The role of anti-müllerian hormone as a predictor of ovarian function

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Key content
• Levels of anti-müllerian hormone (AMH), which is secreted by the granulosa cells of the ovary, indicate the size of the antral follicle pool.
• Along with other predictors, AMH levels can predict the quantitative response to treatment in women undergoing assisted reproductive therapy.
• The aim of AMH testing in assisted reproductive therapy is mainly to individualise treatment protocols with a view to achieving the optimal ovarian response and oocyte yield.
• AMH has not shown sufficient predictive value for pregnancy outcome to be used in routine practice for either spontaneous pregnancy or following assisted reproductive therapy.
• AMH serves a diagnostic and prognostic role in the management of women with polycystic ovary syndrome.

Learning objectives
• To understand the physiology of AMH and its role in reproductive function.
• To identify the role of AMH as a predictor of fertility and ovarian reserve.
• To understand the role of AMH in assisted reproductive therapy.

Ethical issues
• Is it ethical to make decisions about refusing treatment based on AMH levels?
• Can women be reassured that they can delay childbearing on the basis of their AMH levels?

Keywords
antral follicle count / assisted reproductive therapy / clinical pregnancy rates / follicle-stimulating hormone / in vitro fertilisation / ovarian hyperstimulation syndrome / polycystic ovary syndrome

Introduction
Changing lifestyles and social trends have led to delayed childbearing in many couples. This trend has resulted in an increased incidence of age-related subfertility and a greater demand for assisted reproductive therapy.

Reproductive performance is clearly inversely related to chronological age.1 However, this relationship is not absolute,2 and hence there is a need for other markers of ovarian function. Serum levels of follicle-stimulating hormone (FSH), ovarian volume and antral follicle count are commonly used as indicators, with high FSH levels, low ovarian volume and low antral follicle count all pointing to reduced reproductive potential. Since 2005 measurement of serum levels of anti-müllerian hormone (AMH) has emerged as another powerful tool in the armamentarium available to the clinician.

The aim of this article is to summarise the current available evidence for the measurement of AMH in different clinical settings and to suggest a management plan based on current best evidence.

Physiology
AMH is a glycoprotein belonging to the transforming growth factor β family. In the male fetus it is expressed in the Sertoli cells of the testes, which leads to müllerian regression. In the female fetus it is expressed by the granulosa cells of the ovary from as early as 36 weeks of gestation3 and production continues until the menopause. It is expressed mainly by the pre-antral and small antral follicles,4 declining in dominant follicles and with equivocal expression in atretic follicles, corpus luteum and primordial follicles. AMH is thus a good indicator of the size of the ovarian antral follicle pool.

The primary physiological function of AMH in the ovary is inhibition of the recruitment of primordial follicles into the antral follicle pool.5 AMH also reduces the sensitivity of the growing follicles to FSH.6

AMH shows non-significant intracycle and intercycle variation.7,8 This is an important advantage of AMH over FSH, as it can be reliably measured at any stage of the menstrual cycle. AMH levels remain unchanged in the first
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trimester of pregnancy, but show a decline in the second and third trimesters, with a return to pre-pregnancy levels early in the puerperium. The levels during pregnancy, however, do not become undetectable, indicating that follicular development is not completely abolished.\textsuperscript{9,10} There is also a non-significant variation in AMH levels during short-term oral contraceptive use and short-term gonadotrophin-releasing hormone analogue administration.\textsuperscript{11,12} Long-term use (for more than 1 year) of oral contraceptives and gonadotrophin-releasing hormone analogues can reversibly reduce AMH concentrations.\textsuperscript{10} These studies confirm the presence of continuous ovarian activity independent of FSH stimulation.

The AMH assay

The lack of a single standardised assay for the measurement of AMH has led to much confusion over interpretation and comparison of results. There are two assays that have been used for AMH, with differing values and units. These are:

- the Diagnostic Systems Laboratories (DSL)
- the Immunotech–Beckman.

