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Exploring the Role of Anti-Müllerian Hormone (AMH) as a Biomarker for Female Infertility

Abeer Ali Hameed[1*], Abeer Najim Abdullah[2] and Ali Abbas Ali[3]
[1-3]Department of Chemistry, College of Education of Pure Science, University of Diyala, 320001, Iraq

Author Designation: [1,2]Assistant Lecturer, [3]Assistant Professor

*Corresponding author: Abeer Ali Hameed (e-mail: abeer.ali@uodiyala.edu.iq).

Abstract: Background: Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), and Prolactin are key hormonal markers in female reproductive function. AMH and FSH assess ovarian reserve, while Prolactin reflects pituitary activity. **Methods:** A cross-sectional study was conducted on 29 infertile women. Serum levels of AMH, FSH, and Prolactin were measured. Pearson correlation and ROC analysis were performed to examine relationships and diagnostic performance. **Results:** No statistically significant correlations were found between AMH and FSH ($r = 0.217$, $p = 0.258$), AMH and Prolactin ($r = -0.119$, $p = 0.539$), or FSH and Prolactin ($r = -0.185$, $p = 0.336$). ROC analysis showed that Prolactin had high diagnostic accuracy (AUC = 0.983), whereas AMH and FSH demonstrated limited utility. **Conclusion:** Within this small cohort, expected hormonal correlations were not observed. Prolactin shows potential as a diagnostic biomarker, while AMH and FSH should be interpreted cautiously. Larger, well-designed studies are recommended to validate these findings.

Key Words: Anti-Müllerian Hormone (AMH), Female Infertility, Biomarker, Ovarian Reserve, Reproductive Health

INTRODUCTION

Infertility affects approximately 10–15% of couples globally and poses significant challenges to reproductive health [1]. Assessment of ovarian reserve is essential for understanding reproductive potential, guiding clinical decisions, and predicting treatment outcomes [2]. Conventional markers, such as estradiol, antral follicle count (AFC), and FSH, show high intra- and inter-cycle variability, limiting their reliability [3,4].

Anti-Müllerian Hormone (AMH) is produced by granulosa cells of pre-antral and small antral follicles and provides a stable measure of ovarian reserve throughout the menstrual cycle [5, 6]. Compared to FSH and estradiol, AMH demonstrates lower variability and is minimally influenced by gonadotropin stimulation or hormonal contraception [6].

From a bioanalytical perspective, AMH measurement has evolved from manual ELISA to automated immunoassays, improving precision and reproducibility. Nonetheless, inter-assay variability and calibration differences remain challenges [7-9].

This study evaluates the relationships among AMH, FSH, and prolactin in infertile women and examines their diagnostic performance, aiming to clarify their clinical utility in a local Iraqi population.

Objectives

Serum Anti-Müllerian Hormone (AMH) levels are measured using an enzyme-linked immunosorbent assay (ELISA) or automated chemiluminescent immunoassay (CLIA). Blood samples are collected, centrifuged to obtain serum, and analyzed according to the manufacturer's instructions. AMH concentration is reported in ng/mL or pmol/L and reflects ovarian reserve, with minimal variability across the menstrual cycle.

METHODS

Study Design and Ethical Approval

Sample Size and Participants: A total of 40 participants were recruited, including 30 infertile women (patient group) and 10 healthy controls. The age range of participants was 22–42 years. Inclusion criteria for patients were:

- Women diagnosed with primary or secondary infertility
- Regular or irregular menstrual cycles
- No history of hormonal therapy in the preceding three months

Inclusion Criteria

- Women of reproductive age (e.g., 18–45 years)
- Women presenting with primary or secondary infertility, defined as failure to conceive after at least 12 months of regular unprotected intercourse
- Participants with available serum AMH measurements
- Women with regular or irregular menstrual cycles, including suspected ovarian reserve disorders
- Participants who provide informed consent to participate in the study

