Comments by the Expert

Anti-Mullerian Hormone in Reproductive Biology – Utility in Clinical Practice

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Introduction

Even 15 years ago, AMH was considered primarily as a mullerian inhibitory substance (MIS) and its function was mainly concerned with mullerian regression and sexual differentiation in males only. But recently its role in controlling and prediction of ovarian function during women's reproductive period is gaining interest very fast. It is secreted in the female as a protein hormone by ***small pre-antral, large pre-antral and small antral follicles in the ovaries***. Apart from predicting ovarian reserve and ovarian responsiveness to stimulation, serum AMH values are being utilized for the diagnosis of pathophysiology of PCOs. Association between AMH and obesity has also been described. The level of serum AMH has also been utilized for recognition and diagnosis of granulosa cell tumor in females. In male, it has a specific indication for recognition of clinical situation of male hypogonadism in the pre-pubertal period.

Importance in Reproductive Medicine

ART, a revolutionary treatment for infertility has brought remarkable popularity of AMH in the field of reproductive medicine. ART is an expensive treatment. Each step of treatment of ART demands precision and perfection. Controlled ovarian stimulation (COS) is an integral step of ART. Since its introduction in early 1980s, there have been many modifications in the stimulation protocol but an ideal protocol has not yet been standardized. Current move is to personalize or tailor the protocol as it suits individual patient's requirement. For this, correct identification of a dependable 'marker' or 'predictor' is desirable for that patient for whom the stimulation protocol is expected to be designed. This refers to 'markers' for identification of 'ovarian reserve' and 'ovarian response' of the particular patient for whom the stimulation regime is to be formulated.

In the initial years of experience of ART practitioners, various markers were used and some of them are still being used today. These are – age of the patient, short menstrual cycle (ovarian ageing), baseline FSH, E2, inhibin, CC challenge test, previous ovarian surgery, poor response in previous IVF (if there is a history of previous IVF) etc. But none of these parameters either alone or in combination could precisely identify the marker of 'ovarian reserve' or type of 'ovarian response' – the patient will have following the specific type of stimulation she is expected to receive.

Changing Concept in 'Marker' Selection

Since identification of anti-mullerian hormone in the pre-antral and small antral follicles of the ovaries and their role in folliculogenesis, serum AMH has been found to be a dependable marker (at least more precise than other known markers) for identification and prediction of the patient's ovarian reserve and more importantly her 'ovarian response' to a particular

stimulation protocol. There are various reasons for this confidence. AMH, - unlike E2 or inhibin, is an autonomous product – not dependant on 'feed-back' mechanism like other ovarian hormones and therefore can be assessed on any day of menstrual cycle and the results of assessment on different day of menstrual cycle will not vary. Therefore, based on this result of AMH, patients may be classified as – ***(a) normal responder (b) hyper responder (c) poor responder***. However, it must be realized that in order to have a more convincing criteria, in addition to AMH, AFC and to a certain extent age of patient should be combined together for the categorization of patients with regard to their ovarian reserve and response. 'Reserve' and 'response' indicate 'quantity' and 'quality' of follicles respectively. AFC (antral-follicle count) denotes quantity (number of follicles) and AMH indicates quality of follicular response. The following table (Table-1) illustrates the practical utility of AMH and AFC for individualization of the treatment protocol in three categories of responders with different ovarian reserve.

Table-1

Low Ovarian Reserve AFC < 5 AMH < 1ng/ml		Normal Ovarian Reserve AFC : 5-15 AMH : 2-5ng/ml		High Ovarian Reserve AFC > 15 AMH > 5ng/ml	
Minimizing treatment burden		Maximizing success rate		Minimizing OHSS risk	
GnRH antagonist	Maximal FSH stimulation	Antagonist/ Agonist Protocol	Average Gn Stimulation	Antagonist protocol	Minimizing FSH stimulation

Comparative Role of Age, AFC and AMH in Predicting Markers as Ovarian Reserve and/or Ovarian Response as well as Selection of Starting Dose of Gonadotropins

