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Anti-Müllerian hormone: clinical implications in Gynecological Endocrinology. An update review

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ABSTRACT

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of the ovary with serum levels that grow until puberty, remain stable up to 30 years and then begin to decline until menopause. It is mainly produced by pre- and early antral follicles with an average diameter of 5-8 mm and it indirectly represents the ovarian reserve (OR). The purpose of this review is to identify what can currently be done with AMH, according to the most recent scientific evidence. AMH does not appear to be a marker for fertility as it does reflect the quantity but not the quality of follicles. It is not able to predict the spontaneous onset of pregnancy, nor the pregnancy rate in cycles of assisted reproduction technology (ART) but is a good predictor of ovarian response to hyperstimulation and it is useful in planning a couple's fertility treatment even in the case of women undergoing chemotherapy, radiotherapy and ovarian surgery. It helps to identify women suffering from mild forms of polycystic ovary syndrome (PCOS) and diagnose and manage menopause and premature ovarian failure (POF). Finally, AMH levels may be used in case of granulosa cells tumors, both for diagnosis and follow up after surgery.

SOMMARIO

L'ormone anti-Mülleriano (AMH) viene secreto dalle cellule della granulosa dell'ovaio ed i suoi livelli sierici aumentano fino alla pubertà, rimangono stabili fino a 30 anni e successivamente si riducono fino alla menopausa. Viene prodotto principalmente dai follicoli pre-antrali ed antrali con diametro medio di 5-8 mm e rappresenta indirettamente la riserva ovarica. Lo scopo di questa review è quello di far emergere l'utilità clinica dell'AMH in accordo con le più recenti evidenze scientifiche. Attualmente l'ormone non sembra un adeguato marker per la fertilità, poiché rappresenta solo la quantità e non la qualità dei follicoli. Non è in grado di predire l'insorgenza spontanea di gravidanza né il tasso di gravidanze nei cicli di riproduzione medicalmente assistita, ma è utile nel predire la risposta ovarica alla stimolazione e nel programmare i percorsi di assistenza alla coppia infertile, anche in caso di donne sottoposte a chemioterapia, radioterapia ed interventi chirurgici sull'ovaio. Inoltre, l'AMH è utile per diagnosticare forme lievi di sindrome dell'ovaio policistico (PCOS), individuare e gestire i casi di esaurimento ovarico prematuro e può aiutare nel predire l'insorgenza della menopausa. Infine, l'ormone può essere utilizzato nella diagnosi e nel follow-up post-chirurgico dei tumori delle cellule della granulosa.

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INTRODUCTION

AMH, which has been known since the 1940s, is a dimeric glycoprotein member of the transforming growth factor-β (TGF-β) superfamily. It has a role in sexual differentiation: it is produced by Sertoli cells of male foetuses and induces regression of the Müllerian duct, allowing the formation of the male reproductive tract from the Wolf ducts (1). Instead, the absence of AMH allows the differentiation of the Müllerian duct into the oviduct, uterus and upper vagina. Subsequently, the hormone is produced by the granulosa cells of the ovary from around 36–38 weeks of gestation (2), with serum levels that grow until puberty, reaching the peak at 20-25 years, and then decline from 30 years up to menopause (3). By immunohistochemistry of human ovarian tissue, it emerged that AMH is mainly produced by primary, secondary, pre-antral and early antral follicles and 60% of the serum AMH comes from follicles with an average diameter of 5-8 mm (4). Instead, follicles greater than 10 mm produced a very small amount of the hormone. Moreover, AMH inhibits follicle response to follicle-stimulating hormone (FSH) and this suggest that it has a role in controlling follicles recruitment (5-7). Indeed, experiments with AMH knock-out mice showed an increase in primordial follicles recruitment and their premature depletion, and lower serum FSH levels, suggesting an increased sensitivity of follicles to this hormone (8). Furthermore, AMH is produced mainly by pre-antral and early antral follicles, which are proportionally related to the primordial follicle pool, so it indirectly represents the ovarian reserve (9,10). This can also be assessed by antral follicle count (AFC) with transvaginal ultrasound, which has a strong positive correlation with AMH levels (11,12). In fact, both have the ability to accurately estimate the pool of ovarian follicles (13). However, AFC is highly dependent on the operator and it has inter- and intracycle variability (14). Moreover, women may perceive it as more invasive than a blood sample. Instead, AMH can be dosed any day of the menstrual cycle, during pregnancy or during a period of amenorrhoea because it is FSH independent (14,15). Although, in women who use oral contraceptives it is still debated whether the values of AMH are modified by these or not (16). In literature, the role of AMH as a predictor of ovarian reserve and response to ovarian hyperstimulation in women undergoing ART is well known (17). Despite this, it does not seem to have the capacity to assess the quality of oocytes nor to predict the likelihood of pregnancy occurrence. Other hypothesis on the use of AMH have been proposed in the literature, such as its role in the diagnosis of PCOS, in the prediction of premature ovarian failure and evaluation of ovarian reserve in women undergoing chemotherapy. But what can we actually do with AMH, according to current scientific evidence? The purpose of this review is to answer this question through a careful analysis of the most recent literature on the subject.

