Serum Level of Anti-Mullerian Hormone in Early Follicular Phase as a Predictor of Ovarian Reserve and Pregnancy Outcome in Assisted Reproductive Technology Cycles

Razieh Dehghani-Firouzabadi MD*, Naeimeh Tayebi MD*, Maryam Asgharnia MD*

Background: Anti-Mullerian hormone is produced by the granulosa cells of preantral and small antral follicles. The objective of this study was to investigate whether anti-Mullerian hormone and antral follicle count can be useful in predicting the ovarian reserve and pregnancy outcome in assisted reproductive technology cycles.

Methods: This prospective study included a total of 60 patients attending an assisted reproductive technology program. Patients with an oocyte count of ≥4 were considered good responders (group A); those with <4 oocytes were considered as poor responders (group B). On day three of the menstrual cycle, blood sample was taken from each woman for the measurement of serum levels of FSH, LH, E2, and anti-Mullerian hormone. Thereafter, ovarian ultrasound scanning was performed to evaluate the number and size of antral follicles.

Results: Parameters such as serum FSH, LH, and E2 levels were not statistically different between the two groups. Meanwhile, the difference between serum anti-Mullerian hormone levels, AFC, HCG day follicle counts, and retrieved oocyte counts were statistically significant in the two groups. The mean±SD serum anti-Mullerian hormone level was 34.22±13.95 and 12.53±9.4 pM/mL in groups A and B, respectively (P=0.002). The number of chemical pregnancies was seven versus three in groups A and B, respectively (P=0.014), whereas the number of clinical pregnancies was six versus two in groups A and B, respectively (P=0.52).

Conclusion: It appears that there is an association between the serum level of anti-Mullerian hormone in early follicular phase and ovarian reserve. Furthermore, a higher serum level of anti-Mullerian hormone on day three is associated with chemical pregnancy success.

Keywords: Anti-Mullerian hormone (AMH) • follicle number • ovarian reserve

Introduction

In assisted reproductive technology (ART), serum levels of several key hormones are used to evaluate the ovarian reserve and to monitor the growth of gonadotropin-stimulated follicle. Traditional methods used to prospectively predict the response to ovarian stimulation have mainly included the measurement on the third day of the cycle of baseline serum concentrations of hormones such as FSH, LH, estradiol, and inhibin, or ultrasonographic indices such as pretreatment ovarian volume and number of early antral follicles.1 Recently, anti-Mullerian hormone (AMH), also referred to as Mullerian-inhibiting substance (MIS), has been proposed as a novel marker for predicting ovarian response to gonadotropin stimulation.2

AMH is a glycoprotein that belongs to the transforming growth factor-B (TGF-B)—a member of the superfamily of growth and differentiation factors. It was identified as a factor which is being synthesized by testicular Sertoli cells, induces regression of the Mullerian ducts during male fetal development. In females, AMH is only expressed by the ovary, and mRNA studies in rat and mouse species revealed specific expression of AMH in...
granulosa cells of early growing, preantral and small antral follicles but not in nonatretic large antral follicles and atretic follicles. In female gonads, it may affect the transition from resting primordial into growing follicles. Furthermore, AMH may be involved in the recruitment of FSH-sensitive follicles in the early antral stage. AMH secretion is probably modulated by the degree of gonadic development, as it increases from barely detectable levels at birth to augment, yet subtle, levels after puberty. During adulthood, although AMH continues to be expressed at basal levels by Sertoli and granulosa cells, its biologic role is poorly understood.

Normal human female serum contains measurable amounts of AMH during the reproductive life span. AMH level varies slightly with the menstrual cycle, reaching a maximum in the late follicular phase.

Since AMH is solely produced by the growing ovarian follicles, serum levels may be used as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool. Recent preliminary reports indeed indicate that AMH levels decline with increasing female age, and that the initial AMH level is associated with ovarian response in *in vitro* fertilization (IVF) patients with normal FSH levels.

In the present study, we prospectively assessed the significance of AMH as a marker for ovarian reserve in ART cycles. We tested whether serum concentration of AMH in early follicular phase is associated with ovarian response and pregnancy outcome in patients undergoing ovulation induction in ART cycle.

**Patients and Methods**

**Subjects**

This prospective study included 60 patients aged <40 years who attended the IVF (n=45) or intracytoplasmic sperm injection (ICSI, n=15) program ran in Clinical and Research Center for Infertility between July 2005 and September 2006.

