Anti-Müllerian hormone—is it a crystal ball for predicting ovarian ageing?

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**Abstract:** Several studies have demonstrated that anti-Müllerian hormone (AMH) is a better marker of ovarian reserve than age, basal FSH, estradiol and inhibin. AMH is very good in (i) predicting both over- and poor-response in the controlled ovarian stimulation environment, (ii) determining the most appropriate stimulation regimen and (iii) pre-treatment counselling for couples to make an appropriate and informed choice. Recent reports are exploring the use of AMH in various other indications, including (i) predicting long-term fertility and guiding how long a woman can delay childbearing without facing the risk of reduced ovarian reserve, (ii) predicting the age of menopause, (iii) prediction of ovarian ageing in women prior to or following chemotherapy, (iv) prediction of long-term fertility following ovarian surgery and (v) screening for polycystic ovaries. However, widespread use of AMH for indications not proved by evidence-based medicine can lead to either false reassurance or distress, leading to unnecessary medical interventions. It also has huge implications for costs. We evaluated the evidence basis for using AMH for various indications to decide how justified it is to promote AMH as a crystal ball, until more evidence is available.

**Key words:** AMH / screening test / ovarian ageing / ovarian reserve

Introduction

The anti-Müllerian hormone (AMH), also known as the Müllerian-inhibiting substance, is a peptide growth factor and a member of the tissue growth factor beta superfamily (Visser and Themmen, 2005). In males, Sertoli cells in the testis produce AMH, which signals the regression of Müllerian duct system (Visser and Themmen, 2005), while Leydig cells in the testis produce testosterone, which stimulates the differentiation of Wolffian ducts into epididymides, vasa deferentia and seminal vesicles (Visser and Themmen, 2005). In females, the Müllerian ducts differentiate into the oviducts, uterus and upper part of the vagina in the absence of AMH (Lee and Donahoe, 1993).

AMH is first produced in females by the granulosa cells from the pre-antral and antral follicles in the human foetus after 36 weeks of gestation (Durlinger et al., 1999; Rajpert-De Meyts et al., 1999; Weenen et al., 2004). AMH continues to be expressed until the follicles reach a size of ~4–6 mm, a differentiation state at which they become receptive for exogenous FSH (Weenen et al., 2004). Some reports state that the responsiveness to exogenous FSH starts more probably around 2 mm (Gougeon, 1996).

What is ovarian ageing?

Ovarian ageing is a reduction in the quantity and quality of the oocytes in the ovaries. Although the average age of menopause is 51 years, approximately 1 in 10 women reach menopause before the age of 45 and approximately 1 in 100 women reach menopause prior to the age of 40 years. It has been postulated that fertility reduces 13 years before menopause, i.e. 1 in 10 women will have reduced fertility by the age of 32 years (Nikolaou and Templeton, 2003). A series of ovarian reserve tests have been used to predict ovarian ageing or poor ovarian reserve. The main purpose of these tests is to determine reduced fertility at a stage when appropriate treatment can be instituted.

AMH as a test of ovarian reserve

Several studies have demonstrated that AMH is a better marker of ovarian reserve compared with age alone or other markers described in the literature such as basal FSH, estradiol and inhibin B (La Marca et al., 2010). AMH is a serum blood test with a stable value obtainable throughout the menstrual cycle, which is a major advantage over other markers such FSH and inhibin, which can only be done at a certain time in the menstrual cycle. In addition, being a blood test, it has advantages over ultrasound (USG) based on antral follicle count (AFC), as trans-vaginal ultrasound is considered an invasive examination. AMH is used most commonly in an IVF setting, being routinely used in certain centres (Barad et al., 2011).

What can AMH predict in an IVF setting?

Approximately 2–30% of women responded poorly to controlled ovarian stimulation (COS). Poor responders have lower rates of
pregnancy compared with normal responders of a similar age (Klinkert et al., 2005; Galey-Fontaine et al., 2005). As a result, couples experience significant psychological stress, which can be avoided by appropriate pre-treatment counselling with the easily available information obtained by AMH (Broer et al., 2009).

