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Original Research Article

To Evaluate AMH as a Predictive Marker of Ovarian Response in Assisted Reproductive Technology Outcome: An Observational Study

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Abstract

Objective: To evaluate AMH as a predictive marker of ovarian response in assisted reproductive technology outcome.

Methods: 70 women (age 25–40 years) selected for in vitro fertilization treatment were included in this study. Analysis of day-2 serum samples was done for the AMH, FSH, Inhibin B, and LH by ELISA kit methods. USG was done for the antral follicle count (AFC) and oocytes'retrieval.

Results: The mean AMH levels of all treated patients were 2.260 ± 0.417. ROC for AMH indicating poor ovarian response with sensitivity of 72 % and specificity of 70 %. A statistically significant positive correlation was observed between the number of oocytes retrieved and the AMH (r = 0.620, p = 0.0001) (Fig. 2). Significant correlation was also seen between the number of oocytes retrieved and AFC (r = 0.400, p = 0.0001).

Conclusion: Our data demonstrated that AMH is an adequate predictor of both high and poor ovarian response, but it does not associate with pregnancy outcomes.

Keywords: Anti-Mullerian hormone, Antral follicle count, Oocytes retrieval count, assisted reproductive technology (ART)

Introduction

The optimization and individualization of controlled ovarian stimulation (COS) for in vitro fertilization (IVF) depends on utilizing patient characteristics and biomarkers to accurately predict ovarian response and tailor intended treatment. The characteristics, such as age, body mass index (BMI), menstrual cycle length, and results from previous IVF cycles are generally considered by clinicians for selection of ovarian stimulation strategies. [1] In addition, several different markers of ovarian reserve, which usually refers to the number of available primordial

follicles as well as the oocyte quality, have been proposed as predictors of ovarian response with varying degrees of success. [2,3] Of these, biochemical measures, such as basal follicle-stimulating hormone (FSH), estradiol (E2) and inhibin concentrations, fluctuate substantially during the menstrual cycle and hence their use has been limited. [4,5] Ovarian imaging, particularly antral follicle count (AFC), is largely affected by sonographers' intra- and inter-observer reproducibility and its sensitivity may differ from the resolution of transvaginal ultrasonography equipment. [2,6]

The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of oocytes therein, declines with increasing age, resulting in the decrease of women's reproductive function. [7] Diminished ovarian reserve has been recognized as an increasingly important cause of infertility. With age, ovarian reserve declines principally due to apoptotic loss of primordial follicles and not due to ovulation. [8]

The only effective treatment for decreased ovarian reserve is early attempt at pregnancy; and therefore, identification of accurate predictors of ovarian reserve is a must. [9]

So far, assessment of the number of antral follicle count (AFC) by ultrasonography best predicts the quantitative aspect of ovarian reserve. [9] With the decline of the follicle pool, serum levels of Inhibin B and E2 decrease and subsequently serum FSH levels rise. These factors are part of a feedback system as their serum levels are not independent of each other. Furthermore, changes in serum levels of FSH, Inhibin B, and E2 occur relatively late in the reproductive aging process when reduction in ovarian reserve is critical and chances of pregnancy are significantly reduced. [10] Age, day-3 FSH, InhibinB, AFC, ovarian volume, and several dynamic tests have been correlated with ovarian response in ART. However, their predictive value remains controversial and disappointing. [11]

Anti-Mullerian hormone, a member of the transforming growth factor-β family, is essentially involved in the regression of Mullerian ducts in the male fetus, the initial step of organogenesis of the male genital tract. In females, it is a product of the granulosa cells from pre-antral and small antral follicles. It has direct or indirect roles in various phases of folliculogenesis from the primordial to the FSH-sensitive follicular stages, probably via AMH II receptors, expressed in granulosa theca cells. Therefore, AMH secretion might reflect the activity of pre-antral and early antral follicles, making it a promising marker in the evaluation of ovarian follicular reserve. [12]

Hence, the objective of this study is to measure the levels of early follicular phase Anti-Mullerian hormone (AMH) in Indian patients of IVF and to evaluate the AMH as a predictive marker of ovarian response in assisted reproductive technology outcome.

Methods:

This study included 70 women (age 25–40 years) attending infertility clinic at the department of Obstetrics and Gynecology, Patna, Bihar for 1 year. These patients were collaborated, during their work up of infertility, with the department of Pathology PMCH, Patna. After diagnosis of infertility these patients were referred to a private IVF center and their response were measured and analyzed.

