign of follicular exhaustion and becoming undetectable at menopause. In women, AMH appears to be ovarian origin, since AMH is undetectable 3 – 5 days following bilateral ovariectomy.

AMH concentration remains stable throughout the menstrual cycle. For this reason, AMH measurement can be done at any time during the menstrual cycle, while on the other hand, FSH and inhibin measurement should only be done at days 3 – 5 of the cycle. AMH level seems to be unmodified under the condition in which endogenous gonadotropin diminished, including during pregnancy, GnRH agonist treatment, and short-term oral contraceptive administration, indicating that non-cyclic FSH independent ovarian activity persists even when pituitary FSH secretion is suppressed.

There is variability in measured AMH concentrations due to pre-analytical, proteolysis and conformational changes of AMH dimer. These discrepancies may be explained by differences in sample handling, sampling time (diurnal variation), transport dan storage conditions. Samples stored at room temperature for 7 days increase AMH serum level. Samples stored at -20⁰C yielded on average 23% higher values, while the same samples stored at -80⁰C showed no change. Sample instability may be the effects of complement binding. It can be avoided or minimized by adding a buffer or pre-mixing samples with assay buffer to potentiate AMH stability at all temperatures. Automatic pipetting or centrifugation of samples within 5 hours seems to reduce the influence of complement binding. Immunological kits are available for AMH determination in body fluids (serum, plasma, follicular fluid). They usually apply sandwich system with two specific antibodies to AMH, the first bound to the solid phase and the second labeled with biotin, which streptavidin-labeled enzyme is bound (usually horse-radish peroxidase). After addition of substrate (chromogenic conjugate which affords a colored product a ter cleavage by the enzyme), its absorbance is measured. There is an urgent need to
establish an international reference preparation to make test results comparable and robust independent evaluation of all commercial assays. Several investigators reported disrupting influences on AMH serum concentrations such as vitamin D deficiency, use of oral contraceptives, smoking, obesity, and race or ethnicity. Vitamin D deficiency was associated with lower ovarian reserve as measured by serum AMH level, and vitamin D supplementation normalized serum AMH levels in these women. Vitamin D changes AMH production pattern in ovarian granulosa cells through steroidogenic factor-1 and altering FSH sensitivity, so it is recommended for both partners to receive vitamin D supplementation in infertility treatment. AMH level is 20% lower during oral contraceptive (OAC) use because OAC reduces the effect of FSH and inhibit the growth of follicles. Smoking is associated with increased FSH levels and early menopause. Active smoking decreased AMH values in late reproductive age and perimenopausal women. There is a direct effect of smoking on ovarian follicle depletion. Racial differences and AMH level related to multiple genetic and environmental factor and genome-wide association studies are required.

REFERENCE RANGE
Serum AMH level is usually given in mass (ng/ml or µg/l) or SI units (pmol/l). The respective converting factor is (pmol/l) = 7.14 x (ng/ml). According to Seifer, both median and mean AMH values decreased steadily in a manner highly correlated with advancing age ($R^2 > 0.95$ for a linear relationship; $R^2 > 0.99$ for quadratic equation fit). The average yearly decrease in median serum AMH value was 0.2 ng/ml/year through age 35 and then diminished to 0.1 ng/ml/year after age 35. The rate of decline in mean AMH values was 0.2 ng/ml/year through age 40 and then diminished to 0.1 ng/ml/year after that.

Figure 6 shows a graph of AMH age-specific median values with mean ±SD AMH values for women ages 24 – 50 at 1-year intervals in US fertility centers. The median was substantially lower than average suggesting abnormal distribution of AMH values by age. AMH values were generated using materials and reagents from Beckman/DSL Generation I AMH system. Values from different laboratories may vary.

Lie Fong et al. stated that serum AMH levels increased slightly during childhood until the end of puberty and reached a maximum at age 15.8 year (95% Confidence Interval: 13.5 – 20.3) and remain constant until early 20s. Before age 15.8 year, serum AMH and age were positively correlated ($r = +0.18$, $p = 0.007$). From the age of 25 years onward, AMH levels decreased with increasing age ($r = -0.47$, $p < 0.001$).

Cut-off levels of AMH values for poor ovarian response reported in the literature vary between 0.1 and 2.97 ng/ml, which is within the range of normal values for AMH in healthy women (La Marca and Sunkara, 2014). Beineke stated that level of AMH in adult women in fertile phase is 1 – 8 µg/l, and values > 1 µg/l indicates adequate residual ovarian function.

CONCLUSION
There are several clinical utilities of AMH measurement including the use of AMH to estimate ovarian reserve and as a useful tool for guiding and monitoring IVF treatment. AMH levels represent a sensitive marker for the inevitable decline in the number of primordial follicles related to aging. In oncology, AMH measurement is a reliable tumor marker of ovarian granulosa cell tumors and has potential as a therapeutic agent for ovarian epithelial tumors. AMH could be used as a supplementary marker of PCOS in cases where the ultrasonographic examination of the ovaries is not feasible. The relation between AMH and obesity will be a future research target in pathogenetic mechanism linking obesity and gonadal dysfunction. It is mandatory to develop an international guideline regarding storage of samples and handling technique as well as collecting and formulating a new age-specific standardized reference value for AMH assay to enhance its clinical applications.

CONFLICT OF INTEREST
The authors declare that they don’t have any conflict of interest regarding manuscript.

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AUTHOR’S CONTRIBUTION
Sianny Herawati contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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