

Review Article

Clinical Utilities of Anti-Mullerian Hormone

Maxwell Omabe¹, Martin Ezeani², Okwuegbu Martin¹

¹School of Biomedical Science, Faculty of Health Science, Ebonyi State University Nigeria

²Department of Neurosciences, University of Sussex, United Kingdom

Corresponding author

Maxwell Omabe

Email: maswello2002@yahoo.com

Abstract: Anti mullerian hormone (AMH) is a homodimeric glycoprotein belonging to the transforming growth factors (TGF- β) super family. AMH plays a fundamental role in the regression of mullerian ducts in male embryo. In boys, it is significantly produced in Sertoli cells of testes until puberty and then slowly decreases to residual values for the rest of the men's life. In female, it is secreted by granulosa cells of small follicles in the ovary. AMH levels accurately reflects the ovarian follicular reserve and could therefore, be considered as extremely sensitive marker of ovarian aging and a valuable tool in the diagnosis and the recognition of recurrence of granulosa cell tumor. AMH evaluation is of clinical importance in predicting of ovarian responsiveness, male hypogonadism and ovarian function cessation and in assisted reproduction. AMH could be a replacement diagnostic marker of polycystic ovary syndrome (PCOS) in cases in which ultrasonographic examination is not possible. The measurement of serum AMH levels during woman's reproductive life represents an ideal tool for the assessment of the ovarian follicular reserve. Special reference is made to the possible implications of AMH in the pathogenesis of PCOS and the relationship between AMH and obesity.

Keywords: Anti-mullerian hormone, ovarian reserve, polycystic ovarian syndrome, Assisted Reproduction Technology

INTRODUCTION

History and background of the study Anti mullerian hormone

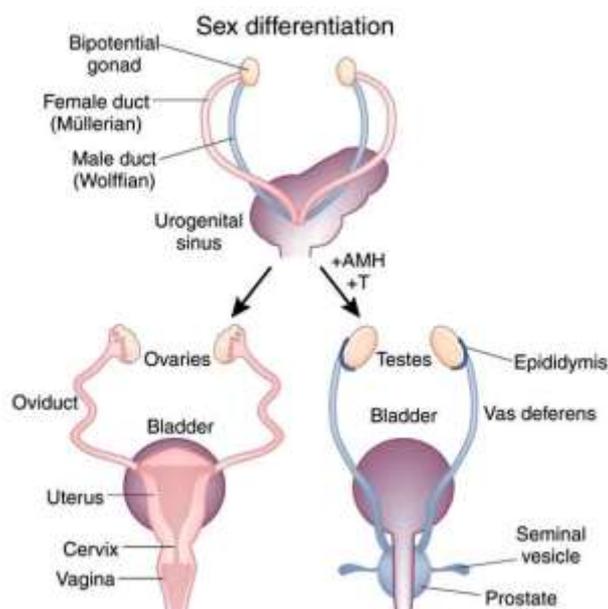
Anti mullerian Hormone (AMH) was for the first reported by A.Jost in 1940. The author described a protein substance formed in testes of mammals including man and different form of testosterone, responsible for regression of mullerian duct. [1]. It is also known as mullerian inhibiting substance. It lasted almost 40 years before the protein was isolation and characterized including the gene which code for it. Soon after its receptors where described [2]. Anti mullerian hormone is a peptide that belong to the family of transforming growth factor-beta (TGF- β). From the chemical point of view its a peptide homodimer of molecular weight 140 kDa that consists of two identical glycoprotein subunit, which is connected by disulphide bridge [1]. The gene for human AMH is localized on the short arm chromosomes 19 and is composed from 2750 nucleotide bases. The fifth exon from the 3'end codes for a biologically active part of the molecular, rich of glycine. The gene encoding for AMH receptor is composed from 11 exons more than 8kb in length and is localized on chromosome 12 [2]. The receptor for the AMH is trans membrane heteromeric proteins, composed from two subunits, denoted type I and II. As all the receptor for growth factors of the TGF- β family, they do not use G- protein and possess intrinsic kinase activity. The type II (better subunit) binds specifically the ligand leading thus to activation of type I, the intracellular part of which acts as threonine kinase. Activation of the letter starts a signal cascade resulting in a respective biological response [1].

Target organ for AMH in male are mullerian ducts and gonads for both sexes. In male the AMH is produced from the Sertoli cells of the testes since the 5th week of the embryonal development and during the whole life [1]. It is also formed in female in ovaries from the 36th week of gestation. From the description and explanation above AMH regress mullerian ducts during embryonal development, leading to initiation of a further development towards the male phenotype. In this study, we reviewed currently available studies concerning AMH to draw a synthesis that brings scientists and clinician to an update on the clinical utility of anti mullerian hormone.