Inclusion criteria:

1. Regular menstrual cycle
2. Presence of both ovaries
3. Age <42 years.

Exclusion Criteria:

1. Women with genital tuberculosis, endometriosis, and autoimmune

disorders were excluded from the study.
2. Endocrine Disorders (Hypothyroidism, Hyperprolactinemia, Cushing' s syndrome)

Hormone Measurement:

Serum AMH was measured by EIA AMH/MIH kit (A Beckman Coulter Company) following the manufacturer's protocol. Serum Inhibin levels were determined by the sandwich ELISA technique using the INHIBIN B DSL-10-84100i kit following the manufacture's protocol. Serum FSH levels were determined by the immune enzymometric assay ELISA technique using the EIAGEN.

Long GnRH Agonist Protocol:

GnRH agonists are started in the mid-luteal phase of the cycle preceding the planned IVF, leading to both pituitary and ovarian desensitization. Following this, ovarian stimulation with gonadotropins are started and GnRH agonist injection is continued until hCG is administered. This is the most widely used method.

Antagonist Protocol:

GnRH antagonists like Cetrorelix or Ganirelix are given either as a single bolus dose of Cetrorelix 3 mg or in multiple doses of 0.25 mg daily. Next, HCG is given to trigger ovulation. Ovum pick-up is done after 34–36 h and inseminated with washed and processed sperms.

Results:

Linear discriminant analysis was done to know the correlation of the AMH with poor ovarian response, and the AMH cut-off level for poor ovarian response was 1.8 ng/ml with the least false positive and false negative results. Seventy patients were included with the aim of having their first IVF attempt. The baseline characteristics of poor and good responder groups are shown in Table 1. The mean AMH levels of all treated patients were 2.260 ± 0.417.

Figure 1 shows the typical ROC for the AMH indicating poor ovarian response with sensitivity of 72 % and specificity of 70%. The patients who responded poorly were older and had less oocyte retrieved with lower AMH than normal responders. ROC curve analysis for poor response showed that the AMH had the largest area under the curve (AUC; 0.812; p = 0.0001) as compared to the FSH (AUC; 0.525 p = 0.04), age (AUC; 0.401; p = 0.05).

The negative correlation was seen between the Oocyte retrieval count and FSH, though less significant (r = -0.481, p = 0.01). No correlation was identified between number of retrieved oocytes and Inhibin B. The AFC and AMH also showed a significant correlation (r = 0.481, p = 0.020). A statistically significant positive correlation was observed between the number of oocytes retrieved and the AMH (r = 0.620, p = 0.0001) (Fig. 2). Significant correlation was also seen between the number of oocytes retrieved and AFC (r = 0.400, p = 0.0001).

Table 1 Baseline characteristics and IVF cycle outcome

	Good responders (C8 oocytes)	Poor Responders (B4 oocytes)
Number of patients	52	18
Age (in years)	31.4 ± 4.7	38.1 ± 6.3
Infertility duration (years)	<10	>15
FSH (IU/l)	4.31 ± 1.63	9.20± 1.18
AMH (ng/ml)	1.278 ± 1.18	0.329 ± 0.48
Inhibin B (pg/ml)	71.82 ± 8.98	53.1 ± 4.1

LH (IU/l)	5.2 ± 0.20	4.0 ± 3.8
Oocytes retrieved	12.3 ± 7.1	2.8 ± 1.03
AFC	15.8 ± 6.1	11.3 ± 5.1

Values are represented as median range. Student's t test is performed to compare good and poor responders. Number of patients is 52 in the good responder group (five cancellations due to high response)

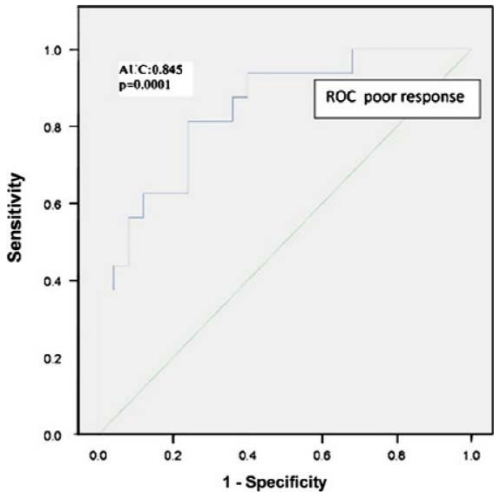

Figure 1 Receiver operating characteristic curve for AMH as an indicator of poor ovarian reserve and oocytes' retrieval

