

VALUE OF SERUM ANTI-MULLERIAN HORMONE IN THE DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting fertility, with diagnostic challenges due to variability in traditional markers like the LH/FSH ratio. This retrospective case-control study aimed to assess the diagnostic performance of anti-Müllerian hormone (AMH) compared to the LH/FSH ratio in women undergoing infertility evaluation at Dong Do Hospital. A total of 178 PCOS cases and 422 controls were identified using the Rotterdam 2003 criteria. Serum levels of AMH, LH, FSH, and testosterone were measured. Women with PCOS showed significantly higher AMH levels than controls (median 7.65 vs. 3.17 ng/mL, $p < 0.001$). Receiver operating characteristic analysis revealed AMH had excellent diagnostic accuracy (AUC = 0.911), with a cutoff of 5.445 ng/mL yielding 84.8% sensitivity and 82.5% specificity. Multivariable logistic regression confirmed AMH as an independent predictor of PCOS (OR = 1.964, $p < 0.001$), along with LH/FSH ratio, testosterone, and BMI. Age was not a significant factor. AMH demonstrated superior diagnostic utility over the LH/FSH ratio, highlighting its potential as a reliable biomarker for PCOS diagnosis.

Keywords: Anti-Müllerian hormone (AMH), Polycystic ovary syndrome (PCOS), LH/FSH ratio, Diagnostic value.

I. INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting approximately 8 – 13% of women of reproductive age.¹ It is a leading cause of anovulatory infertility and amenorrhea. Women with PCOS are at elevated risk for numerous metabolic and reproductive complications, including impaired glucose tolerance, type 2 diabetes, obesity, cardiovascular disease, endometrial cancer, and adverse pregnancy outcomes such as miscarriage, gestational hypertension, preeclampsia, and preterm

birth.² Given these broad health implications, early and accurate diagnosis is essential for improving patient outcomes and reducing long-term morbidity.

However, PCOS diagnosis remains challenging due to the heterogeneity of its clinical presentation. In adolescents, symptoms often overlap with normal pubertal changes, making diagnosis more complex.³ The evaluation of polycystic ovarian morphology (PCOM) using ultrasound is also limited by operator dependence, reduced image quality in obese patients, and the need for transabdominal imaging in sexually inactive women.⁴ Furthermore, PCOM features may vary across the menstrual cycle, affecting diagnostic consistency.⁵ These limitations underscore the need for reliable, objective, and

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Received: 27/05/2025

Accepted: 07/08/2025

accessible biomarkers to support timely PCOS diagnosis.

Anti-Müllerian hormone (AMH), a glycoprotein secreted by granulosa cells of preantral and small antral follicles, has emerged as a promising biomarker for PCOS. Serum AMH levels correlate with the number of small follicles and are significantly elevated in women with PCOS due to increased follicular mass and disrupted folliculogenesis.⁶ Compared to ultrasound-based antral follicle count (AFC), AMH has the advantage of detecting 2 – 5mm follicles, which may be missed on imaging.⁶ Additionally, AMH levels show minimal intra- and inter-cycle variability, unlike gonadotropins and estradiol, which fluctuate with the menstrual cycle.⁵ Consequently, AMH presents a stable and convenient alternative to traditional markers of ovarian reserve and PCOS diagnosis.²

The luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio is another commonly used endocrine marker for PCOS, reflecting the typical LH hypersecretion observed in many patients.⁷ However, this ratio is highly sensitive to the timing of sample collection (typically on days 2 – 3 of the menstrual cycle) and exhibits notable intra-individual variability.^{7,8} As a result, its diagnostic accuracy is generally lower than that of AMH, particularly in cases with ambiguous clinical or ultrasound findings.⁹

Despite the diagnostic potential of AMH, no universally accepted cutoff value exists, due to variations in assay platforms and population characteristics. The 2023 International Evidence-Based Guideline for PCOS recommends assay- and population-specific AMH thresholds.² In Vietnam, research on the diagnostic value of AMH in PCOS remains limited, and proposed thresholds have demonstrated modest performance.¹⁰

At Dong Do Hospital's Reproductive Medicine Center, over 3,000 women undergo infertility screening each year, with PCOS accounting for a significant proportion of all cases. In this context, establishing an AMH cutoff and comparing its diagnostic performance to the LH/FSH ratio could enhance early diagnosis and support individualized treatment strategies, particularly in optimizing ovulation induction for assisted reproductive technologies. This study aimed to evaluate the diagnostic value of serum AMH in identifying PCOS among women at Dong Do Hospital and to compare its diagnostic performance with that of the LH/FSH ratio in this population.