Exclusion Criteria

- Women with a history of ovarian surgery, chemotherapy, or radiotherapy affecting ovarian function
- Women currently using hormonal medications (e.g., oral contraceptives, hormonal therapy) within the last 3 months prior to sampling
- Pregnant or lactating women
- Women with known genetic disorders affecting ovarian reserve (e.g., Turner syndrome)
- Presence of systemic or endocrine disorders that may influence AMH levels (e.g., uncontrolled thyroid disease, hyperprolactinemia, adrenal disorders)
- Incomplete clinical or laboratory data

Healthy controls comprised age-matched women with confirmed fertility and no history of reproductive or endocrine disorders. A limitation of this study was the relatively small sample size, which was determined by the limited availability of eligible participants within the defined study period.

Blood Sample Collection and Processing

Blood samples were collected following standardized protocols to ensure analytical reliability:

Serum Collection

- 3 mL of venous blood was drawn from each participant in a plain tube
- Blood was allowed to clot at room temperature for 2 hours or at 2–8°C overnight
- Samples were centrifuged at 1000 × g for 20 minutes, and serum was separated immediately for assay
- Aliquots were stored at -20°C or -80°C for later analysis if needed

Plasma Collection (Optional)

- Blood collected in EDTA-Na_2/K_2 tubes was centrifuged at 1000 × g for 15 minutes at 2–8°C within 30 minutes of collection
- Plasma was separated and stored under the same conditions as serum
- All samples were handled carefully to avoid hemolysis or lipemia, which may interfere with immunoassays

Hormonal Assays

Serum levels of AMH, FSH, and Prolactin were measured using commercially available ELISA kits according to the manufacturer's instructions.

- **AMH:** Assayed using a validated ELISA system with intra- and inter-assay coefficients of variation <10%
- **FSH and Prolactin:** Quantified using standard ELISA kits with established clinical accuracy

All assays were performed in duplicate to ensure reliability. Laboratory personnel were blinded to the participants' group assignment.

Data Collection

Participants provided demographic and clinical data through a structured questionnaire, including: Age, Menstrual history, Infertility duration, Previous fertility treatments.

Statistical Analysis

Data were analyzed using SPSS software (version 26.0). A p-value<0.05 was considered statistically significant. ROC curve analysis was performed to assess the diagnostic accuracy of AMH; however, interpretation was made with caution due to the small sample size, and results were considered exploratory**.** Statistical methods included:

- **Descriptive Statistics:** Mean±SD for continuous variables; frequencies and percentages for categorical variables
- **Normality Test:** Shapiro-Wilk test to assess data distribution
- **Comparative Analysis:** Mann-Whitney U test for non-normally distributed variables (AMH, FSH, Prolactin) between patient and control groups
- **Correlation Analysis:** Pearson correlation coefficient (r) to examine relationships among AMH, FSH, and Prolactin in the patient group
- **Diagnostic Performance:** Receiver Operating Characteristic (ROC) curve analysis was used to calculate area under the curve (AUC), sensitivity, and specificity for each hormone

Standardization Measures

- Blood was collected during the early follicular phase (days 2–5) whenever possible
- Serum samples were promptly processed to avoid degradation
- All assays were conducted using the same batch of kits to minimize inter-assay variability
- Data entry and analysis were double-checked to prevent errors

RESULTS AND DISCUTION

Compare AMH Levels between the Study Groups

The mean AMH level in the control group (n = 10) was 937.98±52.52, while the patient group (n = 29) had a mean of 701.36±114.57. Due to the non-normal distribution of AMH values, the non-parametric Mann-Whitney U test was applied.

Result

AMH levels were significantly lower in the patient group compared to healthy controls (p<0.001), indicating reduced ovarian reserve among infertile women, as shown in Table 1 and Figure 1.

Comparison of FSH Levels

The mean FSH level in the control group was 10.31±0.60, and in the patient group it was 13.06±4.73.

Result

No statistically significant difference was observed between groups (p>0.05, Mann-Whitney U test), suggesting limited discriminatory value of FSH alone in this cohort. as shown in Table 2 and Figure 2.