On the other end of the spectrum is ovarian hyper-response, which leads to exaggerated ovarian response, called the ovarian hyperstimulation syndrome (OHSS) (La Marca et al., 2010). OHSS range from mild to moderate forms, which occurs in 15–20% of all ovarian stimulation cycles, and to severe form, which occurs in 1–3% of all ovarian stimulation cycles. In addition to producing poor-quality oocytes, hyperstimulated cycles can lead to multi-organ dysfunction. Hence its prediction and therefore modification of stimulation accordingly is of utmost importance in an IVF treatment (La Marca et al., 2010). Basal AMH levels predict OHSS with a sensitivity and specificity of 90.5 and 81.3%, respectively (Lee et al., 2008).

Hence AMH is useful for determining the most appropriate stimulation regimen as well as pre-treatment counselling for couples to make appropriate informed choices, being very good in predicting both over and poor response in the COS environment. It should be noted that AFC is equally good at predicting poor (Broer et al., 2009) and over response. The sensitivity and specificity for predicting over response for AMH are quoted as 82 and 76%, respectively as compared with 82 and 80%, respectively, for AFC (Broer et al., 2011a).

**What AMH cannot predict in an IVF setting?**

Despite being a good marker of ovarian response, AMH fails to predict who will get pregnant (Lamazou et al., 2011; Riggs et al., 2011). This is because AMH or other ovarian reserve tests cannot predict the quality of oocyte. Moreover, a lot more factors (such as age, cause, duration of infertility) are involved, when it comes to prediction of who is going to get pregnant.

Extremely low levels of AMH are associated with non-pregnancy (La Marca et al., 2011), although there have been reports of moderate and reasonable pregnancy rates following extremely low levels of serum AMH (Lamazou et al., 2011; Weghofer et al., 2011) and it has been argued that low AMH should not form the sole basis of denying women ART.

It has been claimed that AMH can provide women with the odds of live births (La Marca et al., 2011). However, looking at the confidence interval (CI) of these studies, several limitations became apparent, i.e. (i) the CI are wide, and (ii) the values were overlapping with the normal values. In addition, such low levels (<0.4 g/dl) of AMH are unusual in women with regular menstruation.

Despite this, media is publicizing AMH as the test to predict pregnancy in an IVF cycle (http://articles.timesofindia.indiatimes.com/2011-06-10/health/29642169_i_ivf-hormone-researchers/).

**AMH testing outside IVF setting**

With success in predicting poor and over response in an IVF setting, better than other markers, measurement of AMH is increasingly recognized to be of clinical value in a range of settings, including polycystic ovarian syndrome (PCOS) and assessment of ovarian reserve following cancer treatment (Nelson et al., 2011). In addition, it has also been used as (i) predictor of how long women can safely delay pregnancy without facing the risk of sub-fertility; (ii) determining age of menopause for an individual and (iii) predictor of damage to ovarian tissue after surgery. We evaluate the evidence basis for using AMH for all these indications and decide how justified it is to promote AMH for these indications.

**Predictor of long-term fertility**

AMH is widely promoted as a predictor of long-term fertility throughout the world (http://www.dailytelegraph.com.au/news/sunday-telegraph/quick-fertility-test-reads-biological-clock/story-e6frewt0-1225832548837) (http://news.bbc.co.uk/1/hi/health/4642698.stm). Similar reports have been published in other parts of the world. Fertility is defined as the ability to produce offspring. There is only one study exploring AMH for natural fecundability in women of reproductive age (Steiner et al., 2011) and concluded that early follicular phase AMH appears to be associated with natural fertility in general population. However, this study was based only on 100 women and had a short follow up of 6 months. They have not even tested if there was any evidence of tubal or male factor infertility. Moreover, odds of pregnancy had very wide CI (0.08–0.91) to be convinced about the results.

A normal AMH level may be reassuring at the time but we do not know how long will it take for AMH to decline in an individual. Moreover, normal AMH does not guarantee conception even in an IVF setting, let alone general population. It is also unclear as to how frequently AMH levels should be tested and at what level should one be worried about rushing for interventions for fertility treatment. Unless these questions are answered and we have a validated long-term data on a general population for prediction of conception, it is premature to use AMH as a measure of long-term fertility (Broekmans et al., 2006).