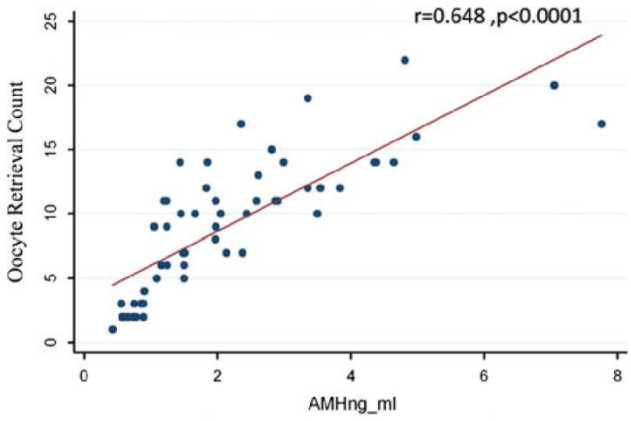

Figure 2 Correlation of number of oocytes after ovum retrieval with AMH in IVF patients. r is Spearman's correlation coefficient followed by the p value

Discussion:

The study evaluates the relationship between serum AMH levels, measured by an ultrasensitive ELISA technique, and the oocytes retrieved after gonadotropins' stimulation in IVF patients and compares the strength of correlations between the various hormonal parameters in predicting the positive outcome of IVF.

Markers of ovarian reserve exhibit comparable predictive value for ovarian response in PPOS (Progestin- primed ovarian stimulation) protocol, in accordance with previous studies indicating that early-follicular phase AFC and AMH have similar correlations to the number of oocytes retrieved. [13] Direct

comparisons of AFC and AMH in ovarian response prediction have generally shown no significant difference, while a few studies demonstrated that AMH or AFC had stronger predictive value than the other. [13]

Previous cohort studies have shown that AMH-tailored stimulation strategies resulted in a decreased incidence of high and poor response, increased pregnancy and live birth rates, as well as a reduction in costs. [14,15] These findings, however, are challenged by two recent RCTs to some extent. [16,17]

While AMH is a predictor of oocyte yield after COS, the literature shows no evidence of AMH being a valid predictor of the chance of achieving pregnancy after COS. Female age is the most accurate predictor for ongoing pregnancy after

IVF. [18]

A meta-analysis including 5764 women with unknown ovarian reserve undergoing IVF explores the association between AMH and live births. They concluded that the ability to predict live birth based on AMH is poor as they find a sensitivity of 83.7% (95% CI 72.5–90.9%) and a specificity of 32.0% (95% CI 21.6–44.6%). In a study based on 749 good-prognosis patients using both fresh and cryopreserved oocytes, an association between AMH level and cumulative pregnancy rate and live birth rate is found; however, the authors conclude that the association is due to a higher oocyte yield and not a better oocyte quality. [19]

Women with low AMH levels are at risk of poor ovarian response and therefore higher doses of gonadotropins are typically applied trying to maximize follicular recruitment and oocyte yield. In contrast, in women with high AMH levels, a milder stimulation protocol with lower doses of gonadotropins are often used to reduce the OHSS risk. [20]

Ovarian response to COS can be defined as the number of growing follicles exceeding10 mm or more or by the number of oocytes retrieved and is dependent on the ovarian reserve, the gonadotropin stimulation dose and the stimulation protocol. The stimulation protocol is chosen according to ovarian reserve markers combined with the woman's age, body mass index and ovarian response to previous IVF attempts. [21] As there is variability in ovarian response to a given dose of gonadotropins, clinicians have tried to identify markers that can predict the ovarian response. The best markers to determine ovarian reserve are AFC and AMH, and both have been shown to predict the ovarian response to COS too. [22]

Poor ovarian response to COS is seen in 10–20% of patients in ART treatment, with increasing prevalence among older women and reaching 50% in the group of women aged 43-44 years. [23, 24]

The Anti-Mullerian hormone can also be a promising marker for the detection of OHSS. Our study shows an elevation of the AMH in the hyper-responders as compared to good responders, although due to the small size, it did not meet statistical significance. [25]

Conclusion

Before fertility treatment the ovarian reserve can be determined via AFC and AMH as these tests predict the ovarian response to COS. This helps clinicians to choose the optimal treatment strategy and to provide women with realistic expectations before treatment. AMH is significantly correlated withthe number of eggs collected and is a good negative predictive marker for the success of ART.

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