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Original Article

Transvaginal ultrasound combined with serum anti-Müllerian hormone, follicle-stimulating hormone, and luteinizing hormone in the evaluation of ovarian reserve function in infertile patients

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Abstract: Objective: This work aimed to evaluate the clinical value of serumanti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and transvaginal ultrasound (TVU) parameters in the diagnosis of ovarian reserve function (ORF) in infertile patients. Methods: Clinical data from 120 infertile patients were retrospectively collected, and divided into an abnormal and a normal ovarian reserve group. The serum AMH, FSH, LH levels, and TVU parameters of the subjects were compared. The receiver operating characteristic (ROC) curve was drawn, and the area under the curve (AUC), sensitivity (Sen), and specificity (Spe) of each parameter diagnosis were calculated. Results: The serum level of AMH was significantly lower, while the levels of FSH and LH were significantly higher in patients with abnormal ovary as compared to patients with normal ovaries (P<0.05). The resistance index (RI), pulsatility index (PI), peak systolic velocity (PSV), and end diastolic velocity (EDV) of the ovarian artery in patients with abnormal ovaries were significantly lower compared with patients with normal ovaries (P<0.05). The AUC, Sen, and Spe of serum AMH, FSH, and LH combined with TVU parameters in the diagnosis of abnormal ORF were markedly higher than those of a single index (P<0.05). Conclusion: Ovarian reserve dysfunction is associated with altered hemodynamics and hormonal profiles. In contrast to a single parameter, serum biochemical indicators plus TVU parameters can more accurately identify the abnormality of ORF in infertile patients.

Keywords: AMH, FSH, LH, TVU, infertility, ORF

Introduction

Infertility is a major health concern affecting many couples at childbearing age worldwide. With the development of society and the change of lifestyle, its incidence is increasing annually [1, 2]. According to the definition of the World Health Organization (WHO), infertility refers to the state in which couples at childbearing age fail to conceive successfully for one year without taking any contraceptive measures and while having a normal sexual life. The causes of infertility are complex and diverse, and the decline of ovarian reserve function (ORF) is one of the important factors of female infertility [3]. ORF refers to the quantity and quality of oocytes, which is an important indicator to evaluate female fertility. With the increase in a women's age, the ORF gradually declines, which results in the reduction of the number of follicles and the quality of oocytes, which directly affects women's ability to conceive [4]. Therefore, accurate evaluation of ORF is meaningful for the diagnosis and treatment of infertility.

At present, the methods to evaluate ORF mainly include hormone level detection and imaging examination. Among them, serum anti-Müllerian hormone (AMH), follicle-stimulating hormone

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(FSH), and luteinizing hormone (LH) are the most commonly used hormone indicators. AMH is a glycoprotein secreted by granulosa cells of follicles, which plays a critical role in the growth and development of follicles. Because AMH level is positively correlated with the number of follicles, it is considered to be a reliable indicator to evaluate ORF [5, 6]. FSH and LH secreted by the anterior pituitary play a key role in regulating follicular development and ovulation. Elevated FSH and LH levels typically indicate diminished ovarian reserve. In imaging examination, transvaginal ultrasound (TVU) is a non-invasive and effective imaging modality, which is widely used in gynecology and reproductive medicine [7-10]. The ovarian structure and the number of follicles can be observed intuitively by TVU, so as to evaluate the ORF. In particular, measurement of the antral follicle count (AFC) provides valuable information for clinicians. Studies have shown that AFC and AMH levels have high consistency, so the combined application of the two can improve the accuracy of assessment [11]. In recent years, more and more studies have begun to focus on the combination of serum AMH, FSH, LH and TVU to comprehensively evaluate ORF. Through the combination of multiple methods, it can comprehensively outline the functional status of the ovary from different angles, and then improve the accuracy of infertility diagnosis and the effectiveness of treatment [12, 13]. For example, the joint evaluation of AMH and AFC can make up for the limitation of a single index and improve the predictive ability of ovarian function status [14]. In addition, the dynamic monitoring of FSH and LH can help identify the changing trend of ovarian function and provide a basis for clinical decision-making. In summary, TVU plus serum AMH, FSH, and LH has important clinical application value for the evaluation of ORF in infertile patients.