AMH does not fulfill the attributes of an ideal screening test (Table I). In addition, ovarian ageing does not fulfill the criteria for a disease for which a screening test can be developed. This is because

### Table I  **WHO criteria for screening**

<table>
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<th>Criteria</th>
<th>Description</th>
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<td>The condition sought should be an important health problem for the individual and community</td>
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<td>There should be an accepted treatment or useful intervention for patients with the disease</td>
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<td>The natural history of the disease should be adequately understood</td>
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<td>There should be a latent or early symptomatic stage</td>
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<td>There should be a suitable and acceptable screening test or examination facilities for diagnosis and treatment should be available</td>
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<td>There should be an agreed policy on whom to treat as patients</td>
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<td>Treatment started at an early stage should be of more benefit than treatment started later</td>
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<td>The cost should be economically balanced in relation to possible expenditure on medical care as a whole</td>
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<td>Case-finding should be a continuing process and not a once and for all project</td>
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neither the natural history of ovarian ageing nor the rate of decline of AMH in an individual female is known. Hence, AMH is unable to predict who will experience early ovarian ageing. AMH is not a screening test for ovarian reserve (Maheshwari et al., 2009), but a diagnostic test for poor ovarian reserve. Unfortunately, by the time poor ovarian reserve is diagnosed, it is too late to provide any fertility treatment using one’s own eggs.

**Prediction of age of menopause**

It has been suggested that premature ovarian failure and early ovarian ageing were associated with very low or undetectable serum AMH levels (La Marca et al., 2006, 2009 de Koning et al., 2008; Knauff et al., 2009). AMH declines with age and continues to decline until it reaches a stage where the serum levels become undetectable after menopause (Rooij, 2004; La Marca et al., 2005; van Rooij et al., 2005; Robertson, 2008; Shin et al., 2008, van Deldorp et al., 2008). It has also been suggested that AMH is the only marker of ovarian reserve showing a mean longitudinal decline over time and that a single AMH measurement may be a good predictor for the onset of menopause in ageing women (van Rooij et al., 2005; Sowers et al., 2008; Tehrani et al., 2009). However, these predictions were tested in small studies and are only valid for women in their mid- and late 1940s, who have bypassed the age for fertility. Although AMH declines with age, the rate of decline is unknown in an individual.

Recently, an age-related normogram for AMH values has been produced but: (i) it is valid only in infertile population (Almog et al., 2011; Shebl et al., 2011), (ii) it is based on cross-sectional data and (iii) it shows a marked variation in the normal values in this population. It is progress in the right direction, but does not have robust enough evidence, yet, for use in routine clinical care to pinpoint exact age of menopause for an individual.

Some data are available from long-term studies as well (Broer et al., 2011b), although on small numbers only, claiming that by using age and AMH, the age range in which menopause will subsequently occur can be calculated individually. It is interesting to note that despite a very low level of AMH (0.33 ng/ml) in young population (age 20–22 years), the median-predicted age at menopause is 48.8, which is not much earlier than average age of menopause at 51 years. This is a classic example of where there are conflicts between statistical and clinical significance. Moreover, it is interesting to note that smoking, which is a well-known predictor of early ovarian ageing, did not have a significant effect in this study.

Other studies have done three measurements of AMH over a time and suggested that AMH measurements in each individual woman could be used in the prediction of age of menopause (Tehrani, 2011b). However, this study is not powered to detect early menopause in younger women. One of the primary clinical values of an AMH measurement (if proven), however, is the potential to predict primary ovarian insufficiency in women younger than 40. There were only 266 study participants with the age range of 20–55 years. This study finding can be applied only to naturally fertile and late reproductive-aged women (between 40 and 50 years) with regular menstrual cycles. In addition, authors themselves claim that their prediction cannot be used for infertile women or those with irregular and unpredictable menstrual cycles because their ovarian ageing process is likely to be affected by hormone disturbances. The utility of AMH testing will continue to be limited until it can reliably predict the age of menopause in the general population, including those who might have been exposed to one or more of the risk factors of diminished ovarian reserve.

Although the current data are intriguing, advance our knowledge and show great promise, it is not yet at a stage where we are able to use AMH testing in routine clinical practice.

**Use in onco-fertility**

With the presumption that AMH is a convenient and non-invasive marker to assess the long-term fertility, AMH is currently being promoted for determining long-term impact of chemotherapy (http://www.medicalnewstoday.com/releases/227540.php).

It has also been recommended as a marker to decide who needs fertility preservation strategies (Decaner et al., 2009; Lie Fong et al., 2009).