AMH FOR PREDICTING SPONTANEOUS PREGNANCY CHANCES IN WOMEN WITH AND WITHOUT INFERTILITY

As we have already said AMH reflects the quantity and not the quality of ovarian follicles, but the OR is represented by both these characteristics (18). That is why many authors have critically analysed its role in predicting the spontaneous onset of pregnancy. One of the most important issues is the absence of a cut-off value of AMH under which we can say with certainty that a woman cannot have a pregnancy. In fact, even if there is a negative association between AMH level and time to pregnancy in fertile women, there is also a wide variability in fecundity in women with similar hormone concentration (19). A recent prospective cohort study has analysed the time to pregnancy in 30-44 years old women without a history of infertility, who had been trying to conceive for 3 months or less, and found no correlation between biomarkers of diminished ovarian reserve, like low AMH and high FSH, and reduced pregnancy rate (20). However, in the study is stressed that they do not look at AMH values lower than 0.1 ng/ml, which reflect a more consistent drop in OR and may negatively affect fecundability. Another study, including 20-35 years old women, found no significant reduction in the pregnancy rate in women with AMH values < 1.4 ng/ml compared to those with higher values (21). Different recent studies reached the same conclusion (22,23) and according to a review by Dawailly and Laven (24) AMH is not a primary marker for

fertility and some authors claim that both AMH and AFC are just expression of woman's "ovulatory potential" (25). Even in women undergoing ovarian surgery for benign ovarian cysts it seems that AMH doesn't have a role in predicting the pregnancy rate. In fact, a study conducted in our department has analysed the pre- and post-operative AMH values of these women and evaluated the spontaneous onset of pregnancy of those who tried to conceive (26). We obtained a live birth of 37% and found no statistically significant difference in reproductive outcome between women with AMH serum levels lower and higher than 1.1 ng/ml. The same result was found also by another study, even with the same live birth rate (27). Moreover, the study conducted in our department found out that AMH levels decline 6 months after surgery in both women with endometrioma and in those with other benign ovarian cysts, but only in case of endometriotic cysts this decline was statistically significant. Anyway, there is a statistically significant recovery at 12 months in women with endometriotic cysts.

Many studies have analysed the general population but only a few observed the AMH role in case of unexplained infertility. One of these, conducted in our department, has included 83 women with unexplained infertility with normal or low ovarian reserve and observed the spontaneous onset of pregnancy and found that markers of OR are similar between women who get pregnant and those who don't (28). Moreover, in the aforementioned study were obtained 5 pregnancy in women with AMH lower than 0.4 ng/ml, a value diagnostic for abnormal ovarian reserve, one of the three diagnostic criteria for Poor Ovarian Response (POR), confirming that POR does not mean sterility and women in this condition could get pregnant (29,30).

An interesting observation is that 2 of these 5 pregnancies resulted in miscarriage and in fact another recent study hypothesized a role of AMH as a risk factor for miscarriage in spontaneous pregnancy (31). They included 460 pregnant women and after adjusting for age, BMI, race and history of recurrent pregnancy loss, they found out that the risk of miscarriage decreased as AMH increased and women with AMH ≤ 0.4 ng/ml have over twice the risk. Other studies confirm this hypothesis and observed that women with an unknown cause of miscarriage have a significantly lower AMH than those with an identifiable cause of pregnancy loss (32,33), so AMH is not a primary marker for fertility but it could be a marker of lower reproductive potential (31). However, there are many other factors that can affect fertility, such as body mass index (BMI), smoking, age and other diseases which we should study more carefully (19).

AMH AS A MARKER FOR THE DIAGNOSIS OF PCOS

PCOS has a prevalence of 8-13% in reproductive age women (34-36) and it is one of the most common gynaecological endocrine disorders, characterized by a wide variety of symptoms.

