# The practical role of anti-Müllerian hormone in assisted reproduction

**C. Siristatidis[1], M. Trivella[2], C. Chrelias[1], N. Vrachnis[3], A. Drakeley[4], D. Kassanos[1]**

*[1]Assisted Reproduction Unit, Third Department of Obstetrics and Gynecology, University of Athens, "Attikon" Hospital, Chaidari*
*[2]Centre for Statistics in Medicine, University of Oxford, Botnar Research Centre, Oxford*
*[3]Second Department of Obstetrics and Gynecology, University of Athens, "Aretaieion" Hospital, Athens*
*[4]Hewitt Centre, Liverpool Women's Hospital, Liverpool (United Kingdom)*

**Summary**
The objective of this study was to offer a brief critical summary of the literature on the role of AMH in the subfertility work up and during ART, while exploring its role in predicting ART success.

*Key words:* IVF/ICSI outcome; Ovarian reserve; AMH/ART.

## Introduction

The primary goal in assisted reproduction is the continuous improvement of the "take home baby" rate. It would be greatly aided by the ability to anticipate how a woman will respond to ovarian stimulation and to predict her chances of pregnancy. The ideal way to achieve this would be to acquire advanced knowledge, that is, to be able to predict the response before a woman enters the cycle of multiple assisted reproduction technology (ART) - especially in vitro fertilization (IVF) - attempts. A meticulous pre-treatment workup would help, but only if a prognostic marker were available. Despite extensive research in the area, such a marker remains elusive [1].

Over the last ten years or so, anti-Müllerian hormone (AMH), has being investigated as a putative marker [1,2]. AMH is a dimeric glycoprotein, acting on tissue growth and differentiation. AMH has shown great potential as a prognostic marker of ovarian reserve and the ability to identify both extremes of ovarian stimulation [2]. Theoretically, AMH could help to dynamically facilitate the planning of women's reproductive life in addition to predicting for whom IVF treatment is more likely to work [2]. There is no reliable proof though that it can directly contribute to assisted reproduction's primary aim, the "take home baby" rate, hence in this context it isn't an efficient marker in its own right [3,4].

### *Current clinical value of AMH*

In clinical practice, AMH is useful in the prediction of poor response and also of hyper-response during ART [2,3,5]. It can additionally provide useful information on the risk of pitfalls during ovarian stimulation for ART, thus saving couples time and heartache, and guiding them fast to the justified "next step" decision of acquiring oocyte donation or adoption.

Many researchers, using a variety of statistical methods, have attempted to determine significant AMH measurements cut points for pregnancy and live births:

Gleicher *et al.* [3] used receiver operating characteristic (ROC) curves and reported that a uniform cut-off value for significantly improved live-birth rates independent of age stands at AMH = 1.05 ng/ml, with values of AMH ≤ 0.04 and 0.41 - 1.05 ng/ml relating to very low and increased pregnancy potential, respectively. Crucially, the authors did not report on which day in the stimulation cycle was AMH measured. Kini *et al.* [4] instead of reporting cut points, compared retrospectively the median AMH levels between women who achieved cumulative ongoing pregnancy and those who did not. They found that in the former, the median AMH level at day 6 was significantly higher. Gnoth *et al.* [5] employed discriminated analyses and used a calculated cutoff point based on minimized false positive and false negative results, concluding that levels of ≤ 1.26 ng/ml were highly predictive of poor ovarian response. In patients with PCOS, Kaya *et al.* [6] reported that the best day-3 AMH cut-off values for fertilization and clinical pregnancy rates were reported at 3.01 and 3.20 ng/ml, respectively, with the sensitivity and specificity of the method exceeding 72% for both. However, the study included only 60 patients and the analysis had *a priori* divided the sample into three groups using the 25$^{th}$, and 75$^{th}$ percentiles as cutpoints. Similarly, Xi *et al.* [7] used these cutpoints and proceeded to make group comparisons of reproductive outcomes in 164 polycystic ovarian syndrome (PCOS) patients.

It would be ideal if derived cut points for early detection of reduced ovarian response were available to clinicians, so that they could advise appropriately and guide the decision-making of treatment options. Unfortunately, none of these techniques stands up to statistical scrutiny for a variety of reasons; the statistical analysis of these is beyond the scope of this communication. Furthermore, different analytical strategies render any comparisons unfeasible.

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In terms of AMH's power to qualitatively assess the response to ovarian stimulation and the outcomes of ART, the literature is contradictory. While a positive correlation between AMH levels in the serum (weaker) or follicular fluid (stronger) with oocyte quality and embryo morphology [2,8] has been reported, the relationship has not been confirmed by others [9].

In summary, the available data on the relationship between AMH and pregnancy prediction are of limited value. This is not surprising since, clinically, there is no known marker reflecting directly the oocyte quality and the ensuing embryo. It is not straightforward to delve into such a relationship as there are a number of parameters involved, the interplay of which is not yet fully understood. So far it can only be quantified retrospectively following a live birth. The clinical value of AMH is certainly getting stronger, but a clinical model based solely on AMH is unlikely to be developed. An ideal strategy would be a systematic review and meta-analysis of all prediction studies, but given the current variability in reporting, this does not seem feasible.