Physiology of Anti mullerian hormone (AMH) in males

In the male, AMH is the earliest Sertoli cell specific protein expressed by the gonad [3]. It is secreted by the testis from the eighth week of pregnancy and remains secreted at high-level until puberty, when Sertoli cell maturation is characterized by decreased AMH production [4]. Paralleling the situation in women, the main physiological role of AMH in the adult male seems to be limited to the paracrine control of testicular function.

In the adult man, AMH is secreted both in serum and in seminal fluid [5] and, being a specific marker of Sertoli cell function; its measurement may be useful to obtain information on spermatogenesis in infertile men.



The changes of AMH expression follow the development of the male reproductive system, first of all changes in the activity of hypothalamo-pituitary-gonadal (HPG) axis. They may be divided into four main stages: fetal and early postnatal period, childhood, puberty and adulthood [6]. Variations in AMH formation are well reflected by its blood levels.

Fetal and early postnatal period

As mentioned above, AMH is synthesized in Sertoli cells of fetal testes already in early stage of embryonal development. In this period hypothalamus already produces gonadoliberin, which stimulates secretion of pituitary gonadotropins - lutropin (LH) and follitropin (FSH). By action of LH on Leydig cells of fetal testes *via* present receptors relatively high amounts of testosterone are formed (as detected for instance in new-born's circulation). Testosterone is responsible for differentiation of Wolffian ducts. At the same time testosterone would inhibit AMH formation in Sertoli cells through androgen receptors (AR). Because, androgen receptor is still not expressed in appropriate amounts, the latter effect does not appear. On the other hand, FSH through its receptors on the membrane of Sertoli cells stimulates AMH expression[7]. FSH utilizes here are the classical mechanism involving binding to the receptor and activation of adenylate cyclase effector through G-protein. Cyclic adenosine triphosphate (cAMP), formed by action of adenylate cyclase, activates a number of kinases, among the first protein kinase A (PKA), one of the most common ones), thus starting a signalling cascade, leading to activation and following translocation of nuclear transcription factors. They bind then to the respective responsive elements (sequences of DNA specifically recognizing activated transforming factors, usually

consisting of 15 nucleotide bases) in the promoter region of the AMH gene, resulting in its expression. [6].Inhibin B is also formed and Sertoli cells, constituting in this stage about one half of the whole testicular tissue, which undergo proliferation [6]. Serum levels of AMH are relatively high in this stage, comparable with the levels in childhood (roughly up to the eighth year of boys, as shown below) [6].

Childhood and early prepubertal period

Childhood, until prepubertal stage is a period of a relative rest of the HPG axis and it is sometimes called a stage of "hypo gonadotropic hypo gonadism". Leydig cells produce only very low amounts of testosterone, almost a half of which in addition originates from adrenals. Sertoli cells are still immature and spermiogenesis is arrested in a premeiotic stage. The latter cells however represent the main portion of testicular tissue and thanks to FSH stimulation produce AMH in amounts, comparable with prenatal period [6-7].

Puberty

With the onset of puberty the secretion of hypothalamic gonadoliberin and both gonadotropins increase again, but the effect of LH is much more pronounced, due to inhibitory effect of inhibin B on FSH secretion. Leydig cells undergo further differentiation and dramatically increases testosterone formation, which invokes also maturation of Sertoli cells. The inhibitory effect of testosterone prevails over FSH stimulation, resulting in down-regulation of AMH expression, the levels of which rapidly sink. Germinal cells undergo meiosis and spermatogenesis begins. In the late puberty stage (Tanner V), the germinal cells, in contrast to Sertoli cells, represent already the major

portion of testicular tissue. The secretion of AMH reaches adult values and it is maintained almost constant until the rest of life [6-7].

Physiology of Anti mullerian hormone (AMH) in Females

In women AMH is produced by granulosa cells, from pre-antral and antral follicles [8] and the main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development [9, 10]. AMH is secreted by the ovary into circulation; hence AMH is measurable in serum. As serum AMH levels essentially reflect the ovarian follicular pool, reduction in the number of small growing follicles may be followed by a reduction in circulating AMH. Recently, AMH has been evaluated by several groups as a potential novel clinical marker of ovarian reserve and of response to gonadotropins [11-20]. In particular in the last few years several large prospective studies have been published reporting extremely interesting new data on the possible clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in assisted reproductive technologies (ART).

