

Original Article

# Role of Chronic Psychological Stress Biomarkers in Triple-Negative Breast Cancer Progression

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## ABSTRACT

**Background:** Polycystic ovary syndrome is a common endocrine disorder and a major cause of anovulatory infertility. Although LH/FSH ratio is traditionally used as a supportive hormonal marker in PCOS, its predictive value is inconsistent and may be influenced by BMI and cycle-related variability. Anti-Müllerian hormone reflects small antral follicle burden and may offer a more stable biomarker for ovulatory dysfunction. **Objective:** To compare the predictive performance of AMH and LH/FSH ratio for anovulation in women with PCOS and to develop a simple clinical scoring tool incorporating hormonal and clinical predictors. **Methods:** This cross-sectional predictive study included 250 women aged 18–35 years diagnosed with PCOS according to Rotterdam criteria at a tertiary hospital in Quetta, Pakistan. Clinical assessment, BMI categorization, serum AMH, LH, FSH, LH/FSH ratio, and pelvic ultrasonography were evaluated. Correlation analysis, ROC curve analysis, BMI-stratified subgroup analysis, and multivariable logistic regression were performed. **Results:** AMH showed stronger predictive accuracy for anovulation than LH/FSH ratio, with AUC values of 0.88 (95% CI: 0.83–0.93) and 0.74 (95% CI: 0.67–0.81), respectively. AMH  $\geq 8$  ng/mL demonstrated 85% sensitivity and 80% specificity, while LH/FSH ratio  $\geq 2.0$  showed 70% sensitivity and 65% specificity. AMH correlated strongly with follicle count ( $r = 0.72$ ,  $p < 0.001$ ), whereas LH/FSH ratio showed a moderate correlation ( $r = 0.41$ ,  $p = 0.020$ ). AMH retained high predictive performance across BMI categories, while LH/FSH ratio declined in obese women. AMH  $\geq 8$  ng/mL was the strongest independent predictor of anovulation (OR = 4.5, 95% CI: 2.7–7.6,  $p < 0.001$ ), and the proposed scoring tool achieved 82% preliminary accuracy. **Conclusion:** AMH is a superior and more BMI-stable predictor of anovulation than LH/FSH ratio in women with PCOS. Integrating AMH into a simple scoring model may improve outpatient risk stratification and fertility counseling. **Keywords:** PCOS, anti-Müllerian hormone, AMH, LH/FSH ratio, anovulation, ovarian follicles, BMI, infertility, predictive biomarker.

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## INTRODUCTION

Polycystic ovary syndrome is among the most common endocrine disorders affecting women of reproductive age and is clinically characterized by variable combinations of oligo- or anovulation, hyperandrogenism, and polycystic ovarian morphology. Its reproductive burden is substantial because ovulatory dysfunction is one of the principal mechanisms through which PCOS contributes to subfertility and infertility, while its metabolic associations, including obesity and insulin resistance, further complicate clinical assessment and treatment planning (1–4). The clinical heterogeneity of PCOS is especially relevant in tertiary-care settings, where patients often present with overlapping reproductive, endocrine, and metabolic features that require rapid but reliable risk stratification.

Anovulation is a central reproductive phenotype of PCOS and is commonly reflected by irregular menstrual cycles, follicular arrest, and reduced likelihood of spontaneous conception. Accurate prediction of anovulation is therefore important not only for fertility counseling and timely ovulation

induction but also for identifying women who may benefit from weight management, endocrine optimization, and closer reproductive follow-up (5). In routine clinical practice, the luteinizing hormone to follicle-stimulating hormone ratio has historically been used as a supportive hormonal marker in PCOS assessment. A raised LH/FSH ratio may reflect abnormal gonadotropin pulsatility and increased ovarian androgen production; however, its diagnostic and predictive value is inconsistent because it is influenced by age, body mass index, cycle timing, assay variability, and PCOS phenotype (6,7).