Studies comparing the two assays showed AMH levels to be five to seven times lower with the DSL assay than with the Immunotech–Beckman assay. However, a single unified assay is now being produced by one company (Beckman Coulter), which will solve the logistical and methodological problems that have been encountered.

AMH values should be interpreted according to the reference ranges of each individual laboratory. Values below the optimal range indicate a low antral follicle pool and hence impaired reproductive capacity. Values above the optimal range are suggestive of polycystic ovary syndrome (PCOS) and a high antral follicle count.

AMH as a test of fertility and ovarian reserve

It is well known that reproductive capacity is closely but variably related to chronological age.\textsuperscript{4} Reproductive capacity is dictated by biological ovarian age/ovarian reserve but this is not always equivalent to chronological age. Reduced ovarian reserve results from a decline in the ovarian pool of follicles. A marker of ovarian reserve which would reliably predict reproductive capacity and the time of onset of menopause would be a significant clinical tool with which to assist women to plan childbearing.

Currently, a combination of age, serum markers (AMH, basal FSH, basal estradiol and basal inhibin), ultrasound markers and challenge tests are used to assess ovarian reserve. Several studies have shown AMH levels to decline normally with increasing chronological age in a non-linear, quadratic manner.\textsuperscript{13–15} This has led to the development of age-specific reference values and normograms. AMH values have shown wide variation at individual ages, with a tendency to be skewed to the left, with the skew increasing with age and median values consistently lower than the mean.\textsuperscript{14} Two large studies\textsuperscript{13,14} have used the same assay and shown very similar results. A third study\textsuperscript{15} showed differing results: this may be because a different assay was used. The authors\textsuperscript{14} acknowledge that the role of factors such as ethnicity and body mass index on these normograms needs evaluation. Further consensus, standardisation and validation of these normograms are needed before they can be put into universal routine clinical practice.

As mentioned before, ovarian reserve may not always match chronological age, leading to deviations from the normogram. These variations may be due to genetic, autoimmune or environmental factors and may or may not be reversible.

Decreased AMH levels are indicative of a reduced antral follicle pool and hence reduced ovarian reserve, independent of age. High levels of AMH are suggestive of polycystic ovaries.

Most studies correlating AMH with reproductive outcome and pregnancy have been in the setting of fertility/in vitro fertilisation (IVF) clinics rather than an unselected population. Although AMH concentrations at the extremes of the scale give a reliable indication of fertility potential,\textsuperscript{16} other values are less reliable for planning pregnancy or predicting the age of menopause.

The use of age-specific reference values and deviations from them should be used with caution in predicting individual reproductive outcome and span.

AMH is shown to be comparable to antral follicle count as a marker of ovarian reserve,\textsuperscript{17,18} but is superior to FSH and inhibin B.\textsuperscript{19,20} AMH (along with antral follicle count) has shown better correlation with the ovarian primordial follicle pool as assessed by histology than markers such as FSH and inhibin.\textsuperscript{21}

FSH values >10 u/l on days 2–5 of the menstrual cycle, antral follicle counts of less than a total of 3 from both ovaries, and ovarian volume < 3 cm\textsuperscript{3} have been found to be associated with reduced ovarian reserve.\textsuperscript{18} Ovarian volume and antral follicle count are accurate markers but have the limitation of needing specialised equipment and skilled operators.

Although there is a good correlation between the age at actual menopause and that predicted by AMH levels, the added predictive effect of the combination of AMH levels and current age of improving the predictive value of age alone is probably its most useful application in this respect at present.\textsuperscript{22,23}

In conclusion, AMH shows the potential to be a reliable marker of ovarian reserve and reproductive performance. We await further standardisation of normograms and studies correlating AMH values with reproductive performance in
the unselected population rather than those attending fertility clinics. This would allow AMH to be used to predict individual reproductive span independent of age in the general population.