II. MATERIALS AND METHODS

1. Subjects

This retrospective study reviewed medical records of female patients who sought infertility evaluation at the Reproductive Medicine Center, Dong Do Hospital (Hanoi, Vietnam), between June 2023 and March 2025. Patients were categorized into two groups based on the 2003 Rotterdam criteria for PCOS diagnosis.¹¹ The sample included 178 PCOS cases and 422 controls.

Inclusion criteria

The control group comprised infertile women without PCOS but had regular menstrual cycles of 21 – 35 days. They were enrolled during the same period as the PCOS group with exclusion of any existing ovarian disease. The PCOS Group met at least two of the following three criteria: (1) Oligo-ovulation or anovulation (cycles < 8/year, cycle duration < 21 days, or > 35 days). (2) PCOM (≥ 12 follicles measuring 2 – 9mm on transvaginal ultrasound. (3) Clinical and/or biochemical hyperandrogenism. Biochemical hyperandrogenism was defined as circulating total testosterone levels above the

97.5th percentile (≥ 1.7 nmol/L).

Ultrasound was performed on cycle days 3 – 5 in women with regular cycles, or at any time in those with oligomenorrhea or amenorrhea, ideally days 3 – 5 following induced withdrawal bleeding.

Exclusion criteria

Patients were excluded if they had:

- Anatomical causes of infertility (e.g., uterine anomalies, post-surgical adhesions)
- Conditions associated with hyperandrogenism (e.g., congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome).
- Ovarian cysts or neoplasms.
- Premature ovarian failure.
- History of long-term exogenous hormone use (e.g., contraceptives, ovulation-inducing agents) prior to testing.
- Age < 18 or > 45 years.^{12,13}

2. Methods

Assays

Serum levels of AMH, LH, FSH, testosterone, estradiol, progesterone, and prolactin were measured using an electrochemiluminescence immunoassay on the Cobas e601 platform (Roche Diagnostics, Switzerland). Daily quality control materials were analyzed to ensure the reliability of the assays.

Statistical Analysis

Data were analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Comparisons between groups were made using the independent samples t-test for normally distributed variables and the Mann–Whitney

U test for non-normally distributed variables. Diagnostic performance of AMH was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), optimal cutoff point, sensitivity, and specificity were determined using the Youden index. A p-value < 0.05 was considered statistically significant.

III. RESULTS

Clinical and Hormonal Characteristics

Among the 178 women diagnosed with PCOS, 84 were 18 – 29 years old, 51 were 30 – 34 years old, and 43 were 35 – 45 years old. In the control group, the corresponding numbers were 78, 171, and 173, respectively.

Across all age groups, women with PCOS had significantly higher mean serum AMH levels compared to controls ($p < 0.001$ for all comparisons). Specifically, AMH levels were elevated by approximately 2.02-fold in the 18 – 29 age group (9.11 ± 4.59 vs. 4.51 ± 2.17 ng/mL), 2.67-fold in the 30 – 34 age group (9.40 ± 3.37 vs. 3.51 ± 1.93 ng/mL), and 2.47-fold in the 35 – 45 age group (7.71 ± 3.25 vs. 3.12 ± 2.25 ng/mL) (Chart 1).

Overall, women with PCOS had significantly higher median levels of AMH, LH, testosterone, LH/FSH ratio, and BMI compared to controls (all $p < 0.001$; Table 1). In contrast, FSH and progesterone levels were significantly lower in the PCOS group ($p < 0.001$ for both). Estradiol levels were slightly but significantly higher in the PCOS group ($p = 0.006$), while prolactin levels did not differ between groups ($p = 0.879$).