Materials and methods

Study subjects

A retrospective collection of medical records of 120 patients with infertility who visited the Yongkang Women and Children's Health Hospital from May 2022 to May 2024 was conducted. This study was approved by the Ethics Committee of Yongkang Women and Children's Health Hospital.

Inclusion criteria: (1) Complete medical records, including basic information, clinical manifestations, diagnostic results, treatment plans, and follow-up records; (2) No abnormalities found in the partner's semen analysis; (3) No history of assisted reproductive technology; (4) Bilateral ovaries were identifiable via TVU; (5) Normal thyroid function.

Exclusion criteria: (1) Patients with cardiovascular diseases; (2) Patients with hyperprolactinemia; (3) Patients with endometriosis; (4) Patients who had previously received FSH therapy.

Grouping and assessment

Patients were classified into two groups according to a comprehensive clinical gold standard that did not include serum follicle-stimulating hormone (FSH), antral follicle count (AFC), or daily gonadotropin (Gn) dose. The study population was divided into an abnormal ovarian function group (n = 66) and a normal ovarian function group (n = 54).

Definition of the gold standard: Abnormal ovarian function was independently diagnosed by two senior reproductive medicine specialists, based on a comprehensive evaluation of patient age, menstrual history, reproductive history, and long-term assisted reproductive outcomes, as well as overall clinical assessment of ovarian function. Importantly, this diagnostic decision did not incorporate serum FSH, AFC, or mean daily Gn dose.

Serum FSH, AFC, and daily Gn dose were used exclusively as candidate diagnostic variables for subsequent receiver operating characteristic (ROC) curve analysis and were not included in the construction of the gold standard classification, thereby minimizing incorporation bias.

TVU examination

All subjects underwent TVU imaging scans using a GE HealthCare Voluson E10/E8 ultrasound machine. Transvaginal color Doppler ultrasound was employed to measure the mean diameter, antral follicle count, and ovarian artery blood flow. The pulse Doppler was used to display the spectrum, and the resistance index (RI), pulsatility index (PI), and peak systolic velocity (PSV) of the ovarian stromal artery

were measured. The size of the uterus, endometrial thickness, and blood flow in the uterine artery were also measured, with the pulse Doppler displaying the spectrum and measuring the PSV, RI, and PI. Ovulation was monitored within the same month, starting from the 10th day of menstruation, monitoring follicular development and endometrial thickness, and the presence of fluid in the rectouterine pouch (pouch of Douglas). When the maximum follicle diameter was <1.0 cm, monitoring was conducted every 3 days; when the maximum diameter was <1.5 cm, every 2 days; and when the maximum diameter was ≥1.5 cm, daily monitoring was performed until ovulation occurred. If the dominant follicle reached ≥1.8 cm without spontaneous ovulation, HCG (5,000-10,000 IU) was administered to induce ovulation. Each monitoring session measured the above-mentioned indices of the ovarian and uterine artery spectra. The RI, PI, PSV, and EDV of both ovarian arteries were obtained, and their average values were subjected to statistical analysis.

Physiological indicators

(1) Data on all subjects' age, body mass index (BMI), menstrual cycle regularity, age at menarche, duration of infertility, and type of infertility (primary or secondary) were collected. (2) Serum AMH, FSH, and LH data of subjects were collected.

Statistical processing

SPSS 22.0 software was employed. Continuous variables with a normal distribution were presented as mean ± standard deviation (SD), and categorical variables were presented as frequency and percentage (n, %). The Mann-Whitney test was used to analyze continuous variables that did not conform to a normal distribution. One-way ANOVA was adopted for comparisons of continuous variables conforming to a normal distribution. The chi-square test was used for analysis of categorical variables.