It has been suggested that AMH levels are reduced after chemotherapy (Lie Fong et al., 2008; Lie Fong et al., 2009). Decaner et al. (2009) have compared two chemotherapeutic regimens and have shown that the level of AMH decreases immediately after chemotherapy but rises afterwards in some regimes, and suggested that the analysis of AMH during chemotherapy highlights differences between protocols that could contribute to an understanding of ovarian toxicity. However, it is not known whether there is any difference in long-term fertility in those exposed to both these regimes. It is also not known as to how frequently these levels should be monitored. It is interesting that AMH has not been shown to predict fertility in the general population, but it is used to give false reassurance and information to these individuals in this era of evidence-based medicine.

A recent study (Anderson and Cameron, 2011) indicated that AMH can be used as a direct marker of ovarian reserve to predict long-term ovarian activity after chemotherapy. However, this study was limited in size and was not supported by other previous studies (Yu et al., 2010). Therefore, further larger studies will be required in due time. Such information will be of great value to women suffering from breast cancer as well as other malignancies, and also to aid healthcare professionals in determining the need for additional therapies, including fertility preservation strategies. But they have to be done using right end points and outcome measures as it is well known that the return of menstrual cycle is no assurance of return to fertility. Several studies on onco-fertility patients used absence of chemotherapy-related amenorrhea as a predictor of fertility, which is a surrogate marker.

Chemotherapy rapidly decreases ovarian reserve markedly. Although the ovarian secretory function may recover to some extent, and menses may commence after the completion of chemotherapy treatment, ovarian reserve will remain persistently affected. Neither the baseline nor the changing levels of AMH can predict the return of normal menstrual function in women after chemotherapy, suggesting that ovarian reserve and endocrine function may be affected or recover differently after chemotherapy. Even in young cohort studies, age remains an important factor in the recovery of endocrine function. Return of menses is a surrogate sign and may not equate to return of fertility (ability to produce offspring).
Furthermore, American Society of Clinical Oncologists guidelines recommend that oncologists discuss possible fertility preservation options or refer their patients to a reproductive specialist. One could argue that with improved techniques and the success of oocyte cryopreservation (Smith et al., 2010), all women irrespective of their AMH levels should be offered fertility preservation. This is all the more important as one does not know whether a patient of their AMH levels should be offered fertility preservation. This is useful in the prediction of ovarian response to clomiphene citrate (El-Halawaty et al., 2007).

AMH is also being suggested as a screening test for PCOS, which is an advantage for prepubertal girls as it is a non-invasive blood test compared with USG. However, in a recent study on Chinese girls, the best compromise between specificity (70%) and sensitivity (61.7%) for diagnosis of PCOS was obtained with a cut-off value of 8 ng/ml. The area under the ROC curve for AMH was only 0.664 (Li et al., 2010).

Currently, AMH is not part of the Rotterdam criteria for the diagnosis of PCOS. It is unclear whether the higher AMH levels in PCOS are due to the higher number of preantral follicles or a result from a specific disorder in the synthesis of AMH causing follicular arrest in PCOS. (Pehlivanov and Orbetzova, 2011).

AMH was shown to be a useful parameter for functional androgenization classification and it has been recommended that it should be added as a primary variable in this stratification system (Wetzka et al., 2011) in women with polycystic ovaries. However, in other studies, it did not predict insulin resistance in women with PCO ovaries (Lin et al., 2011). One should note that despite Rotterdam consensus, it is still debated whether clinical and/or biological indices of hyperandrogenism (HA) should be present to qualify a patient as having PCOS (Dewailly et al., 2009). Although it may be beneficial for prepubertal girls, AMH has limited value in its use for infertility and in the Menstrual/Gynaecology Clinics, where trans-vaginal ultrasound scan is routinely used and can readily diagnose polycystic ovaries with greater accuracy.

The use of AMH measurement as a diagnostic criterion in substituting/ adding to AFC with regards to the definition of PCOS should be tested in well-designed large trials. In addition, further studies are needed to evaluate the use of AMH in early identification of adolescents at risk for PCOS. One should bear in mind that there will be an overlap with the normal values as AMH levels decrease with smoking (Plante et al., 2010) and increase in overweight and obese with or without PCOS present. It should also be noted that AMH is elevated in PCO but does not signify better ovarian reserve, as the quality of oocytes in PCO is poorer compared with non-PCO ovaries.