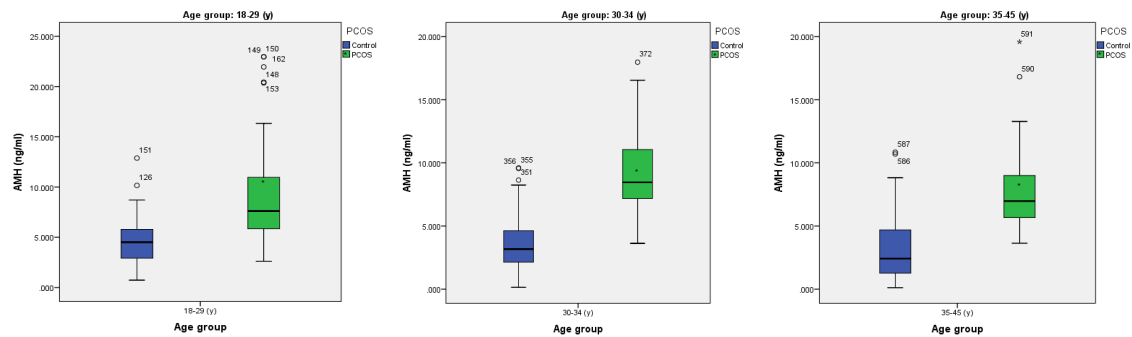


Chart 1. Boxplot comparing serum AMH levels between PCOS and control groups across age subgroups

Table 1. Comparison of hormone levels and BMI between PCOS and control groups

Parameter	PCOS (n = 178)				Control (n = 422)				p-value
	Min	Max	Median (95%CI)	IQR	Min	Max	Median (95%CI)	IQR	
AMH (ng/mL)	2.61	22.96	7.65 (7.23 – 8.46)	5.94 – 10.47	0.12	12.88	3.17 (2.92 – 3.52)	1.84 – 4.86	< 0.001
FSH (IU/L)	0.48	11.41	5.72 (5.57 – 6.08)	4.99 – 6.62	2.33	41.96	6.6 (6.46 – 6.81)	5.68 – 7.68	< 0.001
LH (IU/L)	0.14	29.73	11.08 (10.29 – 12.07)	6.98 – 14.73	0.98	36.08	5.86 (5.62 – 6.12)	4.39 – 7.4	< 0.001
LH/FSH ratio	0.29	5.72	1.91 (1.67 – 2.03)	1.24 – 2.4	0.15	6.08	0.88 (0.83 – 0.93)	0.66 – 1.16	< 0.001
Testosterone (nmol/L)	0.18	3.6	1.30 (1.18 – 1.56)	0.90 – 1.88	0.09	5.05	0.71 (0.67 – 0.76)	0.44 – 1.02	< 0.001
Prolactin (mIU/L)	75.26	1046	301.3 (276.29 – 332.1)	213.8 – 396.6	29.34	2128	300.6 (283.65 – 322.22)	212.8 – 420.2	0.879
Estradiol (pg/mL)	8.25	690.9	40.55 (38.29 – 43.15)	32.57 – 51.00	5.4	260.6	36.68 (35.3 – 38.85)	29.54 – 46.94	0.006

Parameter	PCOS (n = 178)				Control (n = 422)				p-value
	Min	Max	Median (95%CI)	IQR	Min	Max	Median (95%CI)	IQR	
Progesterone (ng/mL)	0.006	2.11	0.20 (0.15 – 0.23)	0.11 – 0.29	0.005	15.07	0.24 (0.23 – 0.26)	0.15 – 0.34	< 0.001
BMI (kg/m ²)	17.6	39.3	23.39 (22.66 – 24.97)	20.8 – 26.2	15.8	29.9	21.1 (20.8 – 21.23)	19.75 – 22.83	< 0.001