A multivariate logistic regression model was constructed to develop a combined diagnostic model incorporating multiple indicators. Variable selection was performed using a forward stepwise method (Forward LR), incorporating AMH, FSH, LH, ovarian artery RI, PI, PSV, and end diastolic velocity (EDV). Regression coefficients, intercept, and the corresponding probability prediction formula were derived from the final model.

Hosmer-Lemeshow test was used to assess goodness-of-fit of the model. ROC curve analysis was performed, and the area under the curve (AUC), sensitivity (Sen), and specificity (Spe) were calculated. The intraclass correlation coefficient (ICC) was used to evaluate the consistency of bilateral ovarian artery Doppler measurement, with ICC >0.75 indicating good consistency.

Sample size of this study was estimated based on diagnostic test methodology for ROC curve analysis, considering an expected AUC of 0.90, a significance level α of 0.05, and a statistical power (1-β) of 0.80. Published literature with similar study designs was also referenced to support sample size estimation. A final sample size of 120 cases was determined to meet the requirements for statistical analysis. A *P*-value <0.05 was considered statistically significant.

Results

Baseline information

The age, BMI, menstrual cycle regularity, age at menarche, duration of infertility, and type of infertility (primary or secondary) were comparable between the two groups (*P*>0.05; **Figure 1**).

Serum AMH, FSH, and LH levels

In the abnormal ovarian function group, serum AMH level was 1.97±0.48 μg/L, FSH was 8.38±1.25 IU/L, and LH was 6.77±0.96 IU/L. In the normal group, AMH was 4.04±0.71 μg/L, FSH was 5.56±1.18 IU/L, and LH was 3.75±0.82 IU/L. Compared with the normal ovarian function group, the abnormal group exhibited significantly lower AMH levels and significantly higher FSH and LH levels (*P*<0.05; **Figure 2**).

TVU parameters

The ICC analysis demonstrated good consistency for bilateral ovarian artery measurements. The ICC values for RI, PI, PSV, and EDV were 0.872, 0.856, 0.893, and 0.869, respectively, all exceeding 0.75, indicating satisfactory agreement between the left and right ovar-

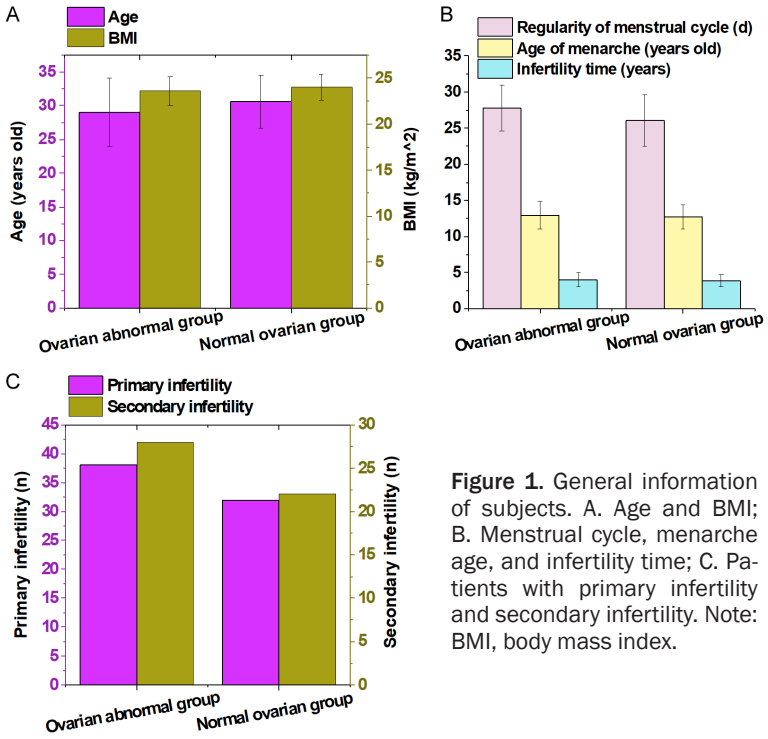

Figure 1. General information of subjects. A. Age and BMI; B. Menstrual cycle, menarche age, and infertility time; C. Patients with primary infertility and secondary infertility. Note: BMI, body mass index.