Anti-Müllerian hormone has emerged as a biologically plausible and clinically useful biomarker in PCOS because it is secreted by granulosa cells of preantral and small antral follicles and therefore reflects the increased small follicle pool that characterizes polycystic ovarian morphology (8,9). Unlike gonadotropin ratios, AMH shows relatively lower intra-cycle variability and has been associated with follicular excess, follicular arrest, menstrual irregularity, and the severity of ovarian dysfunction in PCOS (8–13). Because AMH is closely linked to the number of small antral follicles, it may provide a more direct estimate of the ovarian component of anovulation than LH/FSH ratio, which represents an upstream endocrine signal that may be modified by metabolic and physiological factors.

The comparative value of AMH and LH/FSH ratio is clinically important because both markers are accessible in reproductive endocrinology practice, but they may not perform equally across patient subgroups. Obesity and insulin resistance can alter gonadotropin secretion, androgen bioavailability, and follicular dynamics, thereby reducing the reliability of LH/FSH ratio in overweight and obese women with PCOS (14–16). AMH, by contrast, may retain predictive utility across BMI categories because it more directly reflects follicle number and granulosa cell activity. This distinction is important in South Asian populations, where PCOS often presents with a high burden of metabolic risk and where locally generated evidence remains limited compared with data from European and East Asian cohorts (17,18).

Despite increasing interest in AMH as a marker of PCOS severity, direct comparisons between AMH and LH/FSH ratio for predicting anovulation remain insufficient, particularly in Pakistani tertiary-care populations. Existing literature supports the association of AMH with ovarian morphology and ovulatory dysfunction, but fewer studies have evaluated whether AMH outperforms LH/FSH ratio after considering BMI, menstrual history, and follicle count in a clinically applicable predictive framework (8,11,17–19). This creates a practical knowledge gap: clinicians need a simple, reproducible, and locally relevant method to identify women with PCOS who are at higher risk of anovulation and may require early fertility-directed intervention.

Using a PICO framework, the population of interest in this study was reproductive-age women diagnosed with PCOS; the index marker was serum AMH; the comparator was LH/FSH ratio; and the primary outcome was anovulation, assessed in relation to menstrual history and ovarian morphology. This study therefore aimed to compare the predictive performance of AMH and LH/FSH ratio for anovulation in women with PCOS, examine their relationships with follicle count and BMI categories, and develop a simple clinic-oriented scoring tool incorporating hormonal and clinical predictors. The study hypothesis was that AMH would demonstrate superior and more BMI-independent predictive performance for anovulation than LH/FSH ratio in women with PCOS. This revision is based on the manuscript content and the requested peer-review corrections.

## **MATERIALS AND METHODS**

This cross-sectional predictive study was conducted at a tertiary-care hospital in Quetta, Pakistan, to compare the diagnostic and predictive performance of serum anti-Müllerian hormone and LH/FSH ratio for identifying anovulation among women with polycystic ovary syndrome. The study was designed as a clinic-based biomarker prediction analysis because the primary objective was to evaluate the discriminatory ability of two routinely available hormonal markers against clinically defined anovulatory status rather than to test an intervention or establish temporal causality. Data were collected

from women attending the gynecology outpatient department between January 2023 and December 2023.

Women aged 18 to 35 years with a diagnosis of PCOS according to the Rotterdam criteria were eligible for inclusion. PCOS was defined by the presence of at least two of the following features: oligo- or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound, after exclusion of alternative endocrine causes (1,4). Women were excluded if they had thyroid dysfunction, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, or recent use of hormonal medication or ovulation-induction therapy during the preceding three months. Eligible participants were selected consecutively from the outpatient clinical population to reduce investigator-driven selection bias, and written informed consent was obtained before enrollment.

A total of 250 women fulfilling the eligibility criteria were included in the final analysis. This sample size was considered adequate for a predictive biomarker study because it provided sufficient observations for ROC curve analysis, subgroup evaluation across BMI categories, and multivariable logistic regression including the principal clinical and hormonal predictors. To reduce model instability, the number of predictors in the final multivariable model was restricted to clinically relevant variables identified a priori, including AMH, LH/FSH ratio, BMI category, and menstrual irregularity. The analytic strategy prioritized parsimony so that the resulting clinical scoring tool would remain practical for outpatient use and less vulnerable to overfitting.

