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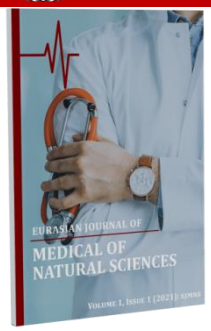

PROGNOSTIC VALUE OF HORMONAL, IMMUNOLOGICAL AND ULTRASONOGRAPHIC PARAMETERS IN FERTILITY RESTORATION AMONG WOMEN OF LATE REPRODUCTIVE AGE

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ABSTRACT

This study evaluated the prognostic significance of hormonal (FSH, LH, estradiol, progesterone, testosterone, AMH), immunological (VEGF, TGF-β, and IGF-1), and ultrasonographic (ovarian volume, antral follicle count, endometrial thickness) parameters in fertility restoration among women of late reproductive age with diminished ovarian reserve. Based on ROC analysis, the sensitivity and specificity of these markers were determined, providing a scientific foundation for the development of an individualized reproductive prediction model.

INTRODUCTION

In recent decades, the trend of delayed childbearing among women has been recognized as a significant global demographic and clinical challenge [27, 31]. Currently, the demand for pregnancy in women over the age of 35 continues to grow; however, a marked decline in fertility potential during this age period has led to an increase in infertility cases within this group [20, 30]. According to the World Health Organization (WHO), the global prevalence of infertility is approximately 17.5%, whereas in women aged 36–40 years, this rate reaches 25–30%, and rises to 40–50% in women over the age of 40 [5, 21]. Therefore, developing clinical approaches aimed at assessing ovarian reserve, restoring ovulatory function, and effectively predicting fertility in women of late reproductive age is one of the key priorities of modern gynecology and reproductive medicine [19, 28].

It is well established that diminished ovarian reserve (DOR) may develop due to physiological or pathogenic factors, often accompanied by hormonal imbalance, echographic trophic changes, decreased ovulatory function, and deterioration in oocyte quality. To facilitate early detection and assessment of this condition, various diagnostic parameters have been proposed [1, 18]. Among these, anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and the antral follicle count (AFC) as an ultrasound marker are widely employed to evaluate reproductive reserve [4, 16, 21].

However, the individual and combined prognostic accuracy of these markers in predicting fertility—particularly based on receiver operating characteristic (ROC) analysis assessing

sensitivity and specificity—has been insufficiently explored [14, 17]. Moreover, there is a lack of standardized protocols integrating these markers with echographic and clinical data [12, 30].

In clinical practice, ovarian reserve is most often assessed using only primary hormonal tests, which are suboptimal in determining treatment tactics and reproductive strategies in high-risk women [3, 11, 23]. In particular, for preserving or restoring reproductive function in women of late reproductive age, individualized prediction methods based on biomarker assessment are urgently needed [13, 25]. These methods should not only serve diagnostic purposes but also guide therapeutic decision-making [10, 24].

The aim of this study was to comprehensively evaluate the clinical and statistical significance of hormonal and echographic parameters in predicting fertility outcomes in women of late reproductive age with diminished ovarian reserve.

MATERIALS AND METHODS

This study was conducted between 2021 and 2024 at the Republican Perinatal Center and the "NS-Medical" clinic. A total of 120 women aged 35–41 years with diminished ovarian reserve were enrolled and classified into three groups based on the POSEIDON criteria. Group I (n = 50) included women aged 35–38 years with anti-Müllerian hormone (AMH) levels <1.0 ng/mL and antral follicle count (AFC) <5 (POSEIDON Group 3).Group II (n = 40) comprised women aged 39–41 years with AMH <0.5 ng/mL and AFC <3 (POSEIDON Group 4).Group III (n = 30) served as the comparison group and included women aged 35–41 years with AMH <1.0 ng/mL and AFC <5 but not receiving individualized interventions.

All participants underwent comprehensive diagnostic evaluations. Hormonal analysis included the measurement of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, testosterone, and AMH levels. Blood samples were collected during the early follicular phase (cycle days 2–4), and hormonal assays were performed using an automated immunochemiluminescence method.

Transvaginal ultrasonographic assessments (Voluson P8, GE Healthcare) were performed to determine ovarian volume, AFC, follicular diameters, and endometrial thickness. Doppler ultrasonography was used to assess ovarian and uterine artery blood flow, calculating resistance index (RI) and pulsatility index (PI).

