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EVALUATION OF ANTI MULLERIAN HORMONE, FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE LEVELS IN WOMEN WITH INFERTILITY

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Abstract

Background: *Infertility is a growing reproductive health concern affecting a significant proportion of women worldwide, with higher prevalence reported in developing countries such as Pakistan. Assessment of ovarian reserve is essential in infertility evaluation, and hormonal markers such as Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH) play a key role in understanding reproductive potential.*

Objective: *To evaluate serum levels of AMH, FSH, and LH in infertile women and to assess their relationship with ovarian reserve and infertility patterns.*

Methodology: *A cross-sectional study was conducted on 80 infertile women aged 18–45 years at Sir Ganga Ram Hospital and associated laboratories, Lahore. Blood samples were collected to measure AMH, FSH levels measured during days 2-5 of the menstrual cycle, and LH levels using standard immunoassay techniques. Data were analyzed using SPSS version 25. Descriptive statistics, Pearson correlation, and independent t-tests were applied, with $p < 0.05$ considered statistically significant.*

Results: *The mean age of participants was 29.4 ± 6.1 years, with primary infertility observed in 65% of cases. The mean AMH level was 2.14 ± 1.87 ng/mL, with 36.3% showing low AMH indicative of diminished ovarian reserve. Elevated FSH levels were found in 41.3% of participants and were significantly higher in older age groups ($p < 0.001$). LH/FSH ratio >2 was observed in 21.3% of women, suggesting PCOS. A significant negative correlation was found between AMH and FSH ($r = -0.62$, $p < 0.001$). Women with primary infertility had significantly lower AMH and higher FSH levels compared to secondary infertility.*

Conclusion: *AMH is a reliable and stable marker of ovarian reserve, while FSH and LH provide complementary information on endocrine function. The combined assessment of these hormones enhances the accuracy of infertility evaluation and supports improved clinical decision-making.*

INTRODUCTION

Infertility, defined as failure to achieve pregnancy after 12 months of regular unprotected intercourse, affects approximately 10–15% of couples globally, with higher prevalence (18–21%) reported in Pakistan due to delayed marriages, untreated reproductive tract infections, and limited healthcare access [1,2]. Female infertility is multifactorial, including ovulatory dysfunction, tubal obstruction, endometriosis, and hormonal imbalances, but ovarian dysfunction remains central [3].

Ovarian reserve—the number and quality of remaining oocytes—declines naturally with age, particularly after 35 years, and is accelerated by genetic predisposition, autoimmune diseases, smoking, obesity, and medical interventions such as chemotherapy [4]. Historically, ovarian reserve has been assessed using basal FSH, estradiol, and antral follicle count (AFC). However, FSH fluctuates significantly across the menstrual cycle and provides unreliable results when interpreted alone, while AFC is operator-dependent and requires ultrasound expertise [5].

In recent years, Anti-Müllerian Hormone (AMH) has emerged as a superior biomarker. Secreted by granulosa cells of pre-antral and small antral follicles, AMH levels remain relatively stable throughout the menstrual cycle, making it a convenient and reliable indicator of the remaining follicular pool [6]. Studies by Peigné et al. (2022) and Nelson et al. (2023) have confirmed that AMH is a consistent marker of ovarian reserve, though it predicts oocyte quantity rather than quality or natural conception outcomes [7,8]. Van der Ham et al. (2024) demonstrated that elevated AMH is strongly associated with polycystic ovary syndrome (PCOS) due to increased small antral follicle counts [9].

Despite AMH's advantages, it has limitations. Salemi et al. (2024) found that while AMH and AFC are the best predictors of ovarian response in assisted reproductive technologies (ART), no single marker is sufficient to determine overall reproductive potential [10]. Lensen et al. (2024) warned against overinterpretation of AMH as a measure of natural fertility, emphasizing that anxiety or false reassurance may result from misinterpretation [11]

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) remain valuable complementary markers. Elevated basal FSH traditionally indicates reduced ovarian reserve, while an elevated LH/FSH ratio (>2) is characteristic of PCOS-related anovulation [12]. Das et al. (2024) reported that high FSH correlates with poor ovarian response, but normal FSH does not guarantee normal reserve, reinforcing the need for combined testing [13].