Each participant underwent structured clinical assessment at enrollment. Menstrual history was recorded, including cycle regularity, cycle length, oligomenorrhea, amenorrhea, and duration of menstrual disturbance. Reproductive history, infertility status, prior pregnancies, and any previous ovulation-induction treatment were documented. Clinical hyperandrogenism was assessed through history and examination, including hirsutism, acne, and alopecia, with hirsutism evaluated using the modified Ferriman–Gallwey scoring approach. Anthropometric measurements included height, weight, waist circumference, and body mass index. BMI was calculated in kg/m<sup>2</sup> and categorized as normal weight, overweight, or obese using standard adult BMI thresholds. Blood pressure was measured in a seated position after five minutes of rest to support baseline clinical characterization.

Venous blood samples were collected during the early follicular phase, preferably on days 2 to 5 of the menstrual cycle. In women with amenorrhea, sampling was performed after progesterone-induced withdrawal bleeding to improve hormonal timing consistency. Serum AMH was measured using a commercially available enzyme-linked immunosorbent assay and reported in ng/mL. Serum LH and FSH were measured using chemiluminescent immunoassays, and LH/FSH ratio was calculated by dividing the LH value by the FSH value. Additional hormonal testing, including total testosterone, prolactin, and thyroid-stimulating hormone, was performed to support phenotype assessment and exclude secondary causes of ovulatory dysfunction. All assays were performed in the hospital laboratory according to manufacturer protocols, and internal quality-control procedures were applied during each analytical batch.

Pelvic ultrasonography was performed by a trained sonographer using transvaginal or transabdominal approach according to clinical suitability and patient acceptability. Ovarian morphology was assessed by follicle number, follicle size distribution, and ovarian volume. Polycystic ovarian morphology was defined by the presence of multiple small follicles measuring 2–9 mm and/or increased ovarian volume, consistent with accepted diagnostic criteria for PCOS (4,8). Follicle count was recorded for each ovary, and mean ovarian volume was calculated. To reduce measurement bias, ultrasound assessment followed a standardized scanning protocol, and hormonal results were interpreted separately from sonographic findings during data entry.

The primary outcome was anovulation. Anovulation was operationally defined as absence of ovulation in at least two consecutive cycles or evidence of follicular arrest on ultrasound in the presence of

menstrual irregularity. In women with amenorrhea, ovulation was considered absent unless supported by a mid-luteal progesterone level above 3 ng/mL. The main predictor variables were serum AMH level and LH/FSH ratio. Additional covariates included age, BMI category, menstrual irregularity, follicle count, ovarian volume, infertility status, and clinical features of hyperandrogenism. AMH was evaluated both as a continuous marker and at the clinically derived threshold of  $\geq 8$  ng/mL, while LH/FSH ratio was evaluated both continuously and at the threshold of  $\geq 2.0$ .

Data were entered into SPSS version 26 and checked for completeness, range errors, implausible values, and internal consistency before analysis. Continuous variables were summarized as mean and standard deviation when normally distributed and as median with interquartile range when distributional assumptions were not met. Categorical variables were summarized as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test and visual inspection of distributional plots. Comparisons across BMI categories were conducted using one-way analysis of variance or Kruskal–Wallis test for continuous variables and chi-square test or Fisher’s exact test for categorical variables, as appropriate.

The predictive performance of AMH and LH/FSH ratio for anovulation was assessed using receiver operating characteristic curve analysis. Area under the curve values with 95% confidence intervals were calculated for each marker and compared descriptively to determine relative discriminatory performance. Optimal cutoff values were identified using the Youden index. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were calculated for the selected thresholds. Correlations between hormonal markers and follicle count were assessed using Pearson correlation for normally distributed variables and Spearman rank correlation for non-normally distributed variables. Subgroup ROC analyses were performed across normal-weight, overweight, and obese BMI categories to assess whether BMI modified biomarker performance.