The diagnosis of PCOS is based on the presence of two out of three Rotterdam Criteria, which are oligo-anovulation, polycystic ovaries (PCO) on ultrasound and clinical and/or biochemical signs of hyperandrogenism (HA) (37), but this criteria are not always easy to apply. For example, hyperandrogenism is difficult to define because of interobserver variability of Ferriman–Gallwey hirsutism scoring system (38) and the poor reliability of androgen assay (39). Moreover, 20-40% of women with PCOS have normal androgen levels, in fact some authors wonder if clinical and/or biochemical hyperandrogenism should always be present to diagnose PCOS, since the serum levels of AMH and the number of follicles are considered a substitute for the expression of hyperandrogenism (40).

The reliability of the ultrasound diagnosis of polycystic ovary is also controversial, because it depends strongly on the equipment and the operator, it is not easy to reproduce and it is more invasive than a blood sample. Furthermore, the improvement in ultrasound technology has led to the revision of the previous cut-off value of follicles number to diagnose PCO (41), which could further change in the future. Recently several studies have pointed out that AMH could play a crucial role in the diagnosis of PCOS and may help improve the diagnostic capacity of Rotterdam Criteria. In fact, this hormone is significantly higher in women with PCOS than in healthy women (42,43), reflecting the increased number of early antral follicles.

Furthermore the serum levels of AMH are

positively correlated with the severity of the disease (44).

It also appears that AMH may play a role in the pathogenesis of PCOS, passing through the placenta and influencing embryo development (45,46). Also, as we have already said, AMH is correlated with AFC and also with biochemical hyperandrogenism and according to some authors one can be used instead of the others (47-51). Two studies conducted in our department have analysed the role of AMH in the diagnosis of PCOS, concluding that AMH is more reproducible than AFC and that it can help to identify those women suffering from mild forms of PCOS, better than using only the Rotterdam Criteria (52,53). One of the two studies also identified a serum AMH value for the diagnosis of PCOS equal to 33 pmol/l (4.62 ng/ml), able to predict PCOS with a sensitivity of 95% and a specificity of 95% (52).

The other study conducted in our department also noted that the AMH can help in the diagnosis of the PCOS by reconciling the Rotterdam criteria with the other two often used criteria, those of National Institutes of Health (NIH) and the Androgen Excess and PCOS Society (AE-PCOS), reducing the difference in prevalence of diagnosed PCOS with these different criteria (53). A recent review has analysed several studies that have searched for an AMH cut off value for the diagnosis of PCOS, concluding however that there is too much heterogeneity in the accuracy of AMH in reflecting PCO and in helping the diagnosis of PCOS (54). It also depends too much on age and there is a need for specific cut offs for each age group (55).

Moreover, AMH seems to be more useful in PCOS diagnosis in adults than in adolescents because the hormone levels are higher at this young age, both in the case of girls with PCOS and those who do not have the syndrome (54). This also reflects the controversial use of ultrasound in adolescents for the evaluation of PCO, which is closely related to AMH. In fact the new international guidelines do not recommend ultrasound evaluation in the diagnosis of PCOS until 8 years post-menarche (33-35).

Therefore, AMH can be helpful in the diagnosis of PCOS together with the Rotterdam criteria, but there is a need to standardize AMH assays, improve their accuracy and to identify age specific AMH level cut-offs.

AMH AND ART

Currently AMH is reliably used to predict ovarian response in women undergoing ovarian stimulation for IUI and IVF (56). In fact AMH is a good predictor of ovarian response to ovarian hyperstimulation (10, 57). This led to the use of algorithms to find the right dose of stimulation based on the initial AMH value, reducing both the risk of ovarian hyperstimulation syndrome (OHSS) and POR (58-60). It has also been hypothesized that in women with PCOS AMH may be useful in deciding the initial dose of stimulation to start with, in fact some authors argue that if AMH is high then stimulation should be started with higher doses (61). On the contrary, it seems there is no correlation between AMH serum levels and gonadotropin sensitivity in patients with PCOS, and the only real role of AMH in these patients is to predict the risk of hyperstimulation syndrome, to which they are more exposed (24). Moreover, a recent Cochrane review show that current evidence does not provide a justification for adjusting the standard dose of FSH in the case of poor or normal responders, indeed this would only lead to using a higher total dose of FSH and increasing the costs of stimulation. Instead, a decreased dose in predicted high responders may reduce the risk of OHSS (62). However, like in the case of spontaneous onset of pregnancy, it seems that AMH has little ability to predict pregnancy chances in women undergoing ART (57).