In Pakistan and other South Asian populations, limited local data on combined AMH, FSH, and LH profiles hinders accurate clinical decision-making. Jaffar et al. (2023) reported significant ethnic differences in AMH levels between South Asian and Western populations, emphasizing the need for region-specific reference ranges [14]. This study therefore aimed to evaluate serum levels of AMH, FSH, and LH in infertile women and to assess their relationship with ovarian reserve and infertility patterns in a local Pakistani setting.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted at the Department of Gynecology, Sir Ganga Ram Hospital, and associated diagnostic laboratories (Green Life Medical Lab, Mughal Lab) in Lahore, Pakistan, over 6 months after synopsis approval.

Participants

Using non-probability consecutive sampling, 80 infertile women were enrolled. Sample size was calculated using Cochran's formula (Z=1.96, estimated infertility prevalence in Pakistan=20%, margin of error=0.09), yielding a minimum of 47; 80 were recruited to increase statistical power.

Inclusion criteria: Women aged 18–45 years, diagnosed with primary or secondary infertility per WHO definition, regular or irregular

menstrual cycles, and willing to provide informed consent.

Exclusion criteria: Hormonal contraceptive or ovulation-inducing drug use within 3 months; history of chemotherapy, radiotherapy, or ovarian surgery; known endocrine disorders (thyroid disease, hyperprolactinemia, Cushing syndrome); pregnancy; severe systemic illness.

Data Collection and Hormonal Assays

After written informed consent, demographic and clinical data (age, infertility duration, type, menstrual regularity) were recorded. Venous blood (3–5 mL) was collected on days 2–5 of the menstrual cycle for FSH and LH; AMH was measured on any cycle day. Serum was separated by centrifugation. AMH was measured using enzyme-linked immunosorbent assay (ELISA), while FSH and LH were measured using chemiluminescent immunoassay (CLIA) on an automated analyzer per manufacturer instructions.

Ethical Considerations

Ethical approval was obtained from the Ethical Committee of Superior University, Lahore. All participants provided written informed consent. Confidentiality and anonymity were maintained, and participants were informed of their right to withdraw at any time.

Statistical Analysis

Data were analyzed using SPSS version 25. Descriptive statistics (mean ± SD) were calculated for AMH, FSH, and LH. Pearson correlation tested relationships between hormones. Independent t-test compared hormonal levels by infertility type. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 80 infertile women were enrolled in this cross-sectional study. The mean age of the study participants was 29.4 ± 6.1 years. The majority of women (52.5%) were in the 26–35-year age group. Primary infertility was observed in 65% of cases, while secondary infertility was present in 35%.

Table 1: Demographic characteristics of study participants (n = 80)

Variable	Category	n	Percentage (%)
Age group (years)	18–25	25	31.3
	26–35	42	52.5
	36–45	13	16.3
Type of infertility	Primary	52	65.0
	Secondary	28	35.0
Duration of infertility	1–2 years	22	27.5
	3–5 years	35	43.8
	>5 years	23	28.8
Menstrual regularity	Regular	48	60.0
	Irregular	32	40.0