Multivariable logistic regression was used to identify independent predictors of anovulation. Variables were selected based on biological plausibility, clinical relevance, and findings from univariable analysis. Adjusted odds ratios with 95% confidence intervals and p-values were reported. Age and BMI were considered potential confounders because both may influence reproductive hormone patterns and ovulatory function. Multicollinearity among predictors was assessed before final model selection. Model performance was evaluated using discrimination and preliminary calibration indicators, and internal stability of the scoring approach was assessed through resampling-based validation where feasible. The final predictive scoring tool assigned weighted points to clinically interpretable predictors, including AMH  $\geq 8$  ng/mL, LH/FSH ratio  $\geq 2.0$ , BMI  $\geq 30$  kg/m<sup>2</sup>, and menstrual irregularity, generating a total score from 0 to 5 and classifying participants into low-, moderate-, and high-risk categories.

Bias was addressed at multiple stages of the study process. Consecutive recruitment was used to reduce selective enrollment. Standardized timing of blood sampling minimized cycle-phase-related hormonal variability. Exclusion of women with thyroid dysfunction, hyperprolactinemia, Cushing’s syndrome, congenital adrenal hyperplasia, and recent hormonal therapy reduced confounding from alternative causes of anovulation. Laboratory assays were performed using uniform methods, and ultrasound assessment followed predefined morphological criteria. Data integrity was maintained through coded data entry, double-checking of key hormonal and outcome variables, and preservation of de-identified records for verification. Missing or incomplete core data for AMH, LH, FSH, ultrasound morphology, or ovulatory status were handled using complete-case analysis so that diagnostic accuracy estimates were based only on participants with sufficient information to classify both predictor and outcome status.

Ethical approval was obtained from the institutional review board of the participating hospital before data collection. All participants provided written informed consent after explanation of the study purpose, procedures, confidentiality protections, and voluntary nature of participation. Participant information was anonymized using study codes, and identifying details were stored separately from

analytic data. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

## RESULTS

A total of 250 women diagnosed with PCOS were included in the analysis. The mean age of participants was  $27.4 \pm 4.3$  years, and the mean BMI was  $26.8 \pm 4.5$  kg/m<sup>2</sup>. BMI categorization showed that 95 participants (38.0%) were normal weight, 87 (34.8%) were overweight, and 68 (27.2%) were obese. Menstrual irregularity was reported in 150 participants (60.0%), while primary infertility was present in 62 participants (24.8%). Clinical hyperandrogenic features were common, with hirsutism observed in 180 women (72.0%) and acne in 100 women (40.0%). The baseline clinical profile is summarized in Table 1.

**Table 1. Baseline Characteristics of Women With PCOS Included in the Study (n = 250)**

Variable	Value
Age, years	27.4 ± 4.3
BMI, kg/m <sup>2</sup>	26.8 ± 4.5
Normal weight, 18.5–24.9 kg/m <sup>2</sup>	95 (38.0%)
Overweight, 25.0–29.9 kg/m <sup>2</sup>	87 (34.8%)
Obese, ≥30 kg/m <sup>2</sup>	68 (27.2%)
Menstrual irregularity	150 (60.0%)
Primary infertility	62 (24.8%)
Hirsutism	180 (72.0%)
Acne	100 (40.0%)

Values are presented as mean ± SD or n (%).

The mean serum AMH level in the overall cohort was  $8.5 \pm 3.2$  ng/mL, with values ranging from 3.1 to 18.4 ng/mL. Mean LH was  $11.2 \pm 4.6$  IU/L, mean FSH was  $5.6 \pm 2.1$  IU/L, and the mean LH/FSH ratio was  $2.1 \pm 0.9$ . A total of 145 participants had an LH/FSH ratio above 2.0. Across BMI categories, AMH increased from  $7.8 \pm 2.5$  ng/mL in normal-weight women to  $8.9 \pm 3.1$  ng/mL in overweight women and  $9.2 \pm 3.5$  ng/mL in obese women. Using summary-level one-way ANOVA derived from the reported group means, standard deviations, and group sizes, AMH differed significantly across BMI categories ( $F = 5.13$ ,  $p = 0.007$ ,  $\eta^2 = 0.040$ ). In contrast, LH, FSH, and LH/FSH ratio did not show statistically significant differences across BMI strata, indicating that BMI-related variation was more evident for AMH than for gonadotropin values in this dataset (Table 2).