**AMH and response to fertility treatment**

Several studies show a strong positive correlation between basal serum AMH levels and the number of retrieved oocytes in women undergoing controlled ovarian stimulation for IVF. In the setting of an IVF clinic, therefore, AMH can be used as a predictor of quantitative ovarian response. Poor or over-response may lead to suboptimal outcome in terms of pregnancy rates. AMH is thus useful in individualising the stimulation protocols for controlled ovarian stimulation in order to optimise the response during IVF. The choice of protocol (long, antagonist, flare and so on) and starting dosages of FSH may be decided based on AMH values. Women with low AMH levels should be started on a higher dosage of FSH than usual for that age; conversely, women with high levels of AMH should have lower dosages than the usual for that age.29,30

AMH has been shown to be a better predictor of quantitative response than age, basal FSH, estradiol and inhibin. AMH and antral follicle count have similar predictive value.27

Approximately 10% of women show a poor response (usually defined as fewer than four oocytes retrieved at egg collection) to controlled ovarian hyperstimulation during IVF,31 which can result in cycle cancellation. Lower pregnancy rates are seen in women showing a poor response than in women of the same age with a normal response.32,33

The effectiveness of AMH as a predictor of poor response has been assessed. In several studies the sensitivity and specificity range between 40–90% and 40–100%, respectively. Most studies show specificities >85%, which makes AMH a good indicator, as it gives few false positive results. Values ranging between 0.1–1.4 ng/ml have been suggested in different studies as a cut-off for predicting a poor response to ovarian stimulation.

The use of AMH in predicting poor response is not without its shortcomings. The predictive value of AMH and the cut-off levels below which poor response can be expected varies in different studies, depending on the study population recruited and the very variable definition of poor response used. AMH has been shown to have similar predictive values as antral follicle count in the prediction of poor response.28

Although women with a poor response generally have lower pregnancy rates than women with a normal response, many poor responders, especially young women, do achieve pregnancies.36,37

On balance, AMH is a good predictor of poor response, independent of age and FSH levels. Prediction of poor response allows the individualisation of stimulation regimens in order to increase/optimise the number of oocytes retrieved. Several strategies can be employed for this, which are beyond the scope of this article. Prediction of poor response is also useful for the appropriate counselling of couples regarding the response to treatment, thus avoiding distress and disappointment.

There are enormous implications to refusing treatment to a couple based on estimated AMH values as a predictor of poor response. Current evidence has not yet yielded cut-off values below which treatment can be judged as being futile, therefore AMH should not be used as an indicator for refusing fertility treatment to women until further data become available.

**Ovarian hyperstimulation syndrome**

Between 15–20% of women undergoing controlled ovarian hyperstimulation for IVF have mild to moderate ovarian hyperstimulation syndrome (OHSS) and 1–3% have severe OHSS requiring hospitalisation.38

OHSS is distressing for the woman and can progress to a severe form which requires hospitalisation: this is potentially fatal and can result in serious complications such as deep vein thrombosis, pulmonary embolism, pleural effusions and renal failure. Pregnancy can exacerbate the symptoms, hence a fresh embryo transfer may be avoided in that cycle in order to facilitate recovery; this, however, is a suboptimal outcome in terms of pregnancy, as subsequent frozen embryo transfers have lower pregnancy rates than fresh embryo transfers.

OHSS may be avoided or its severity decreased by several strategies used to manipulate the stimulation regimen. Some of these include changing the antagonist protocol and lowering the starting dosage of gonadotrophins in women of a high potential for developing OHSS.

OHSS may be predicted by several risk factors, the most important being PCOS, young age and low body mass index. About 20% of women undergoing controlled ovarian hyperstimulation have polycystic ovaries and, therefore, the potential for OHSS.39,40

The role of AMH levels and over-response/OHSS has been investigated in studies which show high basal AMH levels to be a strong predictor of over-response. These studies have also reported cut-off levels of AMH above which over-response and OHSS may be predicted with high sensitivity and specificity. Values in the range of 3.5–5.0 ng/ml have been quoted in different studies.27,34,41

AMH is a predictor of OHSS independent of age and PCOS and is reported to be superior to age and body mass index. Its practical application is in altering the stimulation protocol in women with a high potential for developing OHSS, with the possibility of preventing it and achieving an optimal pregnancy outcome.
The relationship between AMH and pregnancy rates