Diagnostic Value of AMH and LH/FSH Ratio

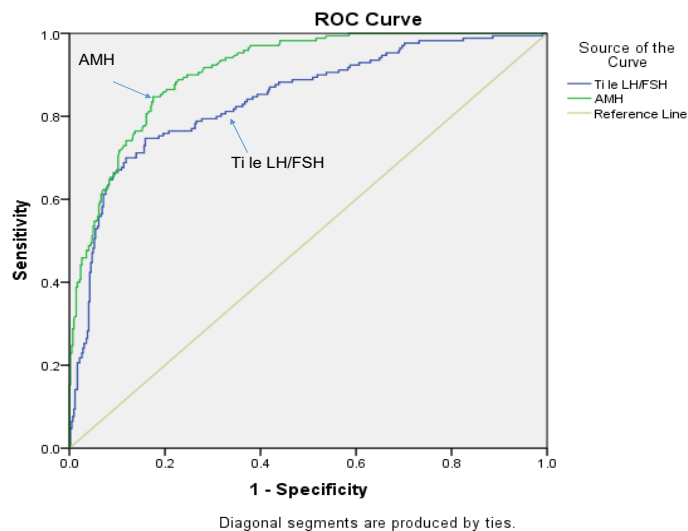


Chart 2. ROC curves for the diagnostic performance of AMH and LH/FSH ratio in distinguishing PCOS from controls

Receiver operating characteristic (ROC) curve analysis showed that AMH had superior diagnostic performance compared to the LH/FSH ratio. AMH yielded an area under the curve (AUC) of 0.911 (95% CI: 0.889 – 0.934), with a cutoff value of 5.445 ng/mL, corresponding to 84.8% sensitivity and 82.5% specificity. In comparison, the LH/FSH ratio had an AUC of 0.841 (95% CI: 0.805 – 0.878), with a cutoff of 1.279, achieving 74.7% sensitivity and 84.1% specificity ($p < 0.05$).

AMH demonstrated strong diagnostic utility for PCOS across all three age groups (AUC

> 0.85 , $p < 0.001$). In the 18 – 29 age group, AMH yielded an AUC of 0.850 (95% CI: 0.793 – 0.908). At a cutoff of 5.035 ng/mL, sensitivity was high (86.9%), but specificity was moderate (66.7%), indicating some overlap in AMH values between PCOS and control individuals. Raising the cutoff to 6.16 ng/mL improved specificity to 83.3%, at the expense of lower sensitivity (70.2%).

In the 30 – 34 age group, AMH achieved the highest diagnostic accuracy (AUC = 0.952, 95% CI: 0.925 – 0.980). A cutoff of 6.205 ng/mL provided 90.2% sensitivity and 91.2%

specificity, indicating excellent diagnostic performance.

For women aged 35 – 45 years, despite a smaller sample size ($n = 43$), AMH remained a strong predictor (AUC = 0.903, 95% CI: 0.863 –

0.944). At a cutoff of 4.94 ng/mL, sensitivity was 90.7% and specificity was 78.6%. However, due to the limited sample size in this subgroup, larger studies are needed to validate the clinical utility and stability of this cutoff.

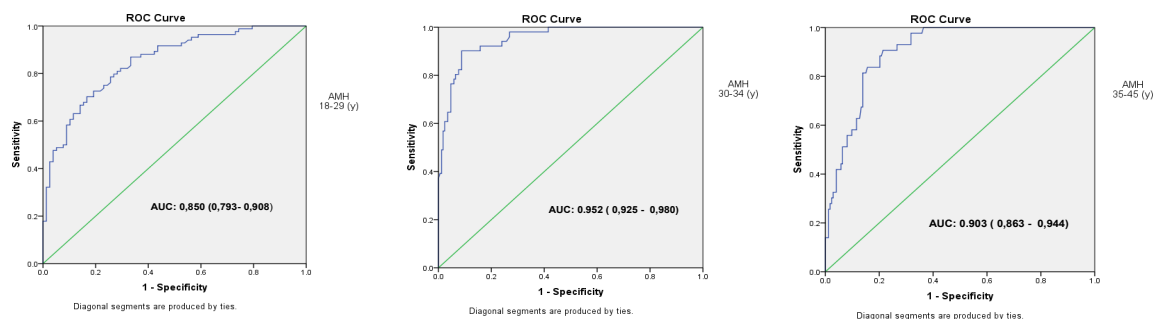


Chart 3. ROC curves for the diagnostic performance of AMH across age groups in distinguishing PCOS from controls