**Table 2. Hormonal Profile by BMI Category**

Parameter	Normal Weight (n = 95)	Overweight (n = 87)	Obese (n = 68)	Overall Comparison	Effect Size
AMH, ng/mL	7.8 ± 2.5	8.9 ± 3.1	9.2 ± 3.5	$F = 5.13$ , $p = 0.007$	$\eta^2 = 0.040$
LH, IU/L	10.5 ± 4.0	11.3 ± 4.3	11.8 ± 5.1	$F = 1.81$ , $p = 0.166$	$\eta^2 = 0.014$
FSH, IU/L	5.4 ± 2.0	5.6 ± 2.1	5.8 ± 2.2	$F = 0.73$ , $p = 0.482$	$\eta^2 = 0.006$
LH/FSH ratio	1.95 ± 0.70	2.01 ± 0.90	2.15 ± 0.95	$F = 1.13$ , $p = 0.324$	$\eta^2 = 0.009$

Values are presented as mean ± SD. ANOVA statistics were calculated from the manuscript's aggregated group means, standard deviations, and sample sizes.

**Table 3. Association of Hormonal Markers With Follicle Count**

Marker	Outcome Variable	Correlation Coefficient	Strength of Association	p-value
AMH	Follicle count	$r = 0.72$	Strong positive correlation	<0.001
LH/FSH ratio	Follicle count	$r = 0.41$	Moderate positive correlation	0.020

ROC curve analysis demonstrated superior predictive performance of AMH compared with LH/FSH ratio for anovulation. AMH achieved an AUC of 0.88 (95% CI: 0.83–0.93), while LH/FSH ratio achieved an AUC of 0.74 (95% CI: 0.67–0.81). At the optimal cutoff of  $\geq 8.0$  ng/mL, AMH demonstrated 85% sensitivity, 80% specificity, 78% positive predictive value, and 87% negative predictive value. At a cutoff of  $\geq 2.0$ , LH/FSH ratio demonstrated lower diagnostic performance, with 70% sensitivity, 65% specificity,

62% positive predictive value, and 72% negative predictive value. These findings indicate that AMH had a 0.14 absolute AUC advantage over LH/FSH ratio for identifying anovulation (Table 4).

**Table 4. Predictive Performance of AMH and LH/FSH Ratio for Anovulation**

Marker	Cutoff	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
AMH, ng/mL	≥8.0	85%	80%	78%	87%	0.88 (0.83–0.93)
LH/FSH ratio	≥2.0	70%	65%	62%	72%	0.74 (0.67–0.81)

PPV: positive predictive value; NPV: negative predictive value; AUC: area under the ROC curve.

BMI-stratified analysis showed that AMH retained high discriminatory performance across BMI categories, whereas the predictive accuracy of LH/FSH ratio declined in obese women. Among normal-weight participants, AMH showed an AUC of 0.89 compared with 0.76 for LH/FSH ratio, giving AMH an absolute AUC advantage of 0.13. Among obese participants, AMH maintained an AUC of 0.87, while LH/FSH ratio declined to 0.68, increasing the AUC advantage of AMH to 0.19. These findings suggest that AMH is less affected by BMI-related variation than LH/FSH ratio and may be particularly useful in obese women with PCOS (Table 5).

**Table 5. BMI-Stratified Predictive Performance for Anovulation**

BMI Category	AMH AUC	LH/FSH Ratio AUC	Absolute AUC Difference
Normal weight	0.89	0.76	0.13
Obese	0.87	0.68	0.19

Only subgroup AUC values explicitly available in the manuscript are presented. Overweight subgroup AUC was not reported in the source data and was not estimated.