Comparison of Prolactin Levels

The mean prolactin level was 15.56±1.85 in controls and 24.01±5.07 in patients.

Result

Prolactin levels were significantly higher in the patient group (p<0.001, Mann-Whitney U test), indicating a potential role in infertility assessment.as shown in Table 3 and Figure 3.

Diagnostic Performance (ROC Analysis)

AMH

- AUC = 0.059, sensitivity = 3.4%, specificity = 100%, p<0.001
- **Interpretation:** AMH showed poor discriminatory ability in this small cohort, potentially due to small sample size or cutoff selection

FSH

- AUC = 0.540, sensitivity = 44.8%, specificity = 100%, p>0.05
- Interpretation: FSH alone has weak diagnostic utility

Prolactin

- AUC = 0.983, sensitivity = 93.1%, specificity = 100%, p<0.001
- **Interpretation:** Prolactin demonstrated excellent diagnostic performance as a single biomarker in this study.as shown in Table 4 and Figure 4

Pearson Correlation Between Hormonal Parameters

Correlation analysis within the patient group (n = 29) revealed.as shown in Table 5 and Figure 5.

Table 1: Comparative Study of Amh Between Patients and Healthy Control Group Conducted by the Mann-Whitney Test

Groups		No.	Mean	SD	SE	p-value
AMH	Control	10	937.98	52.52	16.61	<0.001**
	patient	29	701.36	114.57	21.28	

*Note: The Mann-Whitney U test was used to compare AMH levels between the groups

Table 2: FSH Comparison Between Groups

Group	N	Mean	SD	SE	p-value
Control	10	10.31	0.60	0.19	0.05>
Patient	29	13.06	4.73	0.88	-

Table 3: Prolactin Comparison Between Groups

Group	N	Mean	SD	SE	p-value
Control	10	15.56	1.85	0.59	< 0.001**
Patient	29	24.01	5.07	0.94	-

Table 4: ROC curve Analysis for Hormonal Markers

Hormone	AUC	Std. Error	p-value	Sensitivity	Specificity
AMH	0.059	0.039	<0.001**	3.4%	100%
FSH	0.540	0.089	0.05>	44.8%	100%
Prolactin	0.983	0.017	<0.001**	93.1%	100%