The inclusion criteria were 1) presence of both ovaries and lack of morphologic abnormalities; 2) regular menstrual cycle (cycle length, 25 – 35 days, duration of menstruation, three to eight days); 3) no evidence of endocrine disorders (normal thyroid stimulating hormone, prolactin, testosterone, and androstandione); 4) a body mass index (BMI) ranging from 18 – 27 kg/m²; 5) not on hormone therapy for three months; 6) adequate visualization of ovaries at transvaginal ultrasound scanning; and 7) no endometriosis.

Also, serologic markers (HBsAg, anti-HCV, and anti-HIV) were assessed for all patients.

Etiologies of infertility included sperm abnormalities (72.4%), tubal abnormalities (20.7%), and unexplained infertility (6.9%).

All patients underwent routine explorations before IVF-embryo transfer. Informed consents were obtained from them. The study was approved by the Institutional Research Committee.

**Ovarian stimulation protocol**

The patients were all treated with a long protocol for ovarian stimulation. In the long protocol, pituitary down-regulation was achieved by administering buserelin acetate (Suprefact, Hoechst AG, Germany) (0.5 mg SC) starting from day 21 of menstrual cycle and then decreasing the dose to 0.25 mL/day when the menstrual bleeding happened. Then, stimulation was commenced using human menopausal gonadotropin (HMG) (Menogon, Ferring, Germany) from the second day of their menstrual cycle with a dose of 225 – 300 IU/day. Monitoring was carried out by transvaginal ultrasound (HS-4000 Japan) on day seven of HMG stimulation. After more than three follicles larger than 18 mm in diameter were observed, 10,000 IU of human chorionic gonadotropin (HCG) (Pregnyl®5000, Organon) was administered intramuscularly. Thirty-six hours later, follicles were retrieved under general anesthesia by transvaginal ultrasound-guided aspiration. Mature oocytes were retrieved from follicular fluid and placed in G-fert (version 3; Vitrolife, Goteborg, Sweden) and after fertilization; 2PN zygote was transferred to G-1 media (G-1 TM version 3; Vitrolife, Goteborg, Sweden). All embryo transfers were performed two days after oocyte retrieval using Labotect catheters (Labor-Technik, Germany). Before the transfer, the embryos were evaluated microscopically and the best-quality embryos were selected for the transfer. A maximum of three embryos were transferred.

Luteal phase was supported with Cyclogest (Alpharma, Barnstable, UK) with a dose of 800 mg vaginally per day administered starting on the puncture day.

The primary outcome measures of the study were the number of oocytes retrieved and poor ovarian response. Poor ovarian response was defined as fewer than four oocytes at follicle puncture or absence of follicular growth in
response to ovarian hyperstimulation.13 Secondary outcome measures were chemical pregnancy that was defined as a serum β-HCG >50 mIU/mL, two weeks after IVF or ICSI, clinical or ongoing pregnancy that was determined by detection of fetal heart beat through abdominal ultrasonography eight weeks after the initiation of ART cycles, and miscarriage that was defined as termination of pregnancy before 20 weeks based upon the date of the first day of the last normal menses.13

Hormonal and follicular measurements

On the morning of day three of the menstrual cycle, each woman underwent blood sampling by venipuncture for measurement of serum levels of FSH, LH, E2, and AMH (the normal rang of FSH, LH, and E2 in follicular phase was, respectively 3 – 12 mIU/mL, 0.5 – 10.5 mIU/mL, and 13 – 191 pg/mL). Later in the morning, ovarian ultrasound scanning was performed to evaluate the number and sizes of antral follicles.

On day three of the cycle, 5 mL of blood was drawn; the blood was centrifuged at the 3500 rpm for 10 min, and the serum was stored in 1.5-mL polypropylene tubes at 2 – 8°C if the assay was to be performed within 24 hr. For longer storage, the samples were kept frozen (at ≤18°C). Serum AMH levels were determined using a “second generation” enzyme-linked immunosorbent assay (ELISA) (reference A16507; Immunotech Beckman Coulter Laboratories). Intra- and inter-assay coefficients of variation (CV) were ≤12.3% and ≤14.2%, respectively. The immunoassay was specific for AMH and the analytical sensitivity was 0.7 pMol.

Serum FSH, LH, and E2 levels were measured by the same laboratory technician for all patients on the third day of the cycle by ELISA technical. The technician was blinded to the group the patients belonged to.

The study was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of ≥4 were considered good responders; patients with <4 oocytes were considered poor responders.