Table 2. Multivariable Logistic Regression Analysis of PCOS Risk Factors

Parameter	OR	95% CI for OR	p
AMH (ng/mL)	1.964	1.682 – 2.294	< 0.001
LH/FSH	2.778	1.849 – 4.174	< 0.001
Age	0.943	0.880 – 1.010	0.093
BMI (kg/m ²)	1.593	1.392 – 1.822	< 0.001
Testosterone (nmol/L)	2.850	1.721 – 4.720	< 0.001

Multivariable logistic regression was performed to identify independent predictors of PCOS. Serum AMH, LH/FSH ratio, testosterone, and BMI were all significantly associated with increased odds of PCOS. Each 1 ng/mL increase in AMH was associated with nearly a two-fold increase in PCOS risk (adjusted OR = 1.964, $p < 0.001$). The LH/FSH ratio was also strongly associated (OR = 2.778, $p < 0.001$), consistent with the known role of gonadotropin imbalance in PCOS. Elevated testosterone levels further supported the role of hyperandrogenism (OR = 2.850, $p < 0.001$). Increased BMI was independently linked to PCOS (OR = 1.593, $p < 0.001$), in line with

evidence of its contribution to insulin resistance and reproductive dysfunction.

Interestingly, age was not a statistically significant predictor in the model (OR = 0.943, $p = 0.093$), suggesting that while PCOS is more common in younger women, age alone does not independently predict risk after adjusting for hormonal and metabolic factors.

IV. DISCUSSION

The current study underscores the significant diagnostic value of Anti-Müllerian Hormone (AMH) in identifying polycystic ovary syndrome (PCOS), with an Area Under the Curve (AUC) of 0.911, sensitivity of 84.8%, and specificity of

82.5% at a cutoff of 5.445 ng/mL, as measured by the electrochemiluminescence immunoassay on the Roche Cobas platform. Multivariate logistic regression analysis further confirmed AMH as an independent predictor of PCOS, with an odds ratio (OR) of 1.964 ($p < 0.001$), even when controlling for confounders such as LH/FSH ratio, age, BMI, and Testosterone. These findings, derived from a cohort of 600 women (178 with PCOS, 47.2% aged 18 – 29), align closely with international research and highlight AMH's potential as a reliable biomarker for PCOS. The use of the standardized Cobas assay enhances the comparability of our results with global studies, while forthcoming analyses stratified by BMI and age promise to further refine AMH's diagnostic utility.

Diagnostic Accuracy of AMH

The AUC of 0.911 indicates that AMH is an excellent discriminator of PCOS, outperforming the LH/FSH ratio (AUC = 0.841, 95% CI: 0.805 – 0.878) and closely approximating the combined AMH and LH/FSH model (AUC = 0.926, 95% CI: 0.904 – 0.947). This high diagnostic accuracy is consistent with a 2013 meta-analysis by Iliodromiti et al., which reported an AUC of 0.87 (95% CI: 0.83 – 0.92) for AMH in PCOS diagnosis.¹⁴ Similarly, the 2023 international PCOS guideline, synthesizing 82 studies, reported pooled sensitivity and specificity of 79% and 87%, respectively, with AUC estimates ranging from 0.87 to 0.90.¹⁵ The slightly superior AUC in our study may be attributed to the young age distribution (47.2% of PCOS cases aged 18 – 29), where AMH is highly specific for polycystic ovarian morphology (PCOM), and the use of the Roche Cobas assay, which offers high precision and reproducibility.¹⁶