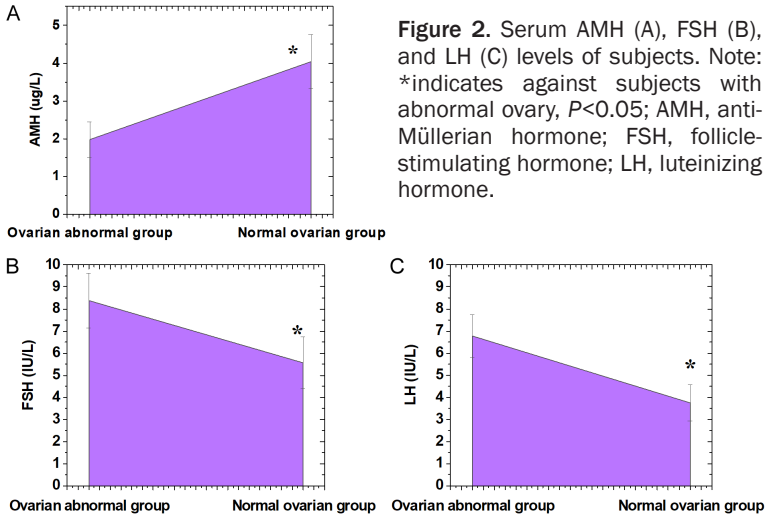

Figure 2. Serum AMH (A), FSH (B), and LH (C) levels of subjects. Note: *indicates against subjects with abnormal ovary, $P<0.05$; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

cantly lower RI, PI, PSV, and EDV values ($P<0.05$).

ROC curve analysis

ROC curve analysis revealed that the AUCs for serum AMH, FSH, and LH in diagnosing abnormal ORF were 0.726, 0.757, and 0.662, respectively. The AUCs for RI, PI, PSV, and EDV were 0.753, 0.818, 0.640, and 0.812, respectively.

Using abnormal ovarian reserve as the dependent variable and AMH, FSH, LH, RI, PI, PSV, and EDV as independent variables, a multivariate logistic regression model was constructed for combined diagnosis:

Regression equation: logit(P) = -0.582-1.265 × AMH + 0.683 × FSH + 0.421 × LH-0.356 × RI-0.724 × PI + 0.215 × PSV + 0.338 × EDV

Goodness of fit: The Hosmer-Lemeshow test yielded χ^2 = 6.124, P = 0.633, indicating a good model fit with no significant overfitting. The AUC of the multi-indicator combined diagnosis was 0.916, which was significantly higher than that of any single indicator ($P<0.05$), demonstrating the best diagnostic performance (**Figures 4**-**6**).

ian arteries. Therefore, the average values of bilateral measurements were used for subsequent statistical analyses. As shown in **Figure 3**, the PI in the abnormal ovarian function group was 2.11±0.45, RI was 0.67±0.18, PSV was 8.05±1.44 cm/s, and EDV was 3.42±0.63 cm/s. In the normal ovarian function group, PI was 3.37±0.65, RI was 0.78±0.11, PSV was 14.25±1.68 cm/s, and EDV was 6.08±0.91 cm/s. Compared with the normal ovarian function group, the abnormal group showed signifi-

Figure 7 illustrates the comparison of the sensitivity and specificity of the combined diagnostic model and single-index diagnostic methods. It was found that both sensitivity and specificity of the combined diagnostic model were markedly higher than those individual parameters ($P<0.05$).