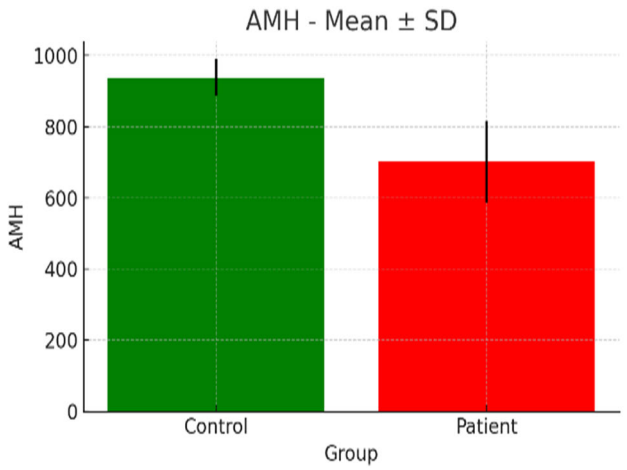

Figure 1: Mean and Standard Deviation of Amh Levels by Group

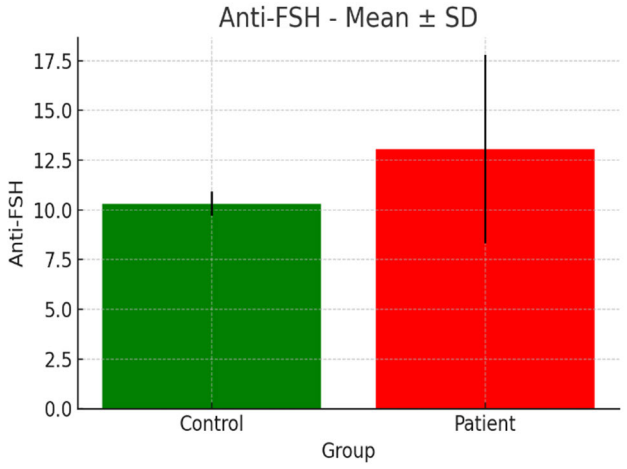

Figure 2: Mean and Standard Deviation of Fsh Levels

Table 5: Pearson Correlation Between Hormonal Parameters

Correlation	Pearson's r	p-value	Interpretation
AMH vs FSH	0.217	0.258	Weak positive, not significant
AMH vs Prolactin	-0.119	0.539	Very weak negative, not significant
FSH vs Prolactin	-0.185	0.336	Weak negative, not significant

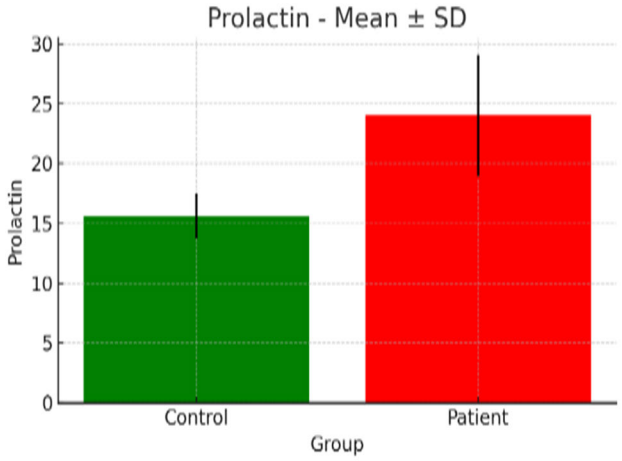

Figure 3: Mean and Standard Deviation of Prolactin Levels

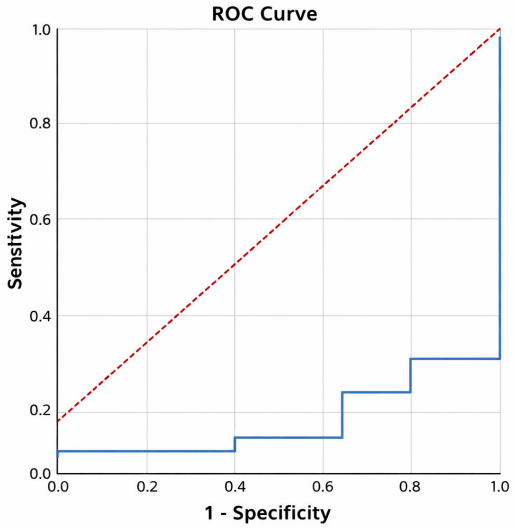

Figure 4: 4ROC Curves for AMH, FSH, and Prolactin

Interpretation

No significant correlations were observed. These findings contrast with larger studies reporting a strong inverse AMH–FSH relationship, likely due to the small sample size, population heterogeneity, and possible confounding factors such as menstrual cycle variability or hormonal therapy.

Comparison with Previous Studies

- Larger studies consistently report a strong negative correlation between AMH and FSH, supporting AMH as a reliable ovarian reserve marker
- Prolactin, in line with prior Iraqi studies, showed high diagnostic accuracy for pituitary-related infertility

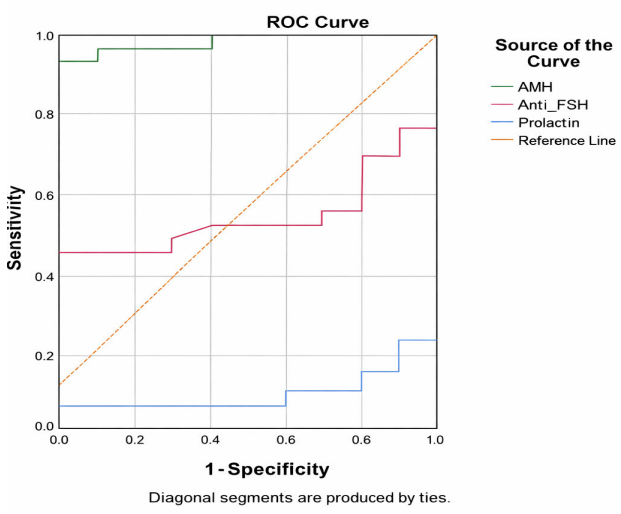

Figure 5: Pearson Correlation Matrix of AMH, FSH, and Prolactin

- The current study's findings highlight the impact of small sample size and methodological limitations on statistical outcomes