CLINICAL RELEVANCE OF ANTI MULLERIAN HORMONE (AMH) IN WOMEN

It is widely accepted that the reduction of AMH levels in serum is the first indication of a decline in the follicular reserve of the ovaries. AMH concentration remains stable throughout the menstrual cycle [21]. Recent data, however, have shown that there are changes throughout the cycle (with lower levels during the early secretory phase) or even between consecutive cycles. Nevertheless, these changes are not considered clinically significant to recommend the measurement of AMH concentrations at a specific phase of the menstrual cycle [22].

The data concerning the impact of oral contraceptives on AMH values are divergent. It has been suggested that AMH concentrations are not influenced by oral contraception [23] but this finding has not been confirmed [24], Contraceptives containing 35mg of ethynylestradiol and 2mg of cyproterone acetate cause a significant suppression of gonadotropins and testosterone levels, a reduction in the number of ovarian small follicles as well as a significant reduction in AMH levels [24]. On the other hand, gonadotropin-releasing hormone (GnRH) agonists do not seem to affect AMH concentrations [25]. Finally, there are no racial differences in the hormone levels between women of comparable age [26].

Physiology Of Anti Mullerian Hormone Actions in Women

The specific actions of AMH on the human ovaries have not yet been fully clarified. It has been suggested that AMH inhibits the recruitment of primordial follicles and diminishes the response of the selectable

follicles to FSH, thus impairing the selection of the dominant follicle [27]. Most of the evidence regarding AMH actions has come from animal studies. [28] showed that AMH knock-out mice had three times more small non-atretic growing follicles and a reduced number of primordial follicles compared with wild mice. Although AMH null mice had low FSH levels, they still presented a large number of growing follicles. This fact led to the hypothesis that, in the absence of AMH, follicles show a tendency to become more sensitive to FSH action [28][29] Furthermore, it has been reported that FSH and estradiol down-regulate AMH gene expression in granulosa cell (GC) of rat follicles[30]. In a recent *in vitro* study, it was ascertained that the bone morphogenic protein-6 (BMP-6) augments AMH expression in GC of human follicles [31]. The researchers assumed that the BMP-6 produced by the GC of developing follicles increases AMH expression, which in turn inhibits the recruitment of primordial follicles, and hence ovarian function sustains its dynamic balance. In a recent prospective study, AMH measurements provided comparable results by both the Immunotech Beckman (IB) and Diagnostic System Laboratories DSL assays [22]

CLINICAL UTILITY OF ANTI-MULLERIAN HORMONE (AMH)

AMH as a tumour marker

Serum AMH is a good marker of tumours originating from granulosa cells, hence it has been proposed to be used as a marker of granulosa cell tumours (GCTs). It has been shown that AMH serum levels are increased in women affected by GCTs. AMH serum levels seem to be elevated in 76%-93% of patients with incidence of GCTs. The mean AMH level was 190-3ng/ml (range of 2-1124ng/ml) in patients with GCTs. In patient in whom serial measurements were made following initial tumour resection, the length of time that on elevation in AMH levels preceded clinical detection of a recurrence was 16 months.

AMH seems to be superior to alpha -inhibin and oestradiol in the follow-up of GCT. It is largely recognized that AMH and alpha-inhibin exhibits a higher degree of sensitivity than oestradiol in progressive GCT. Indeed, oestradiol production by GCT is widely variable. In the follow-up, AMH seems to be a marker of comparable value to alpha-inhibin; AMH is elevated in different types of cancer. Recent research brought evidence that AMH determination may serve as a tool for diagnosis of some other neoplasia, as for instance a prostate cancer and could be used for detection of tumour recurrence. The result however were not definite [15]

Anti müllerian hormone (AMH) as a marker of male hypogonadism

Apart from overt somatic disorders (malformation of external genitalia, gonadal dysgenesis, cryptorchism etc.), male hypogonadism, either central or primary,

may be overlooked in the early as well as in the later childhood, if the diagnosis is based only on investigation of gonadotropins and testosterone [6]. Testicular AMH production increases in response to FSH and is potentially inhibited by androgen. Serum AMH and inhibin B are undetectable in anorchid patients.[32]. Lower AMH levels than those at the corresponding age are thus a very good marker of congenital central hypogonadism. On the other hand, in boys not treated with recombinant FSH doesn't occur a characteristic AMH decline at the age of puberty. Low AMH levels are typical for advanced puberty due to inhibitory effect of testosterone, while persisting high AMH levels at the age of physiological puberty indicate its delay. Besides hypo gonadism AMH may thus help to a more accurate diagnosis of disorders in onset and duration of puberty [6].