Transvaginal ultrasound imaging features

Typical transvaginal ultrasound (TVU) with color Doppler showed significant differences in

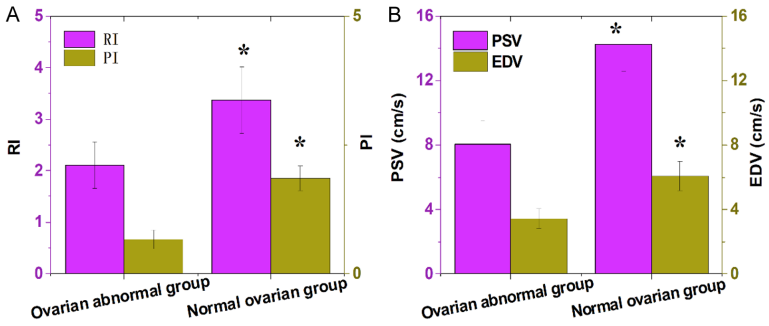

Figure 3. Subject's TVU parameters. A. Ovarian artery RI and PI; B. PSV and EDV. Note: *P<0.05, compared with abnormal group. RI, resistance index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity.

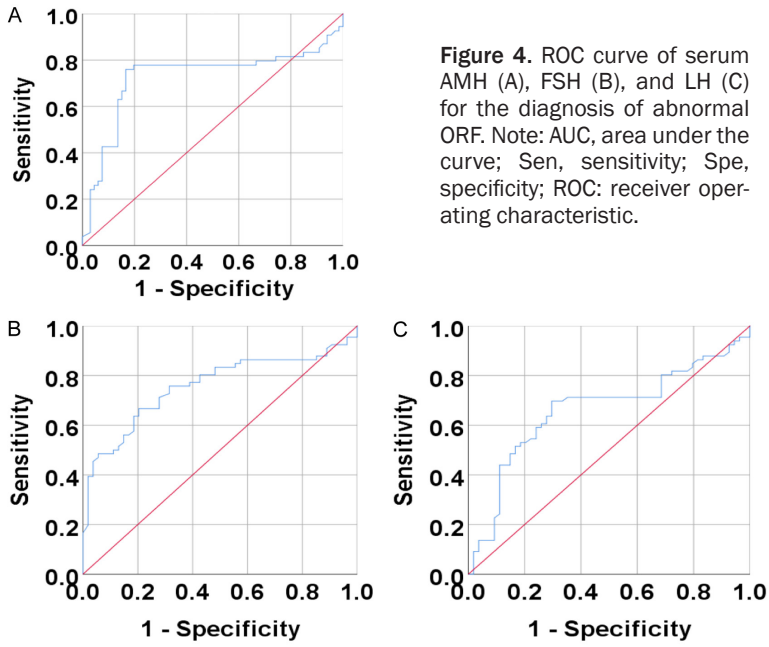

Figure 4. ROC curve of serum AMH (A), FSH (B), and LH (C) for the diagnosis of abnormal ORF. Note: AUC, area under the curve; Sen, sensitivity; Spe, specificity; ROC: receiver operating characteristic.

ovarian morphology and blood flow among infertile patients with different ovarian reserve status. In infertile patients with normal ovarian reserve, the ovarian parenchyma showed homogeneous echotexture with multiple antral follicles, and abundant blood flow signals were observed in the ovarian stroma (**Figure 8A**). In infertile patients with diminished ovarian reserve, the ovarian volume was relatively small, the antral follicle count was significantly reduced (<5), and blood flow signals in the ovarian stroma were decreased (**Figure 8B**).

Discussion

Infertility is defined as the failure to achieve pregnancy after 12 months of regular unprotected intercourse. The ORF refers to the number of eggs remaining in women's ovaries and the quality of these eggs, which is crucial for women's fertility [15-17]. At present, diagnosing infertility is a complex and challenging process because infertility usually involves multiple factors and systems. It may involve male and female reproductive system problems, such as egg quality, sperm quantity and quality, endometrial health [18]. The difficulty of diagnosis is that the existing detection technology may not be able to comprehensively evaluate all potential causes of infertility, and sometimes there may be hidden factors or individual distinctions, such as the influence of immune factors or environmental factors. Therefore, combining multiple diagnostic methods is of great clinical importance [19]. The data of 120 cases of infertility were retrospectively collected. According to whether the ORF of patients was abnormal, they were divided into 66 cases of ovarian abnormal group and 54 cases of ovarian normal group. Firstly, it was found that there was no statistically meaningful distinction in the age, BMI, menstrual cycle, menarche age, infertility time, infertility type (primary infertility, secondary infertility) between the subjects. This means that the ovarian abnormal and the normal groups are basically similar in other important characteristics except ovarian-related parameters. This balance is crucial for the internal validity of the study, which can reduce the confounding caused by other factors, so as to more accurately evaluate the impact of ovarian abnormalities on the occurrence and development of infertility [20].