The role of AMH and AFC in determination of ovarian 'reserve' and 'response' has already been emphasized. However, it is still recognized that apart from AMH, FSH and AFC, woman's age is also a commonly used clinical marker based on which the starting dose of gonadotropin has so long been calculated. Because with advancing age, women's ability to respond to ovarian stimulation usually declines. But women with similar age may have a wide variability in the pool of recruitable antral follicles. Therefore, age may not be the only important criteria based on which the dose of gonadotropin can be calculated. Other markers of ovarian reserve namely FSH, AFC and AMH are equally and may be better predictors for ovarian response. Amongst these three, the performance of AFC and AMH are superior than FSH in predicting the size of 'primordial follicle pool' and 'follicular recruitment rates'. Though age as marker of ovarian reserve has several advantages like lack of variability between cycle and perhaps it is an easy and inexpensive marker but age has the least ability to predict poor and hyper responders. Therefore for all practical purposes both AMH and AFC still stand out to be the most reliable predictors for ovarian response, - and therefore for selection of gonadotropin starting dose. The following diagram illustrates the starting dose of gonadotropin programming based on response predicted by AMH and AFC and classified as hyper, normal and poor responders.

Selection of protocol (antagonist/agonist) and starting dose of gonadotropin based on AMH and AFC values

Prediction based on AHM and AFC (same values as above (pmol/L)		
Expected high response	Expected normal response	Expected poor response

Suggested Treatment		
GnRH antagonist + 150IU FSH	GnRH antagonist + 200IU FSH	GnRH antagonist + 300IU FSH

Reasons for Selecting AMH and AFC Rather than Age and FSH as Superior Quality Markers

- The predictive markers commonly used to select the correct protocol and selection of drug and its dose are decided by:- age of the woman, outcome of previous attempt (if she had any), FSH, AMH, AFC and previous history of ovarian surgery etc
- Of these, who are having the first attempt, - age, FSH, AMH, AFC have been considered to be the reliable markers for ovarian response
- Though woman's ability to respond to stimulation declines with advancing age, age alone is not a dependable marker
- Because women with similar age may have wide variability in the pool of recruitable follicles
- A correlation between individual response to other three markers like FSH, AMH and AFC appears to be stronger and amongst these, AFC

and AMH appear to be more effective than FSH. Therefore, AFC and AMH have been accepted as the most dependable markers of ovarian response

Limitations

Having said so much in favor of AMH, - yet it must be admitted that there are limitations as well.

Even a few years back, AMH values were not standardized. Recently there has been an evolution of AMH assessment from laboratory versions to the commercially available diagnostic systems lab (DSL) and Immuno-tech Beckman Coulter (IBC) assessment. Currently published studies have used either the DSL or IBC assessment methods. But using these two different assay procedures have also created problems because values reported by different authors have varied substantially. IBC assay provides values of AMH which are higher than those provided by the DSL assay. Currently, the problem has been solved to a large extent as Beckman Coulter has purchased the patents of all previous versions and initiated AMH Generation-II assay. AMH Generation-II assay is highly specific and has been developed to standardize the measurement of AMH-between methods. A similar precision and excellent correlation between-assay agreement should be obtained when laboratory change from the DSL (diagnostic system laboratory) to AMH generation-II Elisa assay. Therefore it has been suggested that performance of AMH generation-II assay is ideal for determination of physiological role of AMH in men and women.

At present, in clinical practice, the normal level of plasma AMH has been accepted as 1-3 ng/ml. Levels between 0.7 to 0.9 ng/ml is recognized as low normal, while levels below 0.3 ng/ml is considered as very low level. Level above 3ng/ml is considered very high and may be a diagnostic marker for PCOS women. But still there is a wide variation in the level for clinical interpretation.

Ongoing Research for Better Ovarian Response Predictor

In future, in addition to conventional markers like AMH and AFC, genetic polymorphism, - such as single nucleotide polymorphism (SNP) may be more dependable marker of ovarian reserve or response. Information about polymorphism of the FSH receptor gene (FSH-R) is already available; and they may help in predicting the appropriate dose of FSH for individual woman. Other PCOS genes so far identified include AMH and AMH-receptor genes. From these studies, it appears that future attention in clinical research should focus on genetic prediction for individualization of COS protocol.