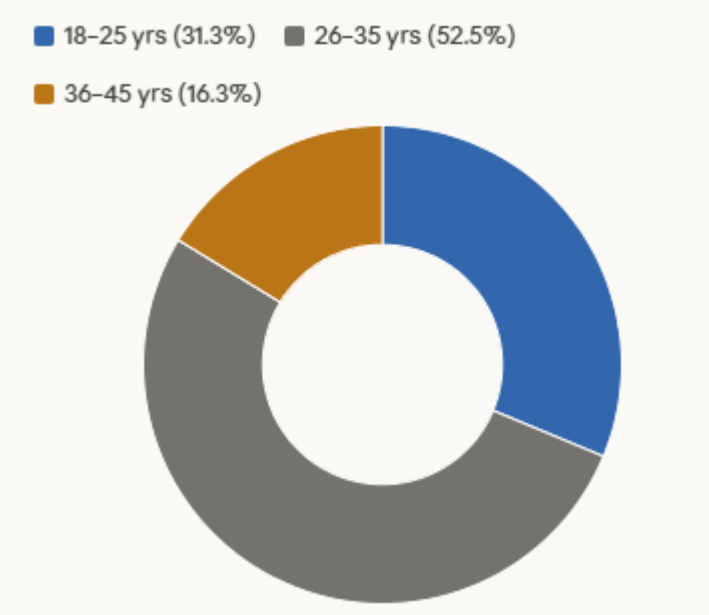

Figure 1: Age group distribution of study participants

Serum AMH levels ranged from 0.10 to 8.90 ng/mL with a mean of 2.14 ± 1.87 ng/mL. According to standard clinical cut-offs, 36.3% of participants had low AMH levels indicating diminished ovarian reserve, 46.3% had normal AMH levels, and 17.5% showed elevated AMH suggestive of PCOS.

Table 2: AMH levels by infertility type (n = 80)

AMH Category	Cut-off	Total (n=80)	Primary (n=52)	Secondary (n=28)
Low	<1.0 ng/mL	29 (36.3%)	21 (40.4%)	8 (28.6%)
Normal	1.0–3.5 ng/mL	37 (46.3%)	24 (46.2%)	13 (46.4%)
High	>3.5 ng/mL	14 (17.5%)	7 (13.5%)	7 (25.0%)
Mean ± SD (ng/mL)		2.14 ± 1.87	1.92 ± 1.74	2.53 ± 2.08

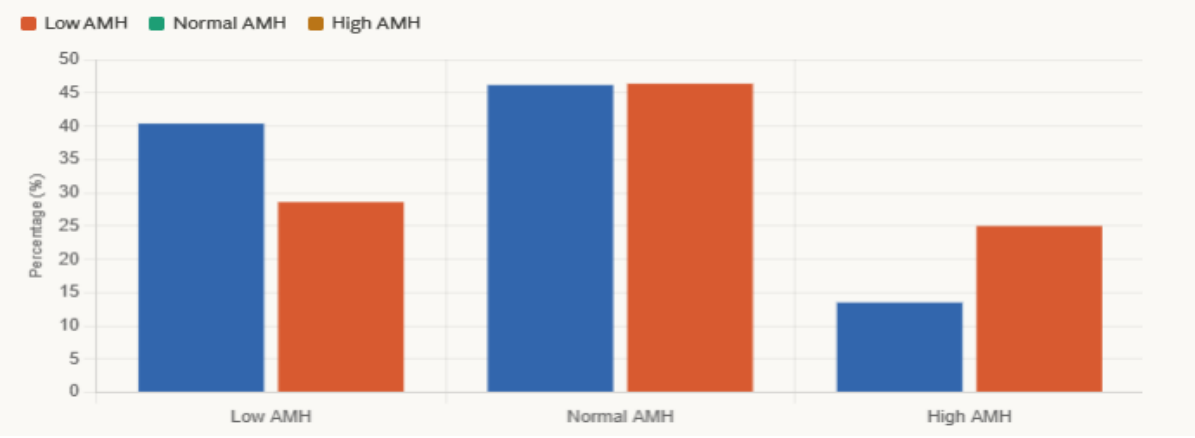

Figure 3. AMH category distribution by infertility type

The mean basal FSH level was 9.82 ± 5.64 mIU/mL. Elevated FSH levels (≥10 mIU/mL) were observed in 41.3% of participants. Women aged 36–45 years demonstrated significantly higher FSH levels compared to younger age groups ($p < 0.001$).