Data were analyzed by SPSS, version 13. The appropriate statistical tests including Student's t-test, Chi-square, and Fisher exact test were used to compare the results. A \( P < 0.05 \) was considered statistically significant.

Results

The AMH levels of two patients could not be measured due to lack of stored serum. Therefore, 58 patients were included in the ART program, 43 of whom underwent IVF and 15 ICSI treatments. Of the remaining 58 couples, the causes of infertility were male factors (42 couples), tubal factors (12 couples), and idiopathic (four couples). The age of women was between 19 and 38 years. The mean±SD menstrual cycle and infertility duration were 28.8±2.9 and 6.78±3.3 years, respectively.

Ovarian reserve was assessed by basal hormonal levels on cycle day three, and antral follicle count on ultrasound (Table 1).

Patient and ovarian reserve test characteristic of good and poor responders are separately presented in Table 1. Characteristics such as age, menstrual cycle duration, infertility period, and BMI were similar between good and poor responders. Parameters such as basal serum FSH, LH, and E2 levels were also not statistically different. Meanwhile, the difference between serum AMH levels, AFC, HCG day follicle counts, and the number of retrieved oocyte were significant (Table 1). Furthermore, day three serum AMH, but not E2 or FSH, was positively correlated with the number of retrieved oocytes (Figure 1).

Good responders had a mean±SD beginning dose of 225±75 IU/day and a total dose of 1200±150 IU that was reached within a mean±SD period of 9±2 days. Poor responders, on the other hand, had a mean±SD starting dose of 300±75 IU/day, and a total dose of 1800±225 IU that was reached within a mean±SD period of 11±2 days \( (P = 0.12) \).

The main parameter of the study, AMH, was found to be considerably higher in good responders; the mean AMH level was 34.22±13.95 pMol/mL, whereas it was 12.53±9.4 pMol/mL in poor responders \( (P = 0.002) \).

In total, 10 out of the 58 couples had a positive β-HCG test. The number of chemical pregnancies was seven in good responders vs. three in poor responders \( (P = 0.014) \); the number of clinical pregnancies was six vs. two in good and poor responders, respectively \( (P = 0.52) \). Two pregnancies were aborted before 20 weeks of gestation (one miscarriage occurred in each group), and the rest of the pregnancies continued as singletons. There was no significant difference in miscarriage
rate between the two groups ($P=0.52$) (Table 1).

No oocyte retrieval took place in three patients with poor response because of insufficient follicle growth. Two patients with poor response had cancelled their cycle due to absence of fertilization.

**Discussion**

Our data demonstrated an association between the serum AMH level in early follicular phase and the number of retrieved oocytes, despite of clinically similar serum FSH, LH, and E2 concentrations on day three of the menstrual cycle. The baseline FSH, LH, and E2 levels are good predictors of ovarian reserve.\(^{20,21}\) Muttukrishna and colleagues found that the levels of baseline FSH were significantly higher in the cancelled group and the mean AMH level was significantly lower in the cancelled group compared to the completed cycle group.\(^{22}\) Another study showed that the levels of baseline FSH and E2, but not LH, were significantly lower in cycles resulting in a normal ovarian response as well as cycles resulting in clinical pregnancy.\(^{23}\) Van Rooij et al. demonstrated that the baseline levels of FSH, but not E2, were higher in poor responders and that AMH levels were lower in the poor responders compared to normal responders.\(^{13}\)

Our results demonstrated that serum AMH levels were highly correlated with the number of antral follicles, HCG day follicles count, and the