Anti müllerian hormone (AMH) as a marker of ovarian reserve

Anti müllerian hormone has been suggested as a marker of ovarian reserve . it was shown to correlate with antral follicle counts outcome from ovarian stimulation and onset of menopause. AMH was considered because it is exclusively produced by the granulosa cell and its only marker that is thought to be stable throughout the menstrual cycle [33]. The levels of AMH reflect the number of preantral follicles and thus are a marker of oocyte pool – a germinal reserve of the ovary for reproduction. Plasma levels of AMH tightly correlated with a number of mature follicles (AFC = antral follicle count), as assessed by transvaginal ultrasonography (correlation coefficient 0.66-0.71) and also with AMH concentrations measured in a follicular fluid [34]. Determination of plasma AMH in women of fertile age enables thus to assess with a greater specificity and sensitivity the extent of ovarian reserve than determination of FSH together with steroid hormones and inhibin, among other because AMH acts first of all paracrine and is not involved in feedback mechanisms of the hypothalamic-pituitary-gonadal axis. That's also the reason that AMH levels are almost independent on the phase of the menstrual cycle, and usually a single measurement is sufficient. It is advantageous for interpretation of the diagnosis and also from the economical point of view [27].

As was demonstrated on a group of women with polycystic ovary syndrome (PCOS) who underwent *in vitro* fertilization (IVF), the effect of IVF on the basis of AMH determination, as a percentage of successful pregnancy could be predicted with sensitivity 75.6 and specificity 77.3 % [27].

Anti müllerian hormone (AMH) as a marker of ovarian aging

The number of primordial follicles decreases with age and is virtually depleted at menopause. AMH serum levels determined over a three-year period in young women with normal menstrual cycles has shown a

significant fall in AMH, while FSH and inhibin B remained stable. Thus, AMH could be used as a marker of ovarian aging given that the reduction in hormone levels reflects the age-dependent fall in the follicular potential of the ovary. The decrease of AMH levels and follicle number with age has been widely accepted [35, 13]. Indeed, AMH values have greater sensitivity than inhibin B, FSH and estradiol [35] values in predicting ovarian follicular reserve. It has been reported that AMH concentrations present a negative linear correlation with basal FSH levels in women who have a poor response to controlled ovarian stimulation with human gonadotropins [36]. Specifically, AMH concentrations of 1ng/ml correspond to FSH values of 10IU/L, whereas 0.5ng/ml of AMH corresponds to 15IU/L.

However, in conditions with high LH and normal or low FSH levels, as in PCOS, AMH concentrations are positively correlated with LH concentrations, while they are not negatively correlated with FSH [37]. The reduction of AMH concentrations has recently been reported as a reliable marker for the evaluation of the ovarian impairment caused by chemo- or radiotherapy [38, 39]. This knowledge could prove particularly helpful in fertility preservation in women subjected to such therapies.

Anti müllerian hormone (AMH) as a marker of ovarian responsiveness

AMH's role as a peripheral signal of the size of the growing follicle pool may have important clinical benefits. In women undergoing treatment for infertility, ovarian aging is characterized by decreased ovarian responsiveness to exogenous gonadotropin administration and poor pregnancy outcome. On the one hand, correct identification of poor responders by assessment of their ovarian reserve before entering an *in vitro* fertilization (IVF) program is important. On the other hand, assessment of the ovarian reserve may also benefit patients that would generally be excluded from IVF programs because of advanced age.

Several studies have shown that AMH is an excellent marker to determine ovarian responsiveness also in an IVF program. Hormone measurements in the early follicular phase (day 3 of spontaneous cycle), retrospectively or in a group of unselected patients, revealed that AMH levels are lower in patients with poor ovarian response than in women with normal response [11, 12], ovarian responsiveness being defined as the number of oocytes retrieved, or as cancellation due to impaired or absent follicular growth. In agreement with the studies described above, AMH serum levels were shown to be highly correlated with the number of antral follicles before treatment and number of oocytes retrieved upon ovarian stimulation [12]. Logistic regression analysis for prediction of poor response showed that serum AMH levels had a better predictive value than serum levels of FSH, inhibin B

and E2, and that the predictive values for AMH and AFC were almost identical (ROCAUC0.85 vs 0.86). Inclusion of FSH and inhibin B together with AMH in a multivariate model improved this predictive value to 0.90 [12]. Similarly, cycle day-5 AMH levels are a better marker of ovarian responsiveness than inhibin B levels [14]. Measurement of serum AMH levels has several advantages over other serum markers such as FSH, inhibin B and E2. To achieve a reliable predictive outcome, one single hormone measurement for AMH seems sufficient [40]. Furthermore, in contrast to FSH, inhibin B and E2, AMH levels remain relatively constant during the follicular phase and entire menstrual cycle [21, 41].

