

EVALUATION OF ANTI-MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFERTILITY PATIENTS IN KARPAGA VINAYAGA MEDICAL COLLEGE HOSPITAL

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ABSTRACT

Background: Infertility and diminished ovarian reserve affect reproductive outcomes and the success of assisted reproductive techniques. Reliable biomarkers are needed to assess the ovarian reserve and predict the response. This study evaluated serum anti-Müllerian hormone (AMH) levels as a marker of ovarian reserve in patients with infertility. **Materials and Methods:** This observational study involved 100 patients with infertility between 2018 and 2019. Blood samples were collected on day 3 of the menstrual cycle for hormonal analysis, including AMH, FSH, LH, oestradiol, prolactin, and thyroid profiles. Ultrasonographic assessment of ovarian volume and antral follicular count was performed using transvaginal and transabdominal ultrasonography. Ovarian reserve assessment included hormonal evaluation, follicular counting, and ovarian volume measurements. **Results:** Most patients were aged 26-29 years (40%), followed by 30-33 years (26%), with obesity in 41%. Primary infertility was more common (77% of cases). High AMH levels (>4.0 ng/mL) were observed in 46% of patients, and normal levels were observed in 38% of patients. AMH levels showed a significant positive correlation with the antral follicular count of the left ($r=0.642$) and right ($r=0.619$) ovaries ($P<0.001$). Linear regression analysis showed a significant positive association between AMH and antral follicular count ($B=0.593$, $\beta=0.632$, $P<0.001$). AMH cut-off values from 5.595 to 6.4 ng/mL showed high sensitivity (93.3%–100%) and specificity (100%) for predicting PCOS in the training set. **Conclusion:** Serum AMH is a reliable predictor of ovarian reserve and response in patients with infertility, offering better predictive utility than conventional hormonal markers for reproductive assessment and treatment planning.

INTRODUCTION

Infertility affects 12.6% to 17.5% of women of reproductive age, affecting 48.5 million couples worldwide. Age and lifestyle contribute to infertility in women. Diminished ovarian reserve (DOR), characterised by a decreased number of ovarian follicles, causes decreased reproductive potential and reduced success rates in assisted reproductive techniques (ART), particularly in vitro fertilization (IVF).^[1,2] Assessment of ovarian reserve is therefore an essential component in the evaluation and management of infertility patients.

Ovarian reserve capacity measures how well the ovaries perform in producing oocytes and provides an estimate of the ovarian reserve response. Poor ovarian reserve response leads to infertility and poor outcomes of ART, especially for women who

respond badly to stimulation.^[1,2] Accurate assessment of ovarian reserve is important for counselling infertile couples, predicting ovarian response, and planning individualized fertility management strategies.

Biomarkers such as antral follicle count (AFC) and Anti-Müllerian hormone (AMH) are reliable for measuring ovarian reserve and predicting ovarian response better than traditional markers. They help identify patients at risk for suboptimal responses, informing protocol and gonadotropin dosing.^[3] For PCOS, where certain markers may be elevated, alterations in protocols should be made to reduce the possibility of OHSS without affecting the results of conception.

Some of the markers that have been used for measuring ovarian reserve traditionally include follicle-stimulating hormone (FSH), luteinizing

hormone (LH), oestradiol, and AFC. FSH and LH are hormones produced by the anterior pituitary gland, which play a role in regulate folliculogenesis and ovulation. Oestradiol is produced by the follicles, and it signifies their activity. AFC uses ultrasonography to count small antral follicles, representing the recruitable follicle pool.^[3,4]

FSH and E2 levels vary significantly, affecting reliability due to menstrual cycle phases and external factors. AFC assessments depend on operator skill and technique, causing inconsistency.^[4] Their limited predictive accuracy for ovarian response complicates the individualisation of treatment protocols. This has led to the search for more reliable ovarian reserve biomarkers. AMH, secreted by granulosa cells of pre-antral and small antral follicles, reflects the non-cyclic follicular pool involved in early follicular genesis. Its minimal variability makes it a stable serum marker measurable anytime during the menstrual cycle.^[5,6]

Compared to conventional hormonal markers, AMH correlates better with ovarian reserve and responsiveness, becoming important in reproductive medicine for evaluating fertility and predicting ovarian response in infertility treatment.^[7-9] Despite its higher usage, variations exist in its predictive utility across different populations and settings. The relationship between AMH and markers like AFC, FSH, LH, and estradiol is still being evaluated. Thus, assessing AMH as an ovarian reserve predictor remains relevant, especially for infertility patients. The study aimed to assess serum AMH levels, their association with ovarian reserve in infertility patients, evaluate AMH levels in women with infertility, determine AMH's predictive value for conception in women with poor ovarian reserve, and explore AMH's utility in guiding infertility treatment planning.