A recent meta-analysis points out that, although AMH levels could be useful in planning a couple's fertility treatment, its predictive accuracy for pregnancies is poor (63). This is because even if AMH has a correlation with the number of follicles that could be fertilized there is no correlation with oocytes and embryos quality (64-66). However, in literature there is still debate on this topic and some studies claim that AMH has a predictive ability for the pregnancy rate (63,67-76), while others do not (77-81). A recent study evaluates the ability of AMH levels, stratified by age, to predict live birth rate in IUI and it concluded that it has a poor predictive value, although they have found a tendency for AMH to be lower in cases of miscarriage and lower pregnancy rates, suggesting that the reduction of the ovarian reserve could be a quantitative but also a qualitative problem (82).

In any case, regardless of the debate in literature, it is certain that the two main factors that influence the success of ART procedures are age and AMH levels (75,83-85) but no AMH value cut-off was identified under which pregnancy was excluded and many different studies have obtained pregnancy with ART in women with very low AMH concentration levels (80,86-88). In conclusion AMH can strongly predict poor ovarian response in ovarian stimulation (89) and it is a useful tool to schedule fertility treatments, but it has little power in predicting pregnancy rate.

MENOPAUSE AND POF PREDICTION WITH AMH

Menopause is defined as the point in time that follows 1 year after the complete cessation of menstruation (90,91), the average age at which it occurs is 51 years, with a range of 40-60 years (92). If cessation of menstruation occurs before age 40 it is called premature ovarian failure or POF (90) and affects 1‰ women of 15-29 years and 1% of women from age 30 up to 39 (93-95). Risk factors are multiple and include hereditary diseases, autoimmune diseases, smoking, alcohol, chemotherapy, ovarian surgery, viruses and others (90,92). POF is diagnosed by two dosage of serum FSH levels, 1 month apart from each other, that measure greater than a threshold range of 30 to 40 mIU/mL (90). The AMH is related to the ovarian reserve and its serum levels decrease with the decrease of this one, therefore it has been hypothesized that it can be used to predict the time of onset of menopause and POF (92,94,96-98). It would be really useful if those hypotheses were confirmed as this could influence women to have pregnancies before the possible cessation of menstrual cycles or apply fertility preservation techniques such as oocyte freezing. In fact, a recent study questioned women about the possibility of performing blood sampling to dose AMH to predict the onset of POF and menopause and women expressed a positive opinion, especially in the case of familiarity for POF (99). Unfortunately, nowadays even if the presence of constant low levels of AMH is a good marker in the diagnosis of POF (92,96,98), the recent developed model to predict the onset of POF and menopause has little accuracy (97,100). In addition, this model involves multiple serial doses over time and requires reliable laboratories to perform the test (101). Moreover, the main issue consists in the absence of a widely accepted cut-off value in AMH serum level to diagnose a decline in ovarian reserve (24). In literature many authors suggest different thresholds, such as 1 ng/ml, but since there is evidence of pregnancies occurring even in women with undetectable levels of AMH, is clear that those thresholds do not predict the chances of spontaneous pregnancy (102,103). A recent study compared the risk of early menopause associated with AMH levels of 1.5, 1.0 and 0.5 ng/ml to an AMH levels of 2.0 ng/ml found out that the risks were respectively 2.6, 7.5 and 23 (104). This highlights that surely the AMH can help in understanding if a woman is going towards the cessation of ovulatory activity but it is not able yet to identify a certain value below which a woman can't get pregnant. Progress in this field could be helpful also in women with Turner syndrome, who are destined to develop POF, since AMH has been found to be higher in those affected women who achieve puberty and represents a marker of the presence of follicle in their biopsied ovarian tissue (105,106). Hence, further studies are crucial to assess if AMH levels dosed at a young age could be used to schedule fertility preservation or pregnancy attempts (10).