The AMH cutoff of 5.445 ng/mL aligns with global studies employing similar assays. For instance, a 2021 study from United States, also

using the Roche Cobas platform, proposed cutoffs of 7.5 ng/mL for non-obese women and 4.4 ng/mL for obese women.¹⁷ Our cutoff is within this range, suggesting its applicability in populations with comparable age and BMI profiles. In contrast, studies using different assays, such as ELISA, reported lower cutoffs (e.g., 3.1 ng/mL in Saudi Arabia, 3.98 ng/mL in Pakistan,), highlighting the assay-specific nature of AMH thresholds.^{18,19} The standardization of our AMH measurements with the Cobas assay ensures greater consistency with high-quality international studies, mitigating variability observed in meta-analyses ($I^2 = 86 - 94\%$).¹⁵

Independent Predictive Value of AMH

Our multivariate logistic regression analysis demonstrated AMH's independent predictive value (OR = 1.964, $p < 0.001$), even when adjusted for LH/FSH ratio (OR = 2.778), Testosterone (OR = 2.850), age, and BMI. This supports AMH's unique role in reflecting PCOM, distinct from other endocrine markers. The 2023 PCOS guideline endorses AMH as a potential substitute for ultrasound in detecting PCOM in adults, particularly when measured with standardized assays like Roche Cobas.^{2,15} A 2024 Chinese study similarly found AMH to be an independent risk factor for PCOS, correlating with LH and Testosterone, consistent with our findings.²⁰ The independent role of AMH is particularly pronounced in our cohort, where 47.2% of PCOS cases were aged 18 – 29, a group in which AMH is more reliable compared to adolescents (sensitivity 66%, specificity 78%) or older women.¹⁵

Influence of age stratification

The age distribution of our PCOS cohort, with 47.2% aged 18 – 29, 28.7% aged 30 – 34, and 24.1% aged 35 – 45, reflects the higher prevalence of PCOS in younger

women, consistent with its epidemiology.¹ This distribution likely enhanced AMH's diagnostic performance, as AMH levels are higher in younger women due to greater ovarian reserve. However, the decreasing prevalence of PCOS with age and the lack of statistical significance for age in our multivariate model (OR = 0.943, $p = 0.093$) suggest that AMH's diagnostic utility may diminish in women aged 35 – 45, as noted in the 2023 meta-analysis.² The stratification of patients by age reveals important differences in the diagnostic performance of Anti-Müllerian Hormone (AMH) for identifying polycystic ovary syndrome (PCOS), highlighting the necessity of age-specific interpretation in clinical practice. While AMH demonstrated consistently high diagnostic value across all age groups (AUC > 0.85), the sensitivity, specificity, and optimal cutoff values varied substantially with age, suggesting that a uniform threshold may not be appropriate for all reproductive-aged women.

In the youngest group (18 – 29 years old), AMH showed good overall discrimination (AUC = 0.850); however, the moderate specificity (66.7%) at a cutoff of 5.035 ng/mL indicates considerable overlap in AMH levels between PCOS and non-PCOS individuals. This is likely due to naturally higher AMH levels in younger women, which reduces its ability to distinguish pathological from physiological ovarian reserve. Increasing the cutoff to 6.16 ng/mL improved specificity (83.3%) but at the cost of lower sensitivity (70.2%), implying that some PCOS cases may be missed-particularly those with milder or atypical phenotypes. This trade-off highlights the challenge of balancing diagnostic sensitivity and specificity in younger populations, where AMH levels are inherently more variable.

In contrast, women at 30 – 34 years old exhibited the best diagnostic performance (AUC

= 0.952), with both high sensitivity (90.2%) and specificity (91.2%) at a cutoff of 6.205 ng/mL. This suggests that AMH is a particularly robust biomarker in this age group, possibly due to a more distinct separation in AMH levels between PCOS and non-PCOS individuals as ovarian reserve begins to physiologically decline with age. The narrower physiological AMH range in this group may enhance its discriminative capacity.