*The power of AMH in predicting outcomes*

From the hormonal tests, AMH's assumed superiority lies on the fact that it directly reflects the number of pre-antral follicles and the earlier stages of follicle development [2,4,10]. Together with antral follicle count (AFC), AMH is considered as the marker with the highest biological plausibility for ovarian reserve [2,11] and demonstrates less individual intra- and inter-cycle variation. However, when predicting poor or high response and pregnancy rates, it has demonstrated a sensitivity of 76% and a specificity of 86% in sub-fertile couples [2,3].

Broekmans *et al.* [1] carried out a comprehensive systematic review of each available putative marker, both separately and as part of a model, with respect to three outcomes of interest; accuracy of poor response prediction, accuracy of non-pregnancy, and clinical value. They found that no marker was significantly better than another, and where models were involved it was not possible to calculate individual model summary statistics for meta-analysis as each model was constructed in a different way, and/or inadequate levels of sensitivity and specificity were chosen. The models, as always, were especially poor in predicting pregnancy.

AMH shows limited power in predicting pregnancy. Surprisingly, a recent retrospective analysis showed that with extremely low serum AMH levels, moderate, but reasonable pregnancy and live birth rates are still possible, indicating that even in the presence of extremely low AMH levels, ART should not be withheld [12].

## The future role of AMH

*Individualization of ART stimulation protocols with or without modeling*

With an increasing number of women delaying motherhood until their thirties, there is a growing need for simple, low-cost biological markers that can offer individual guidance on when is best to plan a family. The future clinical role of any of these markers may be found in the individualization of ART stimulation protocols [13,14]. It is behind this novel field of personalization of treatment that the desired rise in ART outcomes may be hidden. A prospective cohort study by Nelson *et al.* [13] demonstrated the capability of AMH alone in individualized treatment strategies for ovarian stimulation, resulting in reduced clinical risk, optimized treatment burden and maintained pregnancy rates. Similarly, a more recent retrospective study of 769 women receiving IVF, found that individualized protocols resulted in reduced adverse effects and costs [14]. In this respect, AMH appears to have an important role to play. This may even comprise a multitasking role, ranging from helping to discriminate between non- or hyper-response, cycle cancellation, and ovarian hyperstimulation syndrome, to regimen, dose and protocol formation, and possible alteration throughout cycles.

This individualized approach is perhaps a superior avenue not only for utilizing to the maximum AMH's characteristics, but also involving a number of other markers that hitherto proved inadequate prognosticators on their own; woman's age, the hormone-based FSH blood test, estradiol and inhibin B, the ultrasound markers AFC, ovarian volume and blood flow, the clomiphene citrate challenge test, the exogenous FSH and the gonadotropin agonist test from stimulation tests [1]. This arsenal of ovarian reserve and outcome prediction tests, along with AMH has, without much success, been put through its paces using a variety of statistical techniques, often of questionable robustness, either in a univariate or a multivariable setting [2,15-18]. Especially, worrying is the use of a priori chosen cut points in ROC curves, multivariate analyses adjusting for a multitude of combinations of markers (from the list mentioned earlier), discriminant analysis, and adjusted logistic regression. However, there is extensive literature warning against adopting random categorizing levels, or those yielding the best *p*-value [19]. Hence, in this respect individualized models, evolved through a validated process, may well be the best both biologically and statistically.

Finally, construction of new mathematical architectures based on artificial neural networks seems promising. AMH could serve as one of the trustworthiest input factors to build the network, which after proper training could raise the predictive power of the whole model [20]. However, at the moment attempts to combine individual markers into suitable models with, or without AMH, have also proved inconclusive.

*Treatment denial*

There is a lack of adequate data in defining when and how women need to start worrying for their fecundity and runs in parallel to the uncertainty of whether and when medical staff should deny treatment based on AMH values. It has been proposed that AMH should be used only with

very low cut-off values in order to minimize the occurrence of false positive tests [4,13]; in addition, the added value of AMH assay to chronological age is minimal [2,3], although reports are relating it with diminished ovarian reserve in young women [21].

## Conclusion

The current literature of prognostic factors in assisted reproduction is rather diverse and inconclusive. The study variability hence prevents the possibility of combining all prediction studies into a meta-analysis, leaving the data scattered and thus unusable. AMH has emerged as a relatively suitable marker for predicting ART outcomes. It has superseded other traditional tests, but it has definite limitations when used on its own. While acknowledging the limitations is the first step, the combination of AMH with other known prognostic markers, such as woman's age and AFC, into models, preferably individualized, provides a clear direction for the future. There are however certain caveats though that should be adhered to; the hypotheses should be verified through well-designed prospective studies, validated and robust statistical methods should be used for the construction of the models, and a consented attempt to homogenize the reporting mechanisms of such studies should be promoted.

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Address reprint requests to:
C. SIRISTATIDIS, M.D., PH.D.
Rimini 1, Chaidari,
GR-12462, Athens (Greece)
e-mail: harrysiri@yahoo.gr