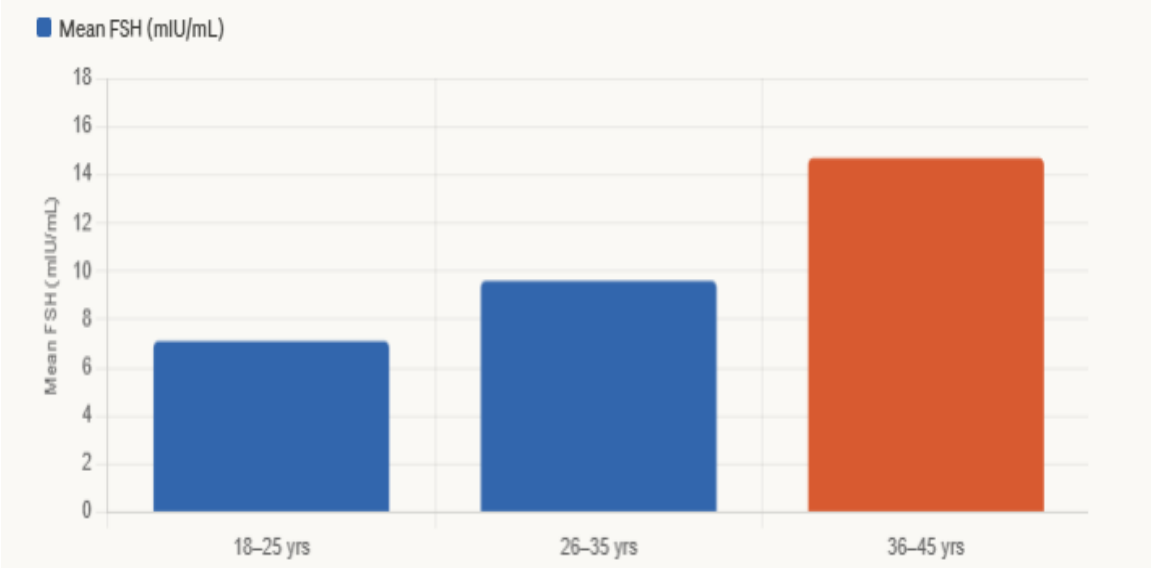

Figure 4. Mean FSH levels across age groups

The mean serum LH level was 7.63 ± 4.51 mIU/mL. An LH/FSH ratio >2 was observed in 21.3% of participants, mainly among women with elevated AMH levels suggestive of PCOS.

Pearson correlation analysis revealed a significant negative correlation between AMH and FSH ($r = -0.62$, $p < 0.001$). A moderate negative correlation was also found between AMH and LH ($r = -0.38$, $p = 0.001$), while FSH and LH demonstrated a moderate positive correlation ($r = +0.44$, $p < 0.001$).

Table 3: Pearson correlation matrix (AMH, FSH, LH

Variable	AMH	FSH	LH
AMH	1.00	−0.62	−0.38
FSH	−0.62	1.00	+0.44
LH	−0.38	+0.44	1.00

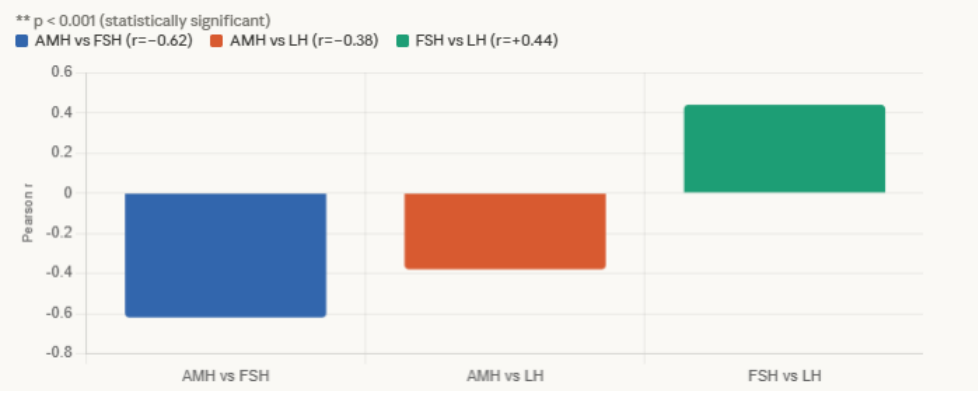

Figure 4: Pearson correlation coefficients between AMH, FSH, LH

Independent t-test analysis showed that women with primary infertility had significantly lower AMH levels (1.92 ± 1.74 ng/mL) and higher FSH levels (10.6 ± 5.9 mIU/mL) compared to women with secondary infertility.