The absence of regulation of AMH by gonadotropins was shown in both rodents and man. Heterozygous AMH null mice present with an ovarian phenotype between that of wild-type and homozygous AMH null mice [28], suggesting that AMH acts as a paracrine rather than a systemic factor, and thus is not part of a negative feedback loop with involvement of gonadotropins. In agreement, treatment of IVF patients with a single, high dose of gonadotropin-releasing hormone (GnRH) agonist, resulting in a rise of endogenous FSH and LH, does not affect AMH serum levels [12]. Similarly, in conditions where FSH levels are suppressed, such as pregnancy, AMH levels remain constant [42]. Thus, AMH is not influenced by the gonadotropic status and reflects only the follicle population. The latter conclusion was confirmed in a more detailed study by [13], who treated women with FSH and human chorionic gonadotropin (hCG) after complete pituitary desensitization with a GnRH agonist. In a normal menstrual cycle, the early antral follicle pool remains intact throughout the follicular phase. However, upon ovarian hyper stimulation, all small antral follicles are stimulated to the pre ovulatory stage, thus providing a model to determine the relationship between AMH levels and follicle dynamics. Serum AMH levels, determined at three days during FSH treatment and at the day of hCG administration, decline significantly at each consecutive measurement [13], reflecting the reduction in number of small antral follicles. A decline in serum AMH was also observed after FSH administration immediately following a spontaneous cycle [43]. Moreover, on day 5 of gonadotropin therapy, levels of serum AMH and estradiol constitute an even better prediction of the ovarian response than cycle day 3 AMH levels [17]. However, from a clinical point of view, poor responders should be identified before treatment; therefore, it is more useful to determine serum AMH levels during a spontaneous cycle.

Throughout the controlled ovarian hyper stimulation protocol, serum AMH levels correlated well with the decrease in number of small antral follicles (≤ 12 mm) [13], reflecting the complete conversion of small antral follicles into large antral follicles in response to FSH

stimulation. Indeed, no correlation with the number of growing follicles (> 12 mm) was observed [13], in line with the low expression of AMH in these follicles [8]. In the days following HCG treatment, AMH serum levels initially declined, possibly as a result of the luteinization of granulosa cells upon HCG treatment that also causes a decline in E2 levels. During the midluteal phase, AMH serum levels slightly increased, probably as a result of the presence of newly developed, small antral follicles [40]. Thus, these changes in serum AMH levels seem to reflect follicle dynamics rather than regulation by gonadotropins.

All combined these studies strongly support a role of serum AMH level as a marker for ovarian responsiveness. However, the application of AMH to predict on going pregnancy seems limited, although day 3 serum AMH levels are higher in patients that become pregnant after IVF treatment than in those who do not [16]. However, data on pregnancy outcome were not stratified for the number of retrieved oocytes, which also in this study showed a positive correlation with AMH levels. Therefore, it is likely that the quantitative aspect of AMH as a marker of the ovarian reserve has contributed predominantly to the association with pregnancy outcome. Indeed, other studies did not observe a predictive value of AMH serum levels for ongoing pregnancy after IVF treatment [12].

Anti müllerian hormone (AMH) in Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is common hormone disorders that affect approximately 5 million women of reproduction age. It is the association of hyperandrogenism with clinical anovulation without underlying disease of the pituitary or adrenal gland. PCOS is characterized clinically by menstrual dysfunction, weight disorders, hirsutism, acne, endometrial hyperplasia, diabetes mellitus, hyperlipidaemia, and metabolic syndrome. [44][45]. Since AMH levels reflect the number of developing follicles, their measurement may be used as a marker of ovarian follicle impairment in polycystic ovary syndrome. PCOS is clinically diagnosed when at least two of the following three features are present: chronic oligo- or anovulation, biochemical hyper androgenemia or hyper androgenism and polycystic ovarian morphology in ultrasound examination (PCO) (the Rotterdam ESHRE/ ASRM 2003). The syndrome, which is diagnosed in 5-10% of women of reproductive age, [46] is the main cause of an ovulatory infertility in developed countries. The common clinical manifestations of PCOS include menstruation disorders and androgen excess, hirsutism and male pattern alopecia [47].

Polycystic ovary syndrome is also associated with metabolic aberrations. The incidence of diabetes mellitus type 2 is 10-fold higher in women with PCOS compared to healthy women in the USA [48, 49].