**Costs involved**

AMH is a blood test that requires a calibrated laboratory set up. It is hardly used by other departments except Reproductive Medicine Units. Therefore, it is not justified to develop AMH testing in laboratory in smaller units, and sending to other units will add to the costs. In contrast, USG is the essential part of providing Reproductive Medicine Services; hence the opportunity costs of AFC determination are negligible, especially when compared with establishing a new service in laboratory. In fact, one cannot do without USG for monitoring ovarian stimulation. There is no difference in AFC and AMH in predicting the outcomes of stimulation and treatments in Assisted Conception Treatments (Broer et al., 2009). As a result, it is difficult to justify additional costs for AMH tests in Reproductive Medicine set up.

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**Predicting damage to ovarian tissue after surgery**

It has also been acknowledged that AMH is sensitive hormonal serum marker of the ovarian primordial follicle pool and that the changes in AMH level may be an early, reliable, direct indicator of declining ovarian function after surgery (Chang et al., 2010; Tsolakidis et al., 2010; Ercan et al., 2011; Hirokawa et al., 2011; Kitajima et al., 2011). These studies are obviously done on the presumption that AMH is a perfect test for predicting ovarian ageing; hence, further exploration with this perfect test is needed to see the effect of surgery on ovarian reserve. Surgery on ovarian cysts is becoming more common with the widespread use of laparoscopy and hence the use of diathermy to control bleeding, which can cause damage to the follicles and reduce ovarian reserve. It is obvious that diathermy application and instrumentation to ovaries will certainly reduce the number of existing primordial follicles but to a variable extent.

Although some authors (Hirokawa et al., 2011; Kitajima et al., 2011) recommend AMH to diagnose the extent of ovarian damage post-operatively in order to predict the future fertility, there is a lack of evidence supporting this. Moreover, AMH is not yet a test for the prediction of future fertility in the general population. Data on spontaneous or treatment-related pregnancy post-operatively do not exist to guide us with the values to determine when intervention is needed. This may just provide a false reassurance to patients. On the other hand, if AMH level is low post-operatively, it will be too late to correct the damage but it does not completely exclude spontaneous or treatment-related pregnancy.

What clinical implications are there for patient counselling and further management of slightly reduced AMH levels immediately post-operatively? When should they be repeated? There are definitely no clear answers to these questions.

In fact as a good medical practice, any surgical procedures should only be performed where indicated and by experienced professionals, especially where future fertility is of major concern. These women should be counselled about the possibility of reduced ovarian reserve following any surgery performed on them.

**AMH to screen/diagnose polycystic ovaries/PCOS**

It has been suggested that AMH measurement offers a relatively high specificity and sensitivity (92 and 67%, respectively) as a diagnostic marker for PCOS (Pigny et al., 2006). It has been proposed that in situations where accurate ultrasound data are unavailable, AMH could be used as a diagnostic criterion for PCOS instead of AFC (Pigny et al., 2006). AMH measurement has also been used to monitor the therapeutic response in PCOS patients (Moran et al., 2007). Studies have also suggested that basal AMH levels may be useful in the prediction of ovarian response to clomiphene citrate (El-Halawaty et al., 2007).
Figure 1  Potential uses of AMH. *Definite evidence available.
Conclusion

The discovery of ART has given many couples the opportunity to conceive despite impaired fertility. It will be ideal to have a marker that can predict who can and cannot conceive spontaneously or under treatment without facing problems in the long run. As a result, AMH has emerged as a suitable marker for ovarian function in women receiving assisted conception. However, it has limitations as to what it can and cannot predict. It is important that we acknowledge those limitations rather than using it indiscriminately to provide false reassurances or discouragements to women and young girls in the era of evidence-based medicine.

Although AMH emerged as the most accurate and stable marker compared with other tests available, further extensive information is required before it can be used as a crystal ball to predict fertility spontaneously, post-chemotherapy or subsequently to IVF.

This test may have the potential for all the explored indications. In fact, AMH has superseded all other tests of ovarian reserve and has become a routine test in some fertility clinics; however, let us prove its validity (both external and internal) and reliability for all the indications prior to allowing commercial interests and media making claims of its usefulness.

It seems that we have rushed in to explore the uses of this test without confirming its validity to determine fertility, i.e. the ability to produce offspring. Let us take a step back and first find a perfect screening test and then use that test to predict what all it can predict and acknowledge that currently it is only very good at predicting poor and over response in an IVF setting (Fig. 1), whereas other uses need more work.

Authors’ roles

J.L. did the literature searches and wrote the initial draft. A.M. conceptualized the idea, cross checked the literature searches and edited the manuscript.

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