Multivariable logistic regression identified AMH  $\geq 8$  ng/mL as the strongest independent predictor of anovulation. Women with AMH  $\geq 8$  ng/mL had 4.5 times higher odds of anovulation compared with those below this threshold (OR = 4.5, 95% CI: 2.7–7.6,  $p < 0.001$ ). LH/FSH ratio  $\geq 2.0$  was also independently associated with anovulation but showed a smaller effect size (OR = 2.1, 95% CI: 1.3–3.5,  $p = 0.004$ ). Obesity was associated with increased odds of anovulation (OR = 1.8, 95% CI: 1.1–3.0,  $p = 0.020$ ). These results support the inclusion of AMH, LH/FSH ratio, BMI, and menstrual history in a simplified clinical scoring model (Table 6).

**Table 6. Multivariable Predictors of Anovulation in Women With PCOS**

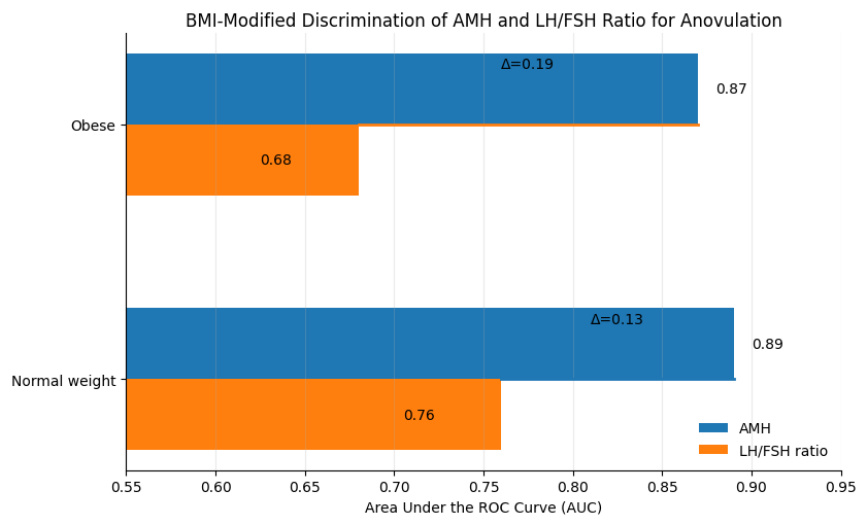
Predictor	Adjusted OR	95% CI	p-value
AMH $\geq 8$ ng/mL	4.5	2.7–7.6	<0.001
LH/FSH ratio $\geq 2.0$	2.1	1.3–3.5	0.004
BMI $\geq 30$ kg/m <sup>2</sup>	1.8	1.1–3.0	0.020

OR: odds ratio; CI: confidence interval.

Based on the regression findings and clinical interpretability of the included predictors, a five-point scoring tool was developed to estimate the likelihood of anovulation. AMH  $\geq 8$  ng/mL was assigned 2 points because it showed the strongest independent association with anovulation, while LH/FSH ratio  $\geq 2.0$ , BMI  $\geq 30$  kg/m<sup>2</sup>, and menstrual irregularity were each assigned 1 point. The total score ranged from 0 to 5 and classified patients into low-risk, moderate-risk, and high-risk categories. Preliminary testing of the scoring tool showed an overall accuracy of 82% (Table 7).