The serum level of AMH was markedly lower, while the levels of FSH and LH were markedly higher in patients with abnormal ovaries as

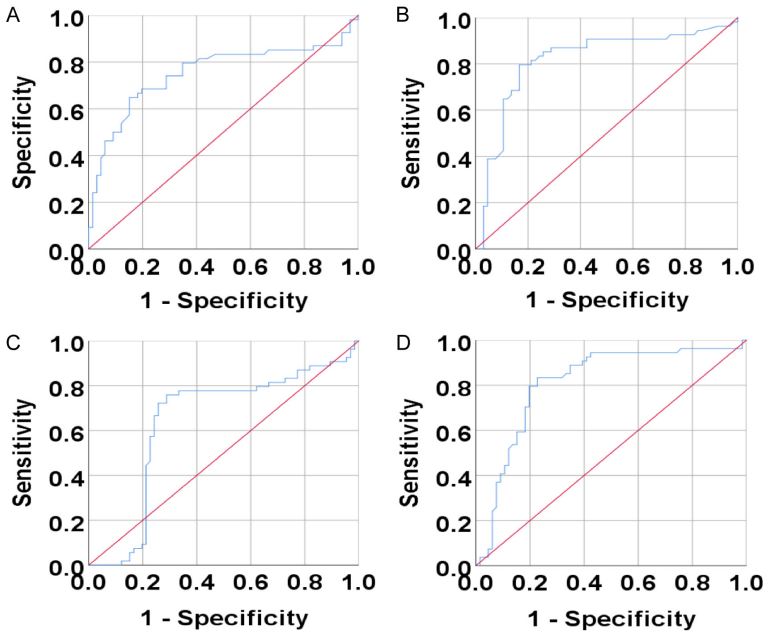

Figure 5. ROC curve of ultrasound parameters for the diagnosis of abnormal ORF. A. RI; B. PI; C. PSV; D. EDV. Note: RI, resistance index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; AUC, area under the curve; ROC: receiver operating characteristic.

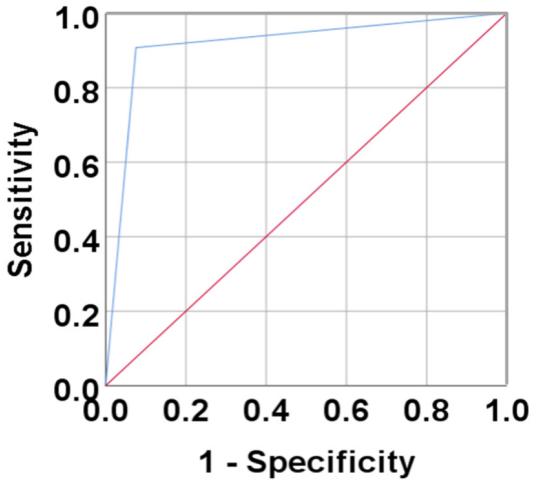

Figure 6. ROC curve of combined diagnosis of multiple parameters on abnormal ORF. Note: AUC, area under the curve; ROC, receiver operating characteristic.

compared to patients with normal ovary. This indicates that ovarian abnormalities may lead to the decline of estrogen levels and gonadal dysfunction in patients, reflecting a compensatory increase in gonadotropin secretion due to decreased ovarian feedback. RI and PI reflect impedance to blood flow; their reduction suggests decreased vascular resistance in the ovarian stroma. The PSV and EDV reflect the peak of blood flow velocity, and their abnormalities may reflect insufficient vascular supply or hemodynamic changes [21]. It was found that the RI, PI, PSV, and EDV of ovarian artery in patients with abnormal ovaries were markedly lower compared with patients with normal ovaries. This result was consistent with the findings of Tian et al. [22], who compared hysterosalpingography (HSG) and hysterosalpingo-contrast sonography (HyCoSy) in assessing tubal patency in women with endometriosis-associated infertility using propensity score matching. Their study confirmed that abnormalities in tubal and ovarian hemodynamic parameters were closely associated with reduced fertility in infertile patients, indicating that these ultrasound parameters could be used in clinical practice to evaluate the condition and treatment outcomes of patients with ovarian disorders or infertility.