Furthermore, 30-50% of women with PCOS develop glucose intolerance or diabetes mellitus type 2 after the age of 30 [49]. The incidence of metabolic syndrome is two to three-folds higher among women with PCOS compared to healthy women of similar age and body mass index (BMI), while 20% of women with PCOS, aged less than 20 years have already manifested the metabolic syndrome [50]. Although there is no prognosis regarding the outcome, especially for women with PCOS, the risk of fatal myocardial infarction is double among postmenopausal hyper androgenemic women with a history of severe oligo menorrhoea, who are actually expected to be PCOS patients, compared to women with regular menstrual cycle [51].

Women with PCOS have a two to six-fold greater number of follicles (primary, secondary and antral) in their ovaries, possibly due to the hyper androgenemia [52]. In an ovulatory woman with PCOS, the follicular development is halted when follicular diameter is 6-9mm that is just before the selection of the dominant follicle [53].

Earlier data showed that in women with PCOS, serum and follicular AMH levels are higher than in healthy controls [54, 55]. Subsequent data confirmed this finding and indicated that the elevated levels of AMH were related to increased number of follicles with a diameter of 2-5mm in women with PCOS [56]. Hence, AMH serum values could be a precise, subsidiary diagnostic marker of the syndrome, particularly in cases in which the transvaginal ultrasound examination is not feasible [57]. The high AMH levels in women with PCOS are attributed to the high number of small antral follicles with a diameter of 2-5mm [58]. Additionally; these high AMH levels are probably related to the follicular arrest, during the selection process of the dominant follicle, through a negative interaction between AMH and FSH. If so, AMH inhibits the production of aromatase which is activated by FSH action on GC [59]. On the other hand, in a large prospective study of adolescent population, although AMH serum levels were higher in adolescents with PCOS or PCO, the hormone was not proven to be a reliable predictor of PCO or PCOS [60].

In a recent *in vitro* study, it was found that AMH production per granulosa cell was increased by up to 75% in women with PCOS compared to controls [61]. According to the authors, the higher levels should be attributed to the increased number of follicles as well as to the intrinsic aberrant follicular function. AMH excess, via endocrine or paracrine paths, would appear to play an essential role in the process of follicular arrest.

AMH concentrations in women with PCOS were independently and positively correlated with testosterone, androstendione and free androgen index

(FAI) values [56, 62]. In a recent study, [63] AMH levels were determined in 200 women (100 normal weight and 100 overweight/obese) with PCOS and 50 controls (C) matched for age and BMI. Women with PCOS were divided into four groups, according to their clinical, biochemical, and sonographic characteristics. Group1 included women with oligo- or amenorrhea (ANOV), hyper androgenemia (HA), and polycystic ovarian morphology (PCO), group 2 included women with ANOV and HA, without PCO, group 3 included women with HA and PCO, without ANOV, and group 4 included women with ANOV and PCO, without HA. In the studied groups, each comprised of 25 PCOS women, we found that AMH levels were significantly higher in women of groups 1 and 2 compared to those of groups 3 and 4 and controls. Additionally, AMH was higher in the women of groups 3 and 4 compared to controls. Finally, AMH values were independently and positively correlated with LH and testosterone levels and the number of small follicles (diameter of 2-9mm). In conclusion, the differences in AMH concentrations between the four phenotypic groups of PCOS reflected the severity of the syndrome. However, a subsequent study failed to prove any correlation of AMH with LH levels [56]. This discrepancy could be attributed to the small number of women included in the latter study.

A great number of women with PCOS have insulin resistance and compensatory hyper insulinemia. A correlation has been reported between AMH levels and HOMA-IR values in women with the syndrome [43], although this finding has not been confirmed by other studies [56, 62, 63] Treatment of obese PCOS women with metformin resulted in the reduction of androgens and AMH levels, without any significant decrease in follicle number. On the other hand, it was recently reported that six-month treatment with dexamethasone and metformin combined with lifestyle modifications did not alter AMH levels [64].

It is known that AMH levels decrease with age in women with normal ovulatory cycles. A similar decline is observed in women with PCOS, but at a slower reduction rate [65]. This could be interpreted as indication that ovarian aging is slowed down in women with PCOS, possibly due to the negative effect of AMH on the recruitment of primordial follicles. High AMH levels were observed in adolescent girls, aged 12-18 years, with polycystic ovary syndrome compared to controls [66]. Furthermore, increased AMH concentrations have been found in girls aged 4-7 years born of mothers with PCOS [67]. Evidence that hereditary factors contribute to the pathogenesis of the syndrome is also found in animal studies showing that prenatal exposure to increased androgen levels can lead to offspring with PCOS features [68]. Although these data support a genetic contribution to the pathogenesis of the syndrome, an environmental component cannot be excluded given the fact that prenatal exposure to

high androgen levels as well as other unknown factors is operative.