Table 7: Comparison of hormonal levels by infertility type

Hormone	Primary infertility (n=52) Mean ± SD	Secondary infertility (n=28) Mean ± SD	t-value	p-value
AMH (ng/mL)	1.92 ± 1.74	2.53 ± 2.08	−2.43	0.018
FSH (mIU/mL)	10.6 ± 5.9	8.4 ± 4.8	+2.16	0.034
LH (mIU/mL)	7.9 ± 4.7	7.1 ± 4.2	+0.95	0.210 (NS)
LH/FSH ratio	0.84 ± 0.49	0.96 ± 0.58	−1.12	0.268 (NS)

DISCUSSION

This study of 80 infertile women in Lahore demonstrates that diminished ovarian reserve (DOR), indicated by low AMH and elevated FSH, is a major contributor to infertility, particularly in primary infertility cases. The mean age (29.4±6.1 years) reflects the prime reproductive years, yet 36.3% already had low AMH, suggesting that age-independent factors such as genetic predisposition, environmental exposures, or delayed healthcare seeking contribute significantly to ovarian dysfunction in our population [14].

The prevalence of low AMH (36.3%) and elevated FSH (41.3%) in our cohort is comparable to international findings. Broer et al. (2014) reported that AMH below 1.0 ng/mL reliably predicts poor ovarian response, while Tal et al. (2015) demonstrated that AMH correlates with cumulative live birth rates independent of age [15,16]. The strong negative correlation between AMH and FSH ($r=-0.62$, $p<0.001$) reflects the physiological feedback mechanism: declining ovarian follicular activity reduces negative feedback on the pituitary, increasing FSH secretion [17].

Age-related FSH elevation in our study (14.7±6.2 mIU/mL in women aged 36–45 years) aligns with Klein et al. (1996), who described accelerated follicular development and monotrophic FSH rise during reproductive aging [18]. Notably, younger women (≤25 years) showed higher LH levels relative to FSH, likely reflecting PCOS-related ovulatory dysfunction rather than diminished reserve.

The finding that 21.3% of participants had an LH/FSH ratio >2, predominantly with irregular cycles and higher mean LH (10.2±5.1 mIU/mL), is consistent with PCOS diagnostic criteria. Adams et al. (2004) reported similar elevated LH/FSH ratios in up to 25% of PCOS populations, and Balen et al. (1995) established that LH imbalance is strongly associated with anovulatory infertility [19,20].

Primary infertility patients had significantly lower AMH and higher FSH than secondary infertility patients, indicating greater ovarian reserve depletion in the primary infertility group. This aligns with Mutsaerts et al. (2016), who reported that primary infertility is more strongly associated with DOR, whereas secondary infertility often involves tubal or uterine factors [21].The absence of significant LH differences between groups suggests LH is more indicative of specific endocrine disorders like PCOS rather than infertility type.

The combined hormonal profile—low AMH, elevated FSH, and abnormal LH/FSH ratio—provides complementary information that individual markers cannot offer alone. The European Society of Human Reproduction and Embryology (ESHRE) recommends this multi-marker approach for comprehensive infertility evaluation [22].

LIMITATIONS

This cross-sectional study cannot establish causality. Long-term outcomes such as pregnancy or live birth were not assessed. BMI, lifestyle factors, and male partner infertility were not included. Future longitudinal multicenter studies are needed to establish population-specific reference ranges for Pakistani women.

CONCLUSION

Diminished ovarian reserve is a major contributor to infertility, especially in primary infertility cases. AMH is a sensitive and stable marker, while FSH and LH provide complementary endocrine insights. Combined hormonal evaluation offers a more accurate and comprehensive approach to infertility assessment than any single marker alone. Routine multi-marker testing should be integrated into infertility workups in Pakistan.

RECOMMENDATIONS

Routine assessment of AMH, FSH, and LH should be incorporated in infertility workup for early detection of ovarian dysfunction. Clinicians should use combined hormonal evaluation rather than relying on a single marker for better diagnostic accuracy.

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