MATERIALS AND METHODS

This hospital-based observational study was conducted in 100 female infertility patients attending the OBG OPD and IVF OPD at Karpaga Vinayaga Institute of Medical Sciences and Research Center from 2018 to 2019. Ethical approval was obtained from the Institutional Ethical Committee and written informed consent was obtained from all participants before study initiation.

Inclusion Criteria

This study included healthy infertile women less than 40 years of age with ovarian causes of infertility and infertility duration of more than 2 years who were willing to participate in the study.

Exclusion Criteria

Patients with acute infections such as pelvic inflammatory disease, endometriosis, history of ovarian surgery, postmenopausal women, thyroid dysfunction, Cushing syndrome, and patients who were not willing to participate in the study were excluded from the study.

Materials

The materials used in the study included a disposable syringe, plastic tubes for sample storage, centrifuge, transvaginal ultrasound probe with 6.5 MHz frequency, transabdominal ultrasound equipment, and laboratory facilities for AMH, E2, FSH, LH, FT3, FT4, TSH, PRL, and estradiol analysis.

Methods

5 ml blood samples were drawn from the median cubital vein on day 3 of the menstrual or progesterone-induced cycle using a plastic syringe. Samples were transferred into tubes, left for 20 minutes to clot, centrifuged at 3500 rpm for 10 minutes, and serum stored at -20°C. AMH, E2, FSH, LH, FT3, FT4, TSH, PRL, and estradiol were analyzed at the central lab. BMI was calculated as weight in kg divided by height in meters squared. Indian guidelines define BMI 23-24.9 as overweight, ≥ 25 as moderate obesity, and ≥ 30 as severe obesity. The Ferriman-Gallway score assessed hirsutism; a score above 8 indicated hirsutism.

Ultrasonography used a 6.5 MHz transvaginal probe for married, transabdominal for unmarried patients. Measurements were real-time B-scan by one physician. Ovarian volume was $0.5 \times \text{length} \times \text{width} \times \text{thickness}$, averaging both ovaries. AFC assessed follicle size and number. Uterine diameters were sagittal from cervix to fundus with maximum anteroposterior diameter. Follicle sizes of 2-6 mm, 2-8 mm, and >10 mm were recorded; 2.0-8.0 mm used for analysis. Ovarian reserve assessment included AMH measurement, FSH and estradiol levels on day 3, ultrasonographic follicle count of 2-10 mm, and ovarian volume. AMH ≤ 1 ng/ml indicated reduced ovarian reserve.

Statistical Analysis: Data were presented as mean, standard deviation, frequency, and percentage. Correlation between continuous variables was determined using Pearson's correlation test. Linear regression analysis was performed to evaluate associations between variables. Statistical significance was defined as $P < 0.05$ using a two-tailed test. Data analysis was performed using IBM-SPSS version 21.0 (IBM-SPSS Science Inc., Chicago, IL, USA).

RESULTS

Among the age groups, most patients were 26-29 years (40%), followed by 30-33 years (26%) and >33 years old (24%), with the fewest in the 22-25 years group (10%). Obesity was present in 41% of patients, overweight in 36%, and normal BMI in 23%. Primary infertility was more common, seen in 77% of patients, while secondary infertility was seen in 23%. High AMH levels (>4.0 ng/mL) were observed in 46% of the patients, and normal levels (1.5-4.0 ng/mL) were observed in 38% of the patients. Low AMH levels (0.5-1.0 ng/mL and 1.01-1.5 ng/mL) were observed in 6% of patients, and very low levels

(<0.5 ng/mL) were observed in 4% of patients. [Table 1]

Table 1: Baseline demographic, clinical characteristics and AMH category distribution among patients

| | | N (%) |
|----------------------|-----------------------------|----------|
| Age group (years) | 22-25 | 10 (10%) |
| | 26-29 | 40 (40%) |
| | 30-33 | 26 (26%) |
| | >33 | 24 (24%) |
| BMI class | Normal | 23 (23%) |
| | Overweight | 36 (36%) |
| | Obese | 41 (41%) |
| Type of infertility | Primary | 77 (77%) |
| | Secondary | 23 (23%) |
| AMH category (ng/mL) | <0.5 (Very low) | 4 (4%) |
| | 0.5-1.0 (Low) | 6 (6%) |
| | 1.01-1.5 (Low normal range) | 6 (6%) |
| | 1.5-4.0 (Normal) | 38 (38%) |
| | >4.0 (High) | 46 (46%) |