Oxidative stress has recently been implicated in the pathogenesis of the anovulatory process [69]. Women with anovulatory PCOS appeared to have increased oxidative [70] stress as well as products of oxidation and advanced glycation end products (AGEs) [71]. Increased serum AGEs is a distinct finding in lean women with polycystic ovary syndrome. A direct relationship between PCOS, anovulatory process and AGEs is supported by finding increased serum AGEs levels and increased expression of their receptors in macrophages (RAGE) [72] as well as elevated deposition in ovarian tissues in PCOS women [73]. More relevant is the finding of significant positive correlation between AMH levels and AGEs in normal weight women with PCOS [74]. In this study, AMH and AGEs concentrations were evaluated in women with ovulatory PCOS, in women with anovulatory PCOS, in anovulatory women without the syndrome and in healthy controls. Their levels were significantly higher in the group of anovulatory PCOS as compared to the other study groups. The authors suggested a possible contribution of AMH and AGEs to the mechanism of anovulation in women with polycystic ovary syndrome [74].

Anti Müllerian Hormone (Amh) In Assisted Reproduction

The clinical significance of AMH determination was first proven in assisted reproduction medicine, as AMH serum levels reflect the ovarian reserve potential with high accuracy. AMH measurement is the best prognostic marker of the ovarian response to controlled ovarian stimulation during IVF cycles, especially when a single marker is determined [75, 76]. AMH levels have prognostic value for both the number of oocytes retrieved during follicular aspiration and the number of arrested cycles [76]. Compared to antral follicle count, AMH concentrations could reliably and equally predict poor response to ovarian stimulation in IVF cycles [77]. Recently, it was reported that AMH levels could recognise those women prone to express ovarian hyper stimulation syndrome (OHSS) during multiple ovulation induction with human gonadotropins [78]. In a prospective study, it was found that the live birth rate, following IVF, was increased when AMH levels were high prior to ovulation induction with human gonadotropins [79]. This could be attributed to the greater number of oocytes retrieved by women with high AMH levels, given that high basal AMH concentrations indicate a great number of selectable follicles. On the other hand, the results of a large meta-analysis showed that AMH levels are very poor predictors of pregnancy outcome [80]. An alternative approach could be the evaluation of AMH levels in the follicular fluid. [81]. AMH follicular fluid levels were strongly associated with pregnancy rates in IVF cycles, these findings also having been confirmed by other

researchers [82]. AMH levels measurement in oocyte donors were not decreased in women who underwent repetitive oocyte donation (three to six cycles), implying that ovarian aging is not advanced in oocyte donors as previously suspected [83].

AMH as an early marker of ovarian function cessation

Hand in hand with the decrease of the number of primary and preantral follicles with age, the production of AMH declines along with its blood levels. Low AMH levels and also their rapid decrease in certain time period is a marker of the drop off of an ovarian function [27]. In women under hormonal anti conception the proper function of ovaries cannot be evaluated without interruption of hormone administration for a period of 2-3 cycles, what brings about difficulties in a daily practice. Determination of FSH, LH and steroid hormone levels need not provide, in contrast to AMH, reliable information because of certain residual gonadal activity.

AMH AND OBESITY

There are very few studies evaluating the impact of obesity and weight loss on AMH levels, the existing studies concerning mostly overweight and obese women with PCOS. In a recent study, it was found that obese women of late reproductive age (35-49 years) had significantly lower AMH levels, (up to 65%), compared to normal-weight women of similar age [84]. This inverse correlation between BMI and AMH levels has not been fully explained. Three hypotheses have been proposed: a) obesity may affect the catabolism of AMH, b) obesity could reduce the ovarian potential, and c) obesity may be related to ovarian dysfunction [85]. To date, none of the three hypotheses could be fully supported or rejected as the data are scarce. Certainly, more studies are necessary to elucidate the impact of obesity on ovarian function. AMH levels were lower in overweight and obese women with PCOS than in normal-weight women with the syndrome and healthy controls [61]. Other studies have also confirmed this finding [61][62]. Furthermore, an independent positive correlation between AMH and LH levels has also been found [86]. Previous research has also shown that normal-weight women with PCOS presented higher LH values than overweight and obese women with the syndrome, [87] a finding which was confirmed by our study [63]. Thus, the lower LH concentrations observed in obese women may be attributed to the increased aromatization of androgens to estrogens which takes place in the peripheral fat tissue, resulting in the suppression of LH [88]. Therefore, higher AMH levels seen in normal-weight women with PCOS compared to obese women with the syndrome could be attributed to the higher LH levels. This is further supported by an *in vitro* study showing that the addition of LH in cultures of granulosa cells from women with PCOS triples the amount of AMH produced [61].