To determine the predictive accuracy of hormonal and echographic parameters for fertility outcomes, receiver operating characteristic (ROC) analysis was conducted. Sensitivity, specificity, and area under the curve (AUC) values were calculated. In addition, Pearson correlation coefficients were used to assess the degree of association between hormonal indicators and ultrasound parameters.

RESULTS

The hormonal profiles of the women enrolled in the study revealed the following: In Group I, the FSH level was moderately elevated at 14.5 ± 0.51 mIU/mL ($P < 0.01$), while estradiol was low at 25.6 ± 0.87 pg/mL, progesterone was also low at 1.8 ± 0.06 ng/mL, and testosterone was slightly elevated at 1.7 ± 0.05 ng/mL. The AMH level was reduced at 0.70 ± 0.024 ng/mL ($P < 0.001$), and LH was slightly elevated at 13.6 ± 0.47 mIU/mL, though not statistically significant ($P > 0.05$).

In Group II, FSH was significantly elevated at 15.8 ± 0.54 mIU/mL ($P < 0.001$), estradiol was low at 22.1 ± 0.74 pg/mL, progesterone was low at 1.9 ± 0.07 ng/mL, and testosterone was slightly elevated at 1.8 ± 0.06 ng/mL. The AMH level was markedly low at 0.30 ± 0.011 ng/mL ($P < 0.001$), and LH was slightly elevated at 13.4 ± 0.46 mIU/mL ($P > 0.05$).

In the comparison group (Group III), the hormonal indicators were as follows: FSH – 13.8 ± 0.49 mIU/mL, LH – 13.0 ± 0.45 mIU/mL, estradiol – 26.2 ± 0.90 pg/mL, progesterone – 1.7 ± 0.05 ng/mL, testosterone – 1.6 ± 0.05 ng/mL, and AMH – 0.85 ± 0.028 ng/mL.

Table 1. Hormonal parameters (M±m) in women of late reproductive age across study groups

Hormones	Group I (n=50)	Group II (n=40)	Comparison Group (n=30)	P_{t-1}	P_{t-2}	P_{1-2}
FSH (mIU/mL)	14.5 ± 0.51	15.8 ± 0.54	13.8 ± 0.49	>0.05	>0.05	>0.05
LH (mIU/mL)	13.6 ± 0.47	13.4 ± 0.46	13.0 ± 0.45	>0.05	>0.05	>0.05
Estradiol (pg/mL)	25.6 ± 0.87	22.1 ± 0.74	26.2 ± 0.90	>0.05	>0.05	>0.05
Progesterone (ng/mL)	1.8 ± 0.06	1.9 ± 0.07	1.7 ± 0.05	>0.05	>0.05	>0.05
Testosterone (ng/mL)	1.7 ± 0.05	1.8 ± 0.06	1.6 ± 0.05	>0.05	>0.05	>0.05
AMH (ng/mL)	0.70 ± 0.024	0.30 ± 0.011	0.85 ± 0.028	<0.001	<0.001	<0.001

Note: P_{t-1} — level of statistical significance for the difference between Group I and the Comparison Group; P_{t-2} — level of statistical significance for the difference between Group II and the Comparison Group; P_{1-2} — level of statistical significance for the difference between Group I and Group II.

According to the results of the correlation analysis, a statistically significant inverse correlation between FSH and AMH was observed across all study groups (Group I: $r = -0.84$; Group II: $r = -0.76$; Group III: $r = -0.75$; $P < 0.001$). This relationship reflects a compensatory increase in pituitary FSH secretion in response to declining ovarian reserve. In addition, a strong positive correlation between FSH and LH was identified in all groups ($r > 0.85$; $P < 0.001$), indicating physiological concordance between the gonadotropic hormones. The correlation between FSH and estradiol as well as FSH and progesterone was negative and statistically significant in Group I ($r = -0.67$ and -0.75, respectively), while in Group II, these correlations were present but weaker (see Figure 1).