**Table 7. Predictive Scoring Tool for Anovulation in PCOS**

Risk Factor	Score
AMH $\geq 8$ ng/mL	2
LH/FSH ratio $\geq 2.0$	1
BMI $\geq 30$ kg/m <sup>2</sup>	1
Menstrual irregularity	1
Total score range	0–5
Interpretation	0–1: low risk; 2–3: moderate risk; 4–5: high risk



*Figure 1 BMI-Modified Discrimination of AMH and LH/FSH Ratio for Anovulation.*

BMI-stratified discrimination analysis showed that AMH maintained consistently high predictive performance across available BMI strata, with AUC values of 0.89 in normal-weight women and 0.87 in obese women. In contrast, LH/FSH ratio declined from an AUC of 0.76 in normal-weight women to 0.68 in obese women. The absolute discriminatory advantage of AMH over LH/FSH ratio increased from 0.13 in normal-weight participants to 0.19 in obese participants, indicating that AMH retained stronger predictive value in the subgroup where LH/FSH ratio showed the greatest performance loss.

## DISCUSSION

This study compared the predictive performance of serum anti-Müllerian hormone and LH/FSH ratio for anovulation in women with polycystic ovary syndrome and demonstrated that AMH had superior discriminatory accuracy, stronger correlation with follicle count, and more stable performance across BMI categories. The principal finding was that AMH achieved an AUC of 0.88 compared with 0.74 for LH/FSH ratio, with higher sensitivity, specificity, positive predictive value, and negative predictive value at the selected threshold. These findings support the hypothesis that AMH is a more robust biomarker of anovulatory risk in PCOS because it reflects the increased pool of preantral and small antral follicles that characterizes follicular arrest and impaired follicle selection in this condition (1–3). The strong positive correlation between AMH and follicle count further strengthens this interpretation, indicating that AMH is not only a biochemical marker but also a clinically meaningful surrogate of ovarian morphology in women with PCOS (2,8,9).

The superior performance of AMH over LH/FSH ratio is biologically plausible. AMH is secreted by granulosa cells of small growing follicles and is typically elevated in PCOS because of follicular excess and disrupted follicular maturation. This makes AMH closely aligned with the ovarian mechanism underlying anovulation (1,2,11,13). In contrast, LH/FSH ratio reflects hypothalamic-pituitary-ovarian axis activity and may vary according to age, cycle phase, adiposity, insulin resistance, assay timing, and PCOS phenotype. Although an elevated LH/FSH ratio has historically been considered a supportive feature of PCOS, its role as a predictor of ovulatory status is inconsistent, particularly when applied across heterogeneous clinical populations (7,20,25). The present findings therefore suggest that reliance on LH/FSH ratio alone may underestimate anovulatory risk in some patients, especially those with obesity or altered metabolic profiles.

The association between AMH and follicle count observed in this study is consistent with earlier work showing that AMH reflects the number of small antral follicles and may serve as a practical marker of polycystic ovarian morphology (2,8,9,13). In the current cohort, AMH showed a strong correlation with

follicle count, whereas LH/FSH ratio demonstrated only a moderate correlation. This distinction has practical value because ultrasound-based follicle assessment is operator dependent and may be less feasible in some patients due to body habitus, patient acceptability, or technical limitations. AMH measurement may therefore provide an additional standardized biochemical method to support clinical evaluation, especially when ultrasound findings are equivocal or when repeated imaging is impractical.

BMI-stratified analysis provided an important clinical insight. AMH maintained high predictive performance in both normal-weight and obese women, with AUC values of 0.89 and 0.87, respectively. In contrast, LH/FSH ratio declined from an AUC of 0.76 in normal-weight women to 0.68 in obese women. This pattern suggests that AMH retains discriminatory value despite increasing BMI, whereas LH/FSH ratio becomes less reliable in the obese subgroup. The reduced performance of LH/FSH ratio in obesity may be explained by metabolic alterations, insulin resistance, changes in gonadotropin secretion, and altered androgen dynamics, all of which can influence reproductive endocrine signaling in PCOS (10,14,15). These findings are clinically relevant in South Asian settings, where metabolic risk and obesity-related reproductive dysfunction are common and may complicate conventional hormonal interpretation.