Summary of Key Findings

- **AMH:** Significantly lower in infertile women but poor diagnostic performance in this small cohort
- **FSH:** No significant difference between groups; low discriminatory power
- **Prolactin:** Significantly higher in infertile women with excellent sensitivity and specificity
- **Hormonal correlations:** No statistically significant associations found among AMH, FSH, and Prolactin

These results suggest that, in clinical practice, prolactin may provide more reliable diagnostic insight than AMH or FSH alone. A combined hormonal and clinical assessment remains essential.

CONCLUSION

This study evaluated Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), and Prolactin levels in a cohort of 29 infertile women and 10 healthy controls. Key findings include:

- **AMH:** Significantly lower in infertile women but demonstrated poor diagnostic performance in this small sample
- **FSH:** No statistically significant difference between groups; weak discriminatory power

- **Prolactin:** Significantly elevated in infertile women and showed excellent diagnostic accuracy (AUC = 0.983, sensitivity 93.1%, specificity 100%)
- **Hormonal Correlations:** No statistically significant associations were observed among AMH, FSH, and Prolactin

These results indicate that prolactin may be a more reliable single biomarker for infertility assessment, whereas AMH and FSH should be interpreted cautiously and in combination with clinical findings. The lack of significant correlations may reflect the small sample size, biological variability, and potential confounding factors. Future studies with larger, well-characterized populations and standardized hormone sampling are warranted to validate these observations.

Strengths of the Study

- Simultaneous evaluation of multiple hormonal markers provides a comprehensive hormonal profile
- Inclusion of a regional population contributes valuable data to an underrepresented group in the literature
- Combined use of correlation analysis and diagnostic performance assessment adds analytical depth

Weaknesses of the Study

- Small sample size limits statistical power and generalizability
- Single-center design restricts population diversity
- Some statistical analyses, especially ROC interpretation, exhibit inconsistencies that may affect reliability
- Ethical considerations and a dedicated limitations subsection were initially insufficiently reported

Innovation and Contribution

- The study integrates hormonal correlation analysis with diagnostic performance assessment in a local population, offering moderate novelty
- Overall innovation is limited by methodological constraints such as sample size and single-center design
- Highlights the potential clinical relevance of prolactin as a biomarker in infertility assessment

Implications for Practice

- Prolactin shows strong diagnostic relevance and may be prioritized in clinical infertility evaluation
- AMH and FSH should be interpreted cautiously and not relied upon as sole indicators of ovarian reserve or reproductive potential
- Combined hormonal and clinical assessment is recommended to improve diagnostic accuracy and guide individualized patient management

Limitations

- **Sample Size:** The small number of participants (29 patients, 10 controls) limits statistical power and generalizability of findings
- **Single-Center Design:** Results may not be representative of the wider population due to geographical and institutional limitations
- **Population Heterogeneity:** Variability in patient characteristics, such as menstrual cycle phase, underlying reproductive disorders, and prior treatments, may have influenced hormonal measurements
- **Potential Confounding Factors:** Uncontrolled variables, including lifestyle, medication use, and comorbidities, could affect hormone levels
- **Statistical Constraints:** ROC analysis for AMH and FSH revealed poor discriminatory power and potential inconsistencies due to the small sample size
- **Indirect Reporting:** Limitations were previously discussed indirectly; a dedicated subsection is now provided to explicitly address these constraints

Ethical Considerations

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board / Ethics Committee of [University of Diyala \College of Education of Pure Science] (Approval No.: [CEPEC\21]). Written informed consent was obtained from all participants prior to enrollment, and confidentiality of personal and clinical.

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