AMH was positively correlated with BMI ($r=0.058$), LH ($r=0.073$), prolactin ($r=0.162$), and AFC of both ovaries (left $r=0.642$, right $r=0.619$), with the latter correlation being statistically significant ($P<0.001$). AMH was negatively correlated with FSH ($r=-0.031$)

and oestradiol ($r=-0.192$) and showed no correlation with TSH ($r=0.000$). Correlations with BMI, LH, FSH, TSH, prolactin, and oestradiol were not statistically significant ($P>0.05$). [Table 2]

Table 2: Correlation of AMH with hormonal and ovarian reserve parameters

| | Pearson correlation coefficient (r) | P value |
|-----------------|-------------------------------------|---------|
| BMI | 0.058 | 0.565 |
| LH | 0.073 | 0.469 |
| FSH | -0.031 | 0.759 |
| TSH | 0.000 | 1.000 |
| Prolactin | 0.162 | 0.107 |
| AFC left ovary | 0.642 | <0.001 |
| AFC right ovary | 0.619 | <0.001 |
| Estradiol | -0.192 | 0.056 |

Linear regression analysis showed a correlation coefficient (R) of 0.661. The coefficient of determination (R square) was 0.437, meaning that 43.7% of the variability in the antral follicular count

was explained by the model. The adjusted R-squared was 0.381, and the standard error of the estimate was 1.38491. [Table 3]

Table 3: Model summary for linear regression analysis for predictors of antral follicular count in left ovary

| Model summary | Value |
|----------------------------|---------|
| R | 0.661 |
| R square | 0.437 |
| Adjusted R-squared | 0.381 |
| Standard error of estimate | 1.38491 |

The linear regression model showed a regression sum of squares of 134.131, a residual sum of squares of 172.619, and a total sum of squares of 306.750. It had

an F value of 7.770 with 9 degrees of freedom and was significant ($P<0.001$). [Table 4]

Table 4: ANOVA for linear regression analysis for predictors of antral follicular count in left ovary

| ANOVA | Value |
|---------------------------|---------|
| Regression sum of squares | 134.131 |
| Residual sum of squares | 172.619 |
| Total sum of squares | 306.750 |
| Degrees of freedom | 9 |
| F value | 7.770 |
| P value | <0.001 |

Multiple linear regression analysis showed that AMH was positively associated with antral follicular count ($B=0.593$, $\beta=0.632$, $t=7.569$, $P<0.001$). Age was negatively associated with AMH levels ($B=-0.065$, $\beta=-0.132$), while BMI ($B=0.027$, $\beta=0.058$), type of

infertility ($B=0.014$, $\beta=0.003$), and LH ($B=0.041$, $\beta=0.030$) showed positive associations. Negative associations with FSH ($B=-0.041$, $\beta=-0.062$), TSH ($B=-0.028$, $\beta=-0.015$), prolactin ($B=-0.020$, $\beta=-$

0.072), and oestradiol (B=0.000, β =-0.006) were not significant (P>0.05). [Table 5]

Table 5: Multiple linear regression coefficients for predictors of antral follicular count in left ovary

| Variable | Unstandardized coefficient (B) | Standard error | Standardized coefficient (Beta) | t value | P value |
|---------------------|--------------------------------|----------------|---------------------------------|---------|---------|
| Constant | 3.805 | 1.819 | - | 2.091 | 0.039 |
| Age | -0.065 | 0.041 | -0.132 | -1.592 | 0.115 |
| BMI | 0.027 | 0.040 | 0.058 | 0.671 | 0.504 |
| Type of infertility | 0.014 | 0.342 | 0.003 | 0.042 | 0.967 |
| LH | 0.041 | 0.113 | 0.030 | 0.365 | 0.716 |
| FSH | -0.041 | 0.055 | -0.062 | -0.751 | 0.455 |
| TSH | -0.028 | 0.153 | -0.015 | -0.180 | 0.857 |
| AMH | 0.593 | 0.078 | 0.632 | 7.569 | <0.001 |
| Prolactin | -0.020 | 0.024 | -0.072 | -0.850 | 0.398 |
| Estradiol | 0.000 | 0.002 | -0.006 | -0.073 | 0.942 |