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**Table 1.** Clinical characteristics and pregnancy outcomes in the total group of ART patients and in good and poor responders separately. Values are given as mean±SD. Student’s t-test, Chi-square, and Fisher exact test were used for statistical analysis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=58)</th>
<th>Good responders (n=35)</th>
<th>Poor responders (n=23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>29.12±4.06</td>
<td>28.51±3.8</td>
<td>30.04±4.2</td>
<td>0.93</td>
</tr>
<tr>
<td>Menstrual cycle duration (days)</td>
<td>28.8±2.9</td>
<td>28.3±2.4</td>
<td>29.5±3.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Duration of infertility (yr)</td>
<td>6.78±3.3</td>
<td>6.46±3.2</td>
<td>7.26±3.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>56</td>
<td>35</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.47±2.6</td>
<td>24.46±3.8</td>
<td>26.48±5.13</td>
<td>0.42</td>
</tr>
<tr>
<td>Causes of infertility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>42 (72.4)</td>
<td>27 (64.3)</td>
<td>15 (35.7)</td>
<td>0.61</td>
</tr>
<tr>
<td>Tubal factor (%)</td>
<td>12 (20.7)</td>
<td>6 (50)</td>
<td>6 (50)</td>
<td>—</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>4 (6.9)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>—</td>
</tr>
<tr>
<td>Day-3 FSH (mIU/mL)</td>
<td>9.38±6.8</td>
<td>9.34±6.09</td>
<td>9.46±4.4</td>
<td>0.38</td>
</tr>
<tr>
<td>Day-3 LH (mIU/mL)</td>
<td>5.64±2.5</td>
<td>5.48±3.67</td>
<td>5.87±3.02</td>
<td>0.66</td>
</tr>
<tr>
<td>Day-3 E2 (pg/mL)</td>
<td>43.51±8.6</td>
<td>40.93±7.02</td>
<td>47.43±8.64</td>
<td>0.55</td>
</tr>
<tr>
<td>Day-3 AMH (pM/mL)</td>
<td>24.62±12.1</td>
<td>34.22±13.95</td>
<td>12.53±9.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of antral follicles</td>
<td>8.37±3.7</td>
<td>9.71±3.04</td>
<td>6.34±3.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Number of day follicles</td>
<td>10.38±5.2</td>
<td>11.77±5.1</td>
<td>8.26±4.76</td>
<td>0.011</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>6.53±5.04</td>
<td>8.74±5.1</td>
<td>2.65±0.81</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of chemical pregnancies</td>
<td>10</td>
<td>7 (70%)</td>
<td>3 (30%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Number of clinical pregnancies</td>
<td>8</td>
<td>6 (75%)</td>
<td>2 (25%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Number of miscarriages</td>
<td>2</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

**Figure 1.** Day three serum AMH, FSH, and E2 levels are compared with the number of retrieved oocytes. Serum AMH, but not E2 or FSH positively correlates with the number of retrieved oocytes.
number of retrieved oocyte than did E2, FSH, or LH on day three of the cycle. In good responders, with increasing AMH levels, the antral follicle, the growing follicle, and oocyte retrieval counts would also increase. These results were in agreement with those of van Rooij, et al.’s study.

Fanchin et al. found that the serum AMH levels were more strongly correlated with antral follicle counts than did the serum levels of inhibin B, E2, LH, and FSH.

AMH expression begins in the third trimester of gestation, actually long before it can be detected in serum. As a result, it is thought to play an important role in early follicular development. Once ovarian cycles begin, serum AMH levels vary slightly from baseline due to the stimulation of a small cohort of follicles and loss of AMH production from corpus luteum. Our results showed an association between AMH level and antral follicles and retrieved oocyte counts. Therefore, serum AMH levels may reflect the size of the antral follicle pool and hence, may provide a marker associated with the anticipated number of oocytes to be retrieved after controlled ovarian stimulation for IVF/ICSI.

Recently, Ficicioglu and associates have found that there is an association between AMH levels, antral follicles count, and the number of retrieved oocyte. Also, the results from Seifer study demonstrated that a higher day three serum AMH concentrations were associated with greater number of retrieved oocytes. For example, in their study, the mean serum AMH concentration was more than two and a half-fold in the group with ≥11 oocytes retrieved compared to the group with ≤6 oocytes.

In our study, we observed that patients with ≤4 retrieved oocytes had lower day three AMH levels, fewer antral follicles, and lower HCG day follicle counts. Thus, the basal antral follicle count and basal AMH levels are good tools to use in counseling patients.

Our results demonstrated that a higher serum AMH level has a relation with chemical pregnancy outcome, and that it does not have any correlation with clinical pregnancy and miscarriage rates.

Deffieux and co-workers demonstrated that day three AMH levels predict the number of oocytes retrieved, but the AMH level cannot predict the likelihood of pregnancy. Another study showed that AFC has a significant association with the number of oocytes retrieved and is predictive of clinical pregnancy.

Lately, Ficicioglu et al. have found that levels of AMH predict the number of oocytes with a positive predictive value of 96%, although it has little value for predicting pregnancy.

In conclusion, it appears that AMH serum levels are associated with ovarian response in ART cycles and can be served as a novel marker for ovarian reserve. Furthermore, with respect to significant difference in chemical pregnancy outcome, serum levels of AMH may be used as a marker for predicting the chemical pregnancy rate. However, further studies are needed to determine whether AMH can accurately predict the ART outcomes or not.

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