Among women at 35 – 45 years old, AMH also maintained strong diagnostic accuracy (AUC = 0.903), although the optimal cutoff value (4.94 ng/mL) was lower than in younger groups, reflecting age-related decline in AMH production. Sensitivity remained high (90.7%), but specificity was moderately reduced (78.6%). This may be due to greater biological variability or the presence of non-PCOS conditions that affect ovarian reserve in this age group. Importantly, the small sample size ($n = 43$) limits the reliability of these findings. Further research in larger cohorts is needed to validate age-specific cutoffs and ensure stable clinical performance in older reproductive-age women.

Overall, these findings support the importance of age stratification when interpreting AMH levels for PCOS diagnosis. Relying on a single cutoff across all age groups risks either overdiagnosis in younger women or underdiagnosis in older women. Age-adjusted AMH thresholds could improve diagnostic accuracy and guide more individualized clinical decision-making.

Our multivariate model identified BMI as a significant predictor (OR = 1.593, $p < 0.001$), and studies like the 2024 Chinese study have shown that AMH cutoffs vary by BMI (5.63 ng/mL for obese vs. 5.06 ng/mL for non-obese women).²⁰ Given our sample size of 178 PCOS cases, stratification into two BMI groups (approximately

71 – 89 cases per group) is statistically feasible for AUC, sensitivity/specificity, and regression analyses, as demonstrated in similar-sized cohorts.²⁰ However, combining age and BMI stratification (e.g., six subgroups) may reduce statistical power due to smaller subgroup sizes (e.g., ~20 – 30 PCOS cases), particularly for the 35 – 45 age group (n = 43 PCOS). These analyses will be essential to tailor AMH cutoffs to specific age and BMI profiles, addressing a key limitation of current global research, which lacks standardized cutoffs.²

Clinical Implications

The high sensitivity (84.8%) and specificity (82.5%) of AMH at a cutoff of 5.445 ng/mL position it as a valuable screening tool for PCOS, particularly in women aged 18 – 29, where its diagnostic accuracy is maximized. The Roche Cobas assay's standardization enhances its clinical reliability, aligning with recommendations to use AMH as a substitute for ultrasound when imaging is unavailable.² However, AMH should be integrated with Rotterdam criteria (oligo-/anovulation, hyperandrogenism, PCOM) for a comprehensive diagnosis, as it alone is insufficient.^{2,11}

Comparison with Global Studies

Our findings are highly consistent with international research. The AUC of 0.911 surpasses the 0.87 reported by Iliodromiti et al.¹⁴ and matches the 0.87 – 0.90 range in the 2023 meta-analysis.¹⁵ The cutoff of 5.445 ng/mL is comparable to international studies using the Cobas assay and slightly higher than the 4.7 ng/mL proposed by Iliodromiti et al., likely due to our younger cohort and assay differences.^{14,15} Studies using non-standardized assays reported lower cutoffs (e.g., 3.98 ng/mL in Pakistan, 3.1 ng/mL in Saudi Arabia), underscoring the importance of assay standardization.^{18,19} The predominance of young women in our study

(47.2% aged 18 – 29) likely contributed to AMH's superior performance, as AMH is less specific in adolescents and older women.²

Limitations

Despite its strengths, our study has limitations. The sample size of 178 PCOS cases is sufficient for overall analyses but may constrain subgroup analyses, particularly for the

35 – 45 age group (n = 43), where statistical power could be limited for sensitivity/specificity or regression. Another limitation is that the study was conducted at a single center; however, it initially indicates that AMH has additional diagnostic value for PCOS and that determining appropriate age-specific cutoffs is necessary to minimize misclassification. While the Roche Cobas assay ensures measurement reliability, the lack of adolescent data restricts applicability to this group, where AMH's diagnostic performance is less robust.² Finally, potential multicollinearity among AMH, LH/FSH ratio, and testosterone warrants further investigation to confirm AMH's independent contribution.

V. CONCLUSIONS

Serum AMH measured using the Cobas platform demonstrated high diagnostic performance for PCOS (AUC 0.911, sensitivity 84.8%, specificity 82.5% at a cutoff of 5.445 ng/mL), outperforming the LH/FSH ratio. AMH should be combined with the Rotterdam criteria to optimize diagnosis.

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