In this article, the clinical data of infertility patients were retrospectively analyzed, and multiple parameters of patients with normal and abnormal ORF were compared. The serum biochemical indexes and TVU parameters of patients with ovarian abnormalities revealed visible distinctions, suggesting that abnormal ovarian function may lead to the decline of estrogen levels and gonadal dysfunction, as well as hemodynamic changes. ROC curve analysis showed that serum biochemical indexes (AMH, FSH, LH) plus TVU parameters had high sensitivity and specificity in the diagnosis of abnormal ORF, which was better than a single index. This article suggests the application value of multiple parameters combined diagnosis in infertility, which can significantly improve the detection of abnormal ORF, providing more accurate diagnosis basis [23].

This study adopted a retrospective design, which carries inherent risks of incomplete data and information bias. Although the sample size

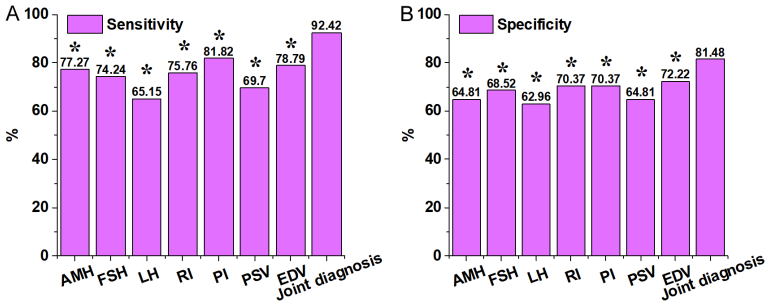

Figure 7. Sensitivity (A) and specificity (B) of combined diagnosis and single index diagnosis. Note: *P<0.05, compared with combined diagnosis. Sen, sensitivity; Spe, specificity.

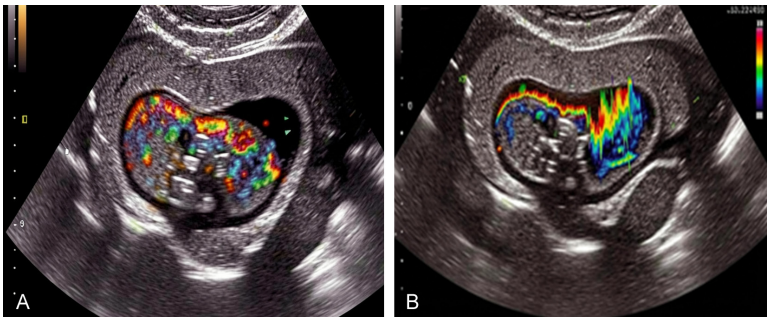

Figure 8. Transvaginal ultrasound images with color Doppler in infertile patients. Note: A. Ovarian image in a patient with normal ovarian reserve, showing abundant blood flow signals in the ovarian stroma. B. Ovarian image in a patient with diminished ovarian reserve, showing relatively reduced blood flow signals in the ovarian stroma.

was calculated based on the expected AUC for the ROC diagnostic test, the significance level, and the statistical power, the final sample of 120 cases is relatively modest, which may limit the external validity of our findings. Furthermore, the study was conducted at a single medical center, and the findings may not apply to other regions or different healthcare systems [24]. Therefore, future research should expand the sample size and be conducted across multiple centers to improve the generalizability and external validity of the results. At the same time, more potential influencing factors, such as immunological factors, environmental factors, lifestyle, and psychological factors, should be comprehensively considered to fully evaluate and understand the complex etiology of infertility.

Disclosure of conflict of interest

None.

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