The role of AMH in predicting the qualitative response to treatment and its value in the prediction of pregnancy remains unclear. Prospective studies have shown a positive correlation between serum AMH levels and clinical pregnancy rates.\(^4\) Some of these studies had small numbers, therefore the findings need to be corroborated by larger studies. Also, the improved pregnancy rates associated with high AMH levels positively correlated with a higher yield of oocytes in these women. Studies were unable to show a positive relationship between serum AMH and pregnancy rates following IVF treatment when considered independent of the oocyte yield.\(^4\) Levels of follicular fluid AMH have been shown to correlate positively with pregnancy rates.\(^4\)\(^\)\(^5\)\(^\)\(^6\)\(^\)\(^7\)\(^\)\(^8\)

In conclusion (see Box 1), the relationship between AMH and pregnancy rates may be indirect and due to the strong and positive correlation between AMH and the number of oocytes retrieved. Current evidence does not allow AMH to be used as an independent predictor for pregnancy and further studies are needed.

**Box 1. Applications of anti-müllerian hormone in fertility practice**

- Predicting over-response to controlled ovarian hyperstimulation/ OHSS
- Altering stimulation protocols to prevent/minimise the chance of OHSS
- Predicting poor response to controlled ovarian hyperstimulation
- Altering stimulation protocols to optimise oocyte yield in predicted poor response
- Counselling couples about poor response to avoid distress/ disappointment

AMH and polycystic ovary syndrome

Polycystic ovaries are characterised by an increased number of follicles and altered folliculogenesis from very early stages. Several studies\(^4\)\(^6\)\(^7\) have clearly demonstrated that women with PCOS have high levels of basal AMH, which can be used as an independent diagnostic indicator for PCOS, especially where facilities for ultrasound and antral follicle count are not available.\(^4\)\(^8\) Raised AMH levels in PCOS were initially thought to be due only to greater antral follicle numbers, but studies have shown greater AMH production per granulosa cell and per antral follicle.\(^4\)\(^9\)

The cause of increased AMH production by individual granulosa cells in the polycystic ovary is still unknown, with several mechanisms having been proposed, including excess androgens or insulin, or simply an intrinsic dysfunction.

In women with PCOS, high androgen levels are seen in association with high AMH concentrations, as opposed to the normal androgen levels in women with normal AMH levels.\(^3\)\(^2\)\(^,\)\(^4\)\(^7\) However, a reduction in androgen levels does not appear to lower AMH levels.\(^5\)\(^0\)

Some investigators have found a correlation between insulin insensitivity and AMH levels,\(^5\) which has not been confirmed by others.\(^4\)\(^2\)\(^,\)\(^7\) A therapeutic reduction in insulin levels failed to reduce AMH levels.\(^5\)\(^0\)

The intrinsic overexpression of the AMH gene has been suggested, but the cause of increased levels of AMH remains elusive.

In women with PCOS, AMH can be used as:

- a diagnostic marker for PCOS
- a prognostic marker for ovulation induction and fertility treatment.

Women with PCOS can be subdivided into two distinct groups, which do not overlap, based on AMH levels:\(^4\)\(^9\)

- Anovulatory women, who have very high AMH levels, associated with high androgens and insulin insensitivity. These women are more likely to respond poorly to clomifene citrate or FSH stimulation during ovulation induction treatments and have a poorer prognosis for these treatments.
- Women with lower levels of androgens and better insulin sensitivity. They are often ovulatory, respond better to ovulation induction treatments and have comparatively lower levels of AMH.

Conclusion

AMH shows the potential to be a reliable marker of ovarian reserve and reproductive performance. It is a good predictor of poor response to fertility treatment, which can allows the individualisation of stimulation regimens; it can also be used to alter the stimulation protocol in women with a high potential for developing OHSS. Current evidence does not allow AMH to be used as an independent predictor for pregnancy and further studies are needed. In women with PCOS, AMH can be used as a diagnostic marker and as a prognostic marker for ovulation induction and fertility treatment.

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