AMH cut-offs from 5.595 to 6.4 ng/mL showed high diagnostic sensitivity and specificity for predicting PCOS. A 5.595 cut-off had 100% sensitivity with a 3.3% false positivity rate, while 5.7 showed 96.7%

sensitivity with the same rate of false positivity. Cut-offs of 5.9 and 6.4 had 100% specificity with no false positivity and sensitivities of 96.7% and 93.3%, respectively. [Table 6]

Table 6: Diagnostic performance of AMH cut-off values for identifying PCOS cases

| AMH cut-off value | Sensitivity | Specificity | False positivity |
|-------------------|-------------|-------------|------------------|
| 5.595 (~6) | 100% | - | 3.3% |
| 5.7 | 96.7% | - | 3.3% |
| 5.9 | 96.7% | 100% | 0% |
| 6.4 | 93.3% | 100% | 0% |

DISCUSSION

In this study, high AMH levels (>4.0 ng/mL) were observed in 46% of patients, whereas normal AMH levels (1.5-4.0 ng/mL) were observed in 38% of patients. Low AMH levels between 0.5-1.0 ng/mL and 1.01-1.5 ng/mL were observed in 6% of patients each, whereas very low AMH levels (<0.5 ng/mL) were observed in 4% of patients, indicating that the majority of patients had preserved ovarian reserve. Bosch et al. found that AMH accurately predicted ovarian response, with cut-offs at 0.89 ng/mL and 1.99 ng/mL.^[10] Similarly, a study by Laqqan and Yassin also showed AMH and age outperforming FSH for prediction.^[11]

AMH levels showed a significant positive correlation with AFC in the left ($r=0.642$, $P<0.001$) and right ($r=0.619$, $P<0.001$) ovaries, confirming the close association between AMH and ovarian reserve. Positive correlations were also observed between AMH and BMI ($r=0.058$), LH ($r=0.073$), and prolactin ($r=0.162$), whereas negative correlations were observed with FSH ($r=-0.031$) and estradiol ($r=-0.192$), although these were not statistically significant. Liu et al. reported both AMH and AFC are reliable predictors of ovarian response depending on population characteristics and cut-off values.^[12] Another study by Laqqan and Yassin in 2022 ranked AFC as the strongest predictor followed by AMH, with AFC cut-offs of ≤ 4.50 for low reserve and ≥ 14.50 for high reserve.^[13]

In our study, linear regression analysis showed a moderate positive association between predictor variables and AFC with an R value of 0.661 and R

square value of 0.437, indicating that 43.7% of the variability in AFC was explained by the regression model. Multiple linear regression analysis demonstrated that AMH had a significant positive association with AFC (B=0.593, $\beta=0.632$, $t=7.569$, $P<0.001$). Age, FSH, TSH, prolactin, and oestradiol showed negative associations, and BMI, LH, and type of infertility showed positive associations that were not statistically significant. Bose and Konar also demonstrated that AMH showed a significant correlation with oocyte yield and ovarian response during controlled ovarian stimulation.^[14]

In the present study, primary infertility was more common and was observed in 77% of patients, whereas secondary infertility was observed in 23% of patients. Most patients belonged to the 26-29 years age group (40%), followed by 30-33 years (26%) and >33 years (24%). Obesity was observed in 41% of the patients, and 36% were overweight. These findings indicate that infertility and altered ovarian reserve are more common among overweight and obese women in the reproductive age group.

AMH cut-off values ranging from 5.595 to 6.4 ng/mL demonstrated high sensitivity and specificity for the prediction of PCOS in the present study. A cutoff value of 5.595 showed 100% sensitivity with 3.3% false positivity, whereas cutoff values of 5.9 and 6.4 demonstrated 100% specificity without false positivity. Friis Petersen et al. demonstrated that AMH-based individualized approaches may be useful for optimizing ovarian response and fertility management despite variability in ovarian stimulation outcomes.^[15]

CONCLUSION

Serum AMH is a reliable marker for assessing the ovarian reserve in patients with infertility. Compared to FSH and oestradiol, AMH had better predictive utility and a significant positive correlation with the antral follicular count. Higher AMH levels indicate better ovarian reserve, whereas lower levels suggest diminished reserve. AMH predicts the ovarian response and aids in personalised fertility counselling. It is useful for evaluating infertility, especially without an antral follicular count assessment. AMH showed good sensitivity and specificity for identifying PCOS at specific cut-off values. Despite low AMH levels indicating reduced reserve, they should not exclude patients from assisted reproductive techniques, as acceptable outcomes may occur. Combining AMH with other parameters offers a comprehensive evaluation of ovarian function. Further large-scale studies are needed to standardise AMH reference values and establish its role in predicting reproductive outcomes and long-term ovarian function.

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