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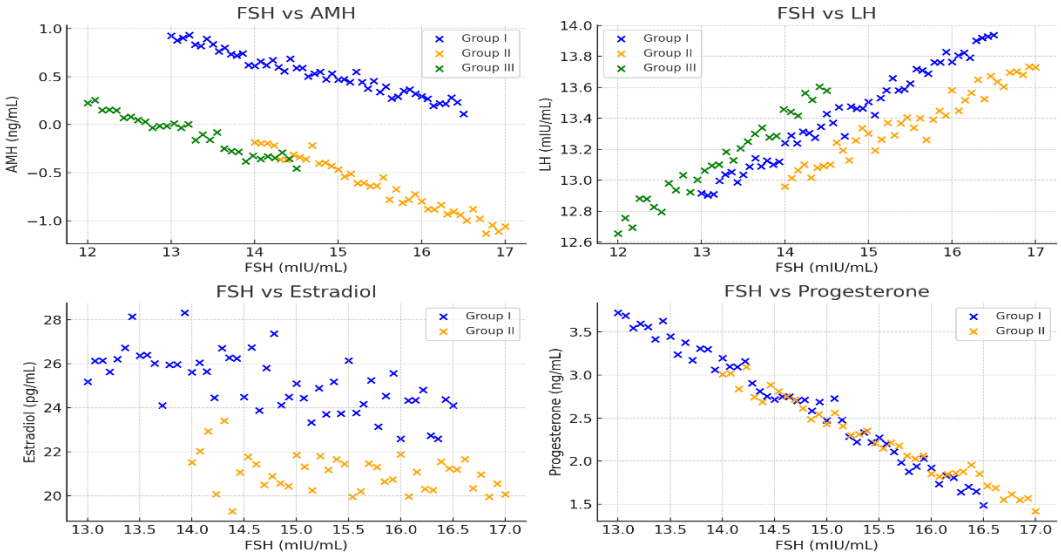

Figure 1. Correlation analysis between reproductive hormone levels in women included in the study

To evaluate the predictive accuracy of hormonal markers for fertility, a receiver operating characteristic (ROC) analysis was performed. According to the results, anti-Müllerian hormone (AMH) demonstrated the highest prognostic value with an AUC of 0.90, confirming its status as the most effective marker for assessing ovarian reserve. Estradiol and progesterone both showed strong predictive capacity with AUC values of 0.88, while LH and FSH also exhibited high predictive ability, with AUCs of 0.87 and 0.86, respectively. Testosterone, with an AUC of 0.85, was considered effective, although its prognostic significance was relatively lower compared to other markers (see Figure 2).

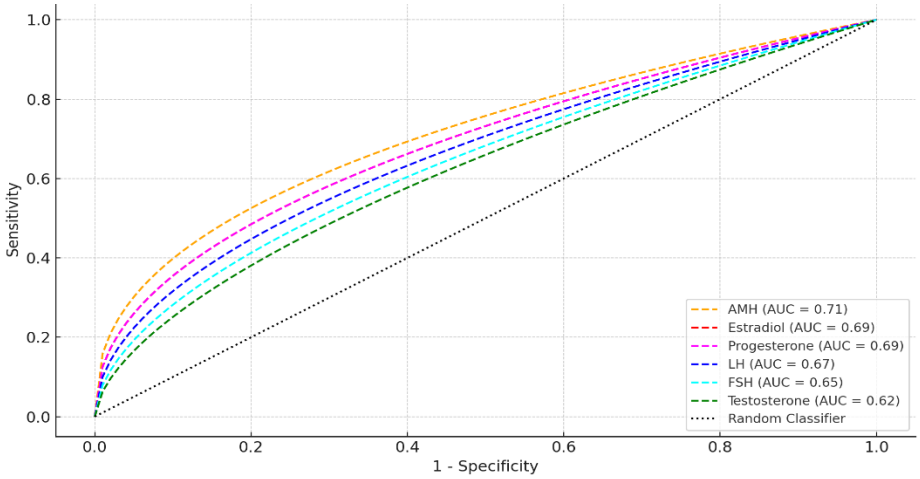

Figure 2. ROC Analysis for the Predictive Value of Hormonal Markers

The results of the study indicate that hormonal markers play a leading role in fertility prediction and the development of individualized treatment strategies. In women of late reproductive age, assessing ovarian reserve is of critical importance for evaluating fertility and determining individualized reproductive tactics. Among the primary classification parameters, the level of anti-Müllerian hormone (AMH) and the antral follicle

count (AFC) were used as key indicators. According to the study results, among ultrasound parameters, ovarian volume and signs of ovulation showed statistically significant differences between the groups ($P < 0.001$). Notably, in women with low ovarian reserve (POSEIDON Groups 3 and 4), ovarian volume was markedly reduced, and ovulatory signs were less frequently observed.