AMH ROLE IN MANAGEMENT OF WOMEN UNDERGOING CHEMOTHERAPY, SURGERY AND RADIOTHERAPY

Along with the increase in cancer survival rate after therapy, the need to improve the quality of life of people who survived has also increased. This topic is very important for women of reproductive age, because it is known that radiotherapy, chemotherapy and surgery damage the ovary (107). AMH could be a useful tool in many aspects of cancer treatment and its outcome. Regarding chemotherapy, the use of the hormone to define the decline of the ovarian reserve has been studied for the first time on childhood cancer survivors (108), find out that they have lower serum AMH levels compared with healthy women. A recent study analysed a group of childhood cancer survivors after 10 years, being in their mid-thirties, showing a decrease in AMH levels according to the gonadotoxic effect of the treatment to which they were exposed

(109). In fact the decrease in AMH level was used to establish the ovarian toxicity power of chemotherapeutic agents (110,111).
In women undergoing treatments for breast cancer AMH levels drastically decrease during chemotherapy, becoming undetectable after six cycles of therapy in most women (112), but it seems that there is a limited recovery after 3-6 months in some cases (113). The damage to the ovarian reserve depends on the type of chemotherapy agent and the age at which the therapy started. For example alkylating agents are associated with the highest risk of gonadotoxicity, amenorrhoea and lower recovery of AMH serum levels (114,115). The ovary is also very sensitive to radiotherapy and the damage depends on irradiation field, therapy dosage, fractionation schedule and whether the patient is pre or post menarche (116), but there are few data about changing in AMH level after radiotherapy. However the hormone could play a role in the management of women undergoing chemotherapy and radiotherapy, especially in deciding whether there is a need to apply fertilization preservation techniques (117,118). The pre-therapy levels of AMH are useful for predicting the loss of ovarian activity, especially when combined with age, providing useful information to plan the available fertility options with the patient. In fact higher levels of AMH before therapy combined with younger age brings a lower risk of chemotherapy-related amenorrhoea (112,115,119) and higher chance of restoration of normal ovarian function (120,121).
Moreover, the higher the pre-treatment AMH, the faster it rises again after therapy (122). Instead AMH recovery is lower in older aged women (123). Thus, in cases where the risk of iatrogenic POF development is high, ovarian tissue cryopreservation can be chosen. Regarding surgery for benign ovarian cysts or endometriomas, it has emerged that AMH undergoes a decline 3-6 months after surgery (26,124), but it seems to be statistically significant only in case of endometriomas and the hormone is unable to predict whether an operated woman may or may not have a pregnancy in the future. Furthermore, in the case of endometriotic cysts there was also a recovery of the AMH values at 12 months from the operation (26,125,126). Salpingectomy does not affect ovarian reserve, while unilateral salpingo-oophorectomy obviously lead to a decline in AMH levels, there are few studies that analyse if women with history of unilateral salpingo-oophorectomy experienced an accelerated loss of oocytes and a premature loss of fertility (127). In any case, as we have already said, we cannot use AMH to predict the pregnancy rate and we cannot predict if a woman, even with very low AMH values, will get pregnant. For example, in case of orthotopic transplantation of ovarian tissue AMH levels are undetectable in most women, probably because of a poor vascularization of the transplanted tissue and a loss of follicles during the procedure of implantation, but pregnancies still have been reported anyway (128,129). Moreover, AMH levels may be used in women with granulosa cells tumors, both for diagnosis and for follow up after surgery, because this cells secrete the hormone (130).
Finally, there is a new hypothesis about the role of AMH in the therapy of epitalial ovarian tumors, given its ability to induce the regression of müller duct cells in the foetus (131).

CONCLUSION

In conclusion AMH reflects the ovarian reserve in terms of quantity but not quality of ovarian follicles. It is a useful tool to predict ovarian response to hyperstimulation in women undergoing ART and it can be used to decide the right dose of FSH to start with in order to avoid OHSS or POR. It has a crucial role in the diagnosis of mild cases of PCOS, when using only Rotterdam criteria is not conclusive. In women of late reproductive age, as in those with familiarity for POF, it can help to predict the onset of menopause, although it does not estimate the likelihood of pregnancy in these women. In the case of ovarian surgery, chemotherapy and radiotherapy, pre-treatment serum levels of AMH can help decide whether to consider techniques such as ovarian tissue cryopreservation, while post-therapy levels may indicate damage to the ovarian reserve. In tumors of granulosa cells that secrete AMH, it can be used in diagnosis and post-surgery follow-up. Moreover, new pathways are being studied to use AMH as a therapeutic agent in epithelial cell tumors. In any case, AMH levels are never able to predict a woman's chance of getting pregnant because, accordingly with the most recent literature, it is not marker of fertility

and many cases of women who achieved a pregnancy with very low AMH values were observed. However, further studies are needed especially to identify a reliable assay to be used in all laboratories to make the AMH values, ob-tained in the different centres, comparable.

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