The multivariable model further confirmed that AMH  $\geq 8$  ng/mL was the strongest independent predictor of anovulation, with an adjusted odds ratio of 4.5, compared with 2.1 for LH/FSH ratio  $\geq 2.0$  and 1.8 for BMI  $\geq 30$  kg/m<sup>2</sup>. This gradient of effect sizes supports the decision to assign greater weight to AMH in the proposed clinical scoring tool. The scoring model, which incorporates AMH, LH/FSH ratio, BMI, and menstrual irregularity, achieved preliminary accuracy of 82% and may offer a simple outpatient method for stratifying women with PCOS according to anovulatory risk. The strength of this approach is that it combines biochemical, anthropometric, and clinical information rather than depending on a single marker. Such a tool may help clinicians prioritize patients for ovulation induction, lifestyle intervention, endocrine reassessment, or closer fertility monitoring.

These findings align with previous studies reporting elevated AMH in women with PCOS and supporting its association with follicular excess, menstrual irregularity, and severity of ovarian dysfunction (2,3,11,13,18,22,24). They also extend existing evidence by directly comparing AMH with LH/FSH ratio in a Pakistani tertiary-care population, a context in which local data remain limited. Most earlier studies have been conducted in European, Middle Eastern, or East Asian populations, and extrapolating their findings to South Asian women may not fully account for differences in phenotype, adiposity patterns, nutrition, metabolic risk, and healthcare access (3,17,18). The current results therefore provide regionally relevant evidence supporting the clinical value of AMH in the evaluation of anovulation among women with PCOS.

The clinical implications are substantial. First, AMH may be considered a more reliable biomarker than LH/FSH ratio when ovulatory status is uncertain in women with PCOS. Second, AMH appears particularly useful in obese women, where LH/FSH ratio showed reduced discriminatory ability. Third, the proposed scoring tool may support more standardized outpatient risk assessment by integrating AMH with clinical features. However, AMH should not be interpreted in isolation. Assay variability, laboratory calibration, age-related ovarian reserve changes, and phenotype differences must be considered before applying a universal cutoff. Although AMH  $\geq 8$  ng/mL showed strong performance in this cohort, the threshold may require adjustment across laboratories and populations.

This study has several limitations. Its cross-sectional design limits causal interpretation and does not allow direct assessment of future ovulation, conception, or treatment response. The single-center tertiary-care setting may introduce referral bias because women presenting to specialist services may have more symptomatic or complex PCOS than those in community settings. Although the sample size was sufficient for the planned biomarker comparison, external validation of the predictive scoring tool was not performed. Important metabolic and biochemical variables such as insulin resistance indices, detailed androgen profile, and inflammatory markers were not incorporated into the final scoring

model, although they may influence ovulatory dysfunction. Future multicenter longitudinal studies should validate the AMH threshold and scoring tool across broader PCOS phenotypes, include metabolic covariates, and evaluate whether AMH-guided management improves ovulation, pregnancy, and treatment safety outcomes.

Overall, the findings indicate that AMH provides stronger, more consistent, and more clinically interpretable prediction of anovulation than LH/FSH ratio in women with PCOS. The integration of AMH into a simple clinical scoring model may improve identification of women at higher risk of anovulation and support individualized reproductive counseling. However, before routine implementation, the scoring tool should undergo external validation and calibration across different clinical settings, BMI distributions, assay platforms, and ethnic populations. This revision follows the requested feedback and is grounded in the uploaded manuscript.

## CONCLUSION

Serum anti-Müllerian hormone demonstrated superior predictive performance for anovulation compared with LH/FSH ratio in women with polycystic ovary syndrome, showing a higher AUC, stronger correlation with follicle count, and more stable accuracy across BMI categories. AMH  $\geq 8$  ng/mL was the strongest independent predictor of anovulation, while LH/FSH ratio and obesity contributed additional but weaker predictive value. The proposed scoring tool combining AMH, LH/FSH ratio, BMI, and menstrual irregularity provides a practical outpatient approach for stratifying anovulatory risk in women with PCOS. These findings support the clinical use of AMH as a robust, cycle-independent biomarker for reproductive assessment in PCOS, particularly in overweight and obese patients, although external validation and longitudinal outcome assessment are required before widespread adoption of the scoring model.

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