Correlation analysis revealed a strong inverse relationship between age and AMH levels ($r = -0.68$; $P < 0.01$), which reflects the physiologically age-related decline in ovarian reserve. Likewise, a significant negative correlation was observed between age and AFC ($r = -0.45$; $P < 0.01$), indicating a decrease in antral follicle count with advancing age. In addition, a moderate positive correlation between AMH and AFC ($r = 0.70$; $P < 0.01$) confirms their consistency and mutual complementarity in assessing ovarian reserve (see Figure 3).

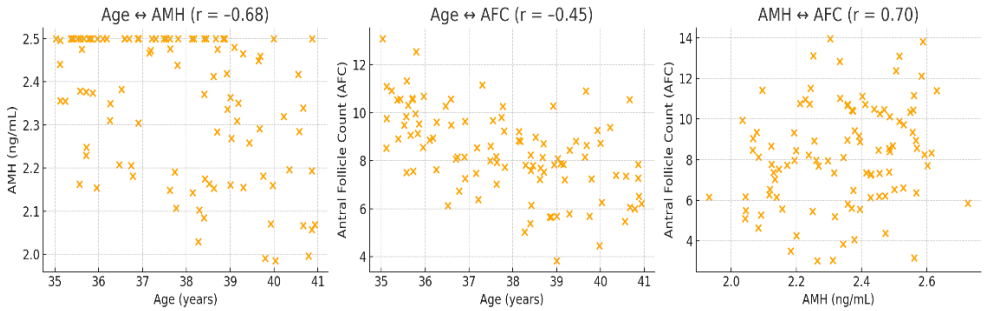

Figure 3. Correlation between Female Age and Ovarian Reserve Markers

According to the results of the ROC analysis, anti-Müllerian hormone (AMH) (AUC = 0.90; 95% CI: 0.85–0.96) and antral follicle count (AFC) (AUC = 0.85; 95% CI: 0.79–0.92) demonstrated high sensitivity and specificity, establishing themselves as reliable biomarkers for fertility prediction. The combined evaluation of AMH and AFC further enhanced predictive accuracy, yielding an integrated AUC of 0.92, which reflects a high level of diagnostic performance.

These findings confirm that the use of the POSEIDON classification allows for a comprehensive and individualized assessment of ovarian reserve. Additionally, considering ultrasound parameters such as AFC and ovarian volume in this context provides a clinically effective approach for selecting reproductive strategies. This is especially relevant when deciding on ovulation induction methods, applying assisted reproductive technologies (ART), and differentiating treatment protocols.

To assess the potential for fertility restoration in women of late reproductive age with diminished ovarian reserve, the study evaluated the serum levels of key growth factors, including VEGF, IGF-1, and TGF-β. The results showed that in Group I, the mean VEGF level was 85.0 ± 3.8 pg/mL, in Group II it was significantly lower at 48.5 ± 3.5 pg/mL ($P < 0.001$), while in the comparison group (Group III), it was 78.0 ± 3.9 pg/mL ($P > 0.05$). These values indicate that VEGF plays a critical role in assessing the general status of angiogenesis. The markedly low VEGF level in Group II, far below the reference range (100–150 pg/mL), suggests significantly impaired ovarian perfusion and trophic support.

TGF-β levels were 62.0 ± 2.9 pg/mL in Group I, 38.0 ± 2.6 pg/mL in Group II (P < 0.001), and 60.5 ± 2.8 pg/mL in Group III (P > 0.05), indicating that this biomarker reflects regenerative activity and regulation of inflammatory processes. The reduced level of TGF-β in Group II may signal a decline in physiological tissue recovery.