In the study of Mehri [89], AMH levels were evaluated in obese women of variable age before and after the performance of bariatric surgery. Despite the small size of the sample, significant AMH reduction was found after the decrease of BMI in the group of young women but not in older age groups. The authors concluded that the small sample as well as the short follow-up period did not permit definitive conclusions. Interestingly, in another study including a small number of obese women with PCOS who followed a program of weight loss and six-month maintenance, it was shown that the women with weight reduction and menstrual cycle improvement were those with significantly lower AMH levels before treatment [89]. In contrast, the menstrual cycles of women who had higher AMH levels before losing weight did not improve. The authors recommended AMH measurement in overweight and obese women prior to weight loss program implementation as a prognostic tool of the menstrual cycle improvement. The same authors have recently published a study regarding the impact of weight loss on AMH concentrations. This study included overweight and obese women with PCOS subjected to a 20-week course of hypocaloric diet [91]. AMH levels were not significantly altered either in the group of women who lost weight or in those who did not respond to the diet. Again, women with lower AMH levels before treatment presented the greater improvement of menstrual irregularities.

CONCLUSION

In advanced countries the determination of AMH belongs to a standard constituent of laboratory examination of the above mentioned states. The aim of this mini review is to inform the scientists about the wide possibilities of utilization of AMH determination. Last but not least an economical advantage of AMH determination over more expensive investigations used at IVF should be mentioned here and also its contribution to early and targeted diagnosis of a number of disorders. One of the reasons of this paper is to support the listing of AMH determination to the laboratory tests reimbursed by health insurance companies.

Recent studies have indicated that AMH may constitute an important novel measure of ovarian reserve. Serum AMH levels show a reduction throughout reproductive life and are undetectable after menopause [42, 94-96]. Similarly, early ovarian ageing and premature ovarian failure have been associated with very low or undetectable serum levels, respectively [97-99]. Furthermore AMH levels do not significantly change during the menstrual cycle [23, 97, 100], whereas all other hormones secreted by the ovary show significant variations throughout the cycle. The stability and consistency of its levels indicate that AMH could be used as the most reliable single marker of ovarian ageing and ovarian response.

For women who want to become pregnant by means of assisted reproductive technologies (ART), it is important to offer counselling about the optimal balance between benefit and risk. Since these outcomes are highly dependent on ovarian reserve, much effort has been put into identifying good clinical markers of ovarian reserve regarding individual prognosis for success and to design appropriate stimulation protocols. Although AMH measurement is of course more expensive than age evaluation as a single marker of ovarian reserve. Furthermore, AMH may also be informative on ovarian reserve in women during GnRH agonist treatment or hormonal contraception that consequently exhibit suppressed FSH levels. Finally, it seems that poor response may be predicted by AMH with a performance which is similar to the AFC. Conversely AMH seems superior to antral follicle count (AFC) in the prediction of hyper response [101]. Although AFC is a very common and useful measurement it may be sometimes technically challenging and operator-dependent. Considering all these peculiar characteristics, it may be concluded that AMH is a candidate proposed as the ideal test for the ovarian reserve evaluation [97, 102].

Comparison of characteristics of the most widely used markers of ovarian reserve. One new interesting field of application for AMH measurement may be its use in the individualization of ovarian stimulation regimens. Some authors have recently proposed adjusting the treatment strategy on the basis of AMH levels [79, 103, 104].

As low and high AMH values are predictive of poor- and high-response to gonadotrophins, respectively, it has been proposed that the daily dose of FSH is tailored according to the pre-IVF AMH levels, and independently of the age of the patient [79, 103, 104].

AMH levels reflect with high accuracy, the ovarian follicle reserve, and this has been demonstrated in numerous studies. Therefore, AMH evaluation has a great clinical importance in predicting the success of IVF cycle. AMH determination can be used in the diagnosis or follow up of woman with tumors of granulosa cell origin. It could be used as well as a marker of polycystic ovary syndrome in a case where ultrasonographic examination of the ovaries is not feasible. AMH may be used postoperatively as a marker of ovarian aging, ovarian responsiveness and as a male hypogonadism. With this research we have been able to establish as well that anti mullerian hormone could be used in assisted reproduction. And it can further serve as ovarian function cessation. Finally, the recent revealed relationship between anti müllerian hormone and obesity.

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