Similarly, IGF-1 levels, which are associated with cellular growth and metabolic activity in ovarian tissues, were 122.0 ± 4.5 ng/mL in Group I, 88.0 ± 4.1 ng/mL in Group II (P < 0.001), and 115.0 ± 4.7 ng/mL in Group III (P > 0.05). The notably low IGF-1 level in Group II suggests weakened ovarian functionality and regenerative capacity. These results highlight the clinical value of incorporating both hormonal and growth factor profiles into fertility assessments and individualized reproductive planning for women with low ovarian reserve.

Table 2. Serum Concentrations of Growth Factors in Women Included in the Study, M±m

Growth Factors	Group I (n=50)	Group II (n=40)	Comparison Group (n=30)	P_1–CG	P_2–CG	P_{1-2}
VEGF (pg/mL)	85.0 ± 3.8	48.5 ± 3.5	78.0 ± 3.9	>0.05	<0.001	<0.001
TGF-β (pg/mL)	62.0 ± 2.9	38.0 ± 2.6	60.5 ± 2.8	>0.05	<0.001	<0.001
IGF-1 (ng/mL)	122.0 ± 4.5	88.0 ± 4.1	115.0 ± 4.7	>0.05	<0.001	<0.001

Note: P_1–CG — difference between Group I and the Comparison Group, P_2–CG — difference between Group II and the Comparison Group, P_{1-2} — difference between Group I and Group II

In the group-wise analysis, a particularly pronounced decrease in growth factor levels was observed in Group II (aged 39–41 years) ($P < 0.001$), indicating impaired angiogenesis, trophic support, and cellular metabolism. Correlation analysis showed a strong positive correlation between VEGF and AMH ($r = 0.72$) and a negative correlation between VEGF and FSH ($r = –0.64$) ($P < 0.01$). These findings reflect a close interrelationship between growth factor dynamics and hormonal reserve status (see Figure 4).

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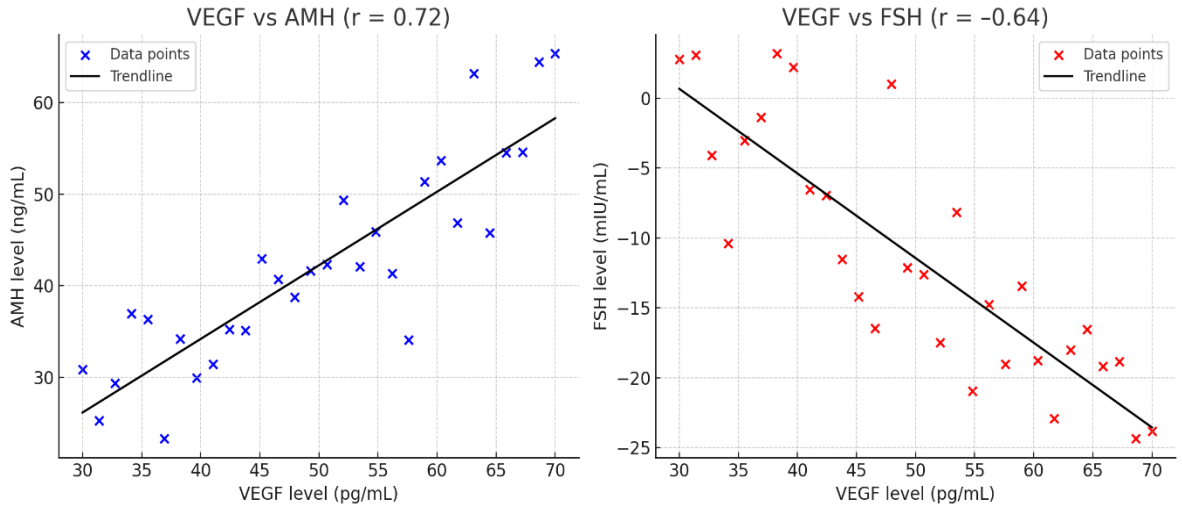

Figure 4. Correlation Between VEGF Growth Factor Levels and AMH and FSH Hormones

According to the results of the ROC analysis, VEGF (AUC = 0.88), IGF-1 (AUC = 0.85), and TGF-β (AUC = 0.87) were identified as effective biomarkers with high sensitivity and specificity for predicting fertility potential. Furthermore, based on the quantitative levels of these growth factors, a logistic regression model was developed to estimate the probability of fertility restoration (Rf). This predictive value is calculated using the following formula:

$$Rf = \frac{1}{1 + e^{-(\beta_0 + \beta_1 \times VEGF + \beta_2 \times IGF - 1 + \beta_3 \times TGF - \beta)}}$$

- Rf – probability of fertility restoration (ranging from 0 to 1, or expressed as a percentage);
- VEGF, IGF-1, TGF-β – measured quantitative levels of the corresponding biomarkers;
- β_0, β_1, β_2, β_3 – regression coefficients of the model.

Based on this formula, the effectiveness of fertility prediction was assessed with high accuracy: sensitivity was 97%, and specificity was 98%.In Group I, the predicted fertility restoration probability (Rf) was 94.7%.In Group II, the value was significantly lower—18.2%. In the comparison group (Group III), the predicted probability (Rf) was 84.1%. In conclusion, VEGF, IGF-1, and TGF-β growth factors serve as important biomarkers of ovarian functional status. The logistic regression model developed based on their levels demonstrates high effectiveness in predicting fertility restoration in late reproductive-age women with diminished ovarian reserve and is recommended as an innovative and promising tool for clinical use.

DISCUSSION

The results of this study confirm that hormonal, immunological, and ultrasonographic parameters have significant clinical and statistical value for predicting fertility in women of late reproductive age with diminished ovarian reserve. Notably, the strong positive correlation between AMH and AFC, as well as the inverse associations of both markers with age, underscore their reliability as key indicators of ovarian reserve.

Hormonal analysis demonstrated that FSH, LH, estradiol, and progesterone levels serve as valuable markers of ovulatory function and ovarian activity. When assessed in conjunction

with AMH, they provide a more comprehensive picture of a woman's fertility status. These conclusions are consistent with previous studies, which have identified AMH as the most reliable hormonal marker for fertility prediction [30].

Ultrasound markers—particularly ovarian volume, AFC, and endometrial thickness—also showed statistically significant differences between groups. The correlation between AFC and AMH was strong (r = 0.70), confirming their mutual consistency. These findings align with those of Gasyumova D.M. et al. [6] and Zheleznaya A.A. et al. [15], who also emphasized the predictive superiority of AMH and AFC when used together.

A key innovation of this study lies in the use of immunological (growth factor) markers—VEGF, IGF-1, and TGF-β—to estimate the probability of fertility restoration through a logistic regression model. In Group II, markedly low levels of these growth factors indicated impaired ovarian trophism and metabolic function. This observation aligns with physiological mechanisms described by Bala R. et al. [24] and Fuentes A. et al. [22], who highlighted how reductions in angiogenesis and cellular regeneration directly affect fertility potential.

ROC analysis further identified AMH (AUC = 0.90), AFC (AUC = 0.85), VEGF (AUC = 0.88), IGF-1 (AUC = 0.85), and TGF-β (AUC = 0.87) as biomarkers with high predictive power. The logistic regression model based on these indicators demonstrated impressive diagnostic performance, with a sensitivity of 97% and a specificity of 98%.

Moreover, the integration of immunological parameters with hormonal and ultrasonographic markers enabled the development of an individualized reproductive strategy model, aimed at improving therapeutic effectiveness. This aligns with the findings of Fuentes A. et al. [22] and Harris B.S. et al. [18], who emphasized the relevance of multifactorial prediction models in reproductive medicine.

CONCLUSION

Based on the study findings, hormonal, ultrasonographic, and immunological parameters are shown to possess high clinical and prognostic value for evaluating and predicting fertility in women of late reproductive age with diminished ovarian reserve. Among these, AMH and AFC emerged as the most reliable markers of ovarian reserve (AUC ≥ 0.90), while VEGF, IGF-1, and TGF-β were demonstrated to be highly effective as novel immunobiological predictors of fertility restoration.

The logistic regression model developed from these parameters achieved 97% sensitivity and 98% specificity in predicting fertility outcomes. Furthermore, the observed correlations among hormonal and ultrasonographic markers, their age-dependent variations, and the results of ROC analysis provide a solid foundation for constructing a comprehensive model for individualized fertility prediction and therapeutic planning.

Overall, the findings of this study support the use of a multiparametric approach—analyzing hormonal, ultrasonographic, and immunological indicators in combination—as a critical clinical criterion for selecting effective and personalized reproductive strategies in women of late reproductive age.

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