ORIGINAL RESEARCH ARTICLE

Prediction of clinical pregnancy in in-vitro fertilization-embryo transfer by transvaginal three-dimensional ultrasonography and serum hormone levels

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Abstract

This study aimed to explore the predictive value of transvaginal three-dimensional ultrasonography combined with serum progesterone and estrogen levels in in-vitro fertilization-embryo transfer. A total of 100 patients undergoing frozen-thawed embryo cycles were investigated. On the day before embryo transfer, two-dimensional ultrasound was used to assess endometrial thickness and blood flow parameters, including the resistance index, pulsatility index, and systolic/diastolic velocity ratio. These measurements were then transitioned to three-dimensional ultrasound, where endometrial volume, flow index, vascularization flow index, and sub-endometrial vascularization index were evaluated. On the day of embryo transfer, serum progesterone and estradiol levels were measured. Based on pregnancy outcomes, patients were divided into pregnant and non-pregnant groups. The results showed no significant differences between the groups in terms of endometrial thickness or two-dimensional ultrasound parameters (P>0.05). However, the pregnant group had significantly higher endometrial volume and sub-endometrial vascularization parameters (P<0.05). The combined predictive value of endometrial volume and serum estrogen was 0.735, with sensitivity of 77.3% and specificity of 90.7%. These findings suggest that three-dimensional ultrasonography, in conjunction with serum estrogen levels, can accurately predict clinical pregnancy outcomes in IVF-ET. *(Afr J Reprod Health 2025; 29 [9]: 54-62).*

Keywords: Transvaginal Three-Dimensional Ultrasound, Estrogen Level, Endometrial Receptivity, Fertilization-Embryo Transfer in Vitro

Résumé

Cette étude visait à explorer la valeur prédictive de l'échographie transvaginale tridimensionnelle combinée aux niveaux sériques de progestérone et d'œstrogène dans le transfert d'embryons par fécondation in vitro. Un total de 100 patientes subissant des cycles d'embryons congelés-dégelés a été étudié. La veille du transfert d'embryons, une échographie bidimensionnelle a été utilisée pour évaluer l'épaisseur de l'endomètre et les paramètres du flux sanguin, y compris l'indice de résistance, l'indice de pulsation et le rapport vitesse systolique/diastolique. Ces mesures ont ensuite été transférées à l'échographie tridimensionnelle, où le volume de l'endomètre, l'indice de flux, l'indice de vascularisation du flux et l'indice de vascularisation sous-endométriale ont été évalués. Le jour du transfert d'embryons, les niveaux sériques de progestérone et d'estradiol ont été mesurés. En fonction des résultats de la grossesse, les patientes ont été divisées en groupes enceintes et non enceintes. Les résultats ont montré qu'il n'y avait pas de différences significatives entre les groupes en termes d'épaisseur de l'endomètre ou de paramètres échographiques bidimensionnels (P>0,05). Cependant, le groupe enceinte avait des paramètres de volume endométrial et de vascularisation sous-endométriale significativement plus élevés (P<0,05). La valeur prédictive combinée du volume endométrial et de l'œstrogène sérique était de 0,735, avec une sensibilité de 77,3 % et une spécificité de 90,7 %. Ces résultats suggèrent que l'échographie tridimensionnelle, en association avec les niveaux sériques d'œstrogène, peut prédire avec précision les résultats cliniques de la grossesse dans le cadre du transfert d'embryons par fécondation in vitro. *(Afr J Reprod Health 2025; 29 [9]: 54-62).*

Mots-clés: Échographie transvaginale tridimensionnelle, Niveau d'œstrogène, Réceptivité endométriale, Transfert d'embryons par fécondation in vitro

Introduction

In vitro fertilization-embryo transfer, or in- vitro fertilization-embryo transfer (IVF-ET), is one of the most significant treatments for infertility [1]. Even though the quality of IVF embryos has significantly improved due to advancements in laboratory techniques, the clinical pregnancy success rate for

IVF-ET remains between 20 and 30 percent. Improving the IVF-ET success rate during the implantation phase is still quite difficult[2]. Previous research has primarily focused on the relationship between two-dimensional ultrasound parameters and IVF-ET outcomes, as well as the use of estradiol (E2), progesterone (P), and the P/E2 ratio in predicting the success rates of IVF-ET. However, these assessments are relatively limited and heterogeneous in scope [3].

A growing body of evidence has consistently demonstrated that three-dimensional ultrasound exhibits excellent reproducibility and high accuracy in volumetric measurements, which has shown superior reliability compared to conventional two-dimensional methods doi:10.1053/ob.1996.v175.a72478; PMID: 8828418. In addition to providing precise assessments of endometrial receptivity, including measurements of endometrial volume and sub-endometrial vascularization parameters, transvaginal three-dimensional ultrasound provides more thorough insights by including traditional two-dimensional ultrasound data, such as endometrial thickness and morphology. In this study, we employed three-dimensional ultrasound in conjunction with serum estrogen and progesterone levels to jointly assess endometrial receptivity. Our objective was to determine the optimal embryo transfer time to boost the clinical pregnancy rate of IVF-ET.

Methods

Study population

For this study, 100 infertile patients who received frozen-thawed embryo transfers at Chun'an Hospital of Traditional Chinese Medicine between September 2020 and March 2021 as a component of their IVF-ET treatment were chosen. The patients ranged in age from 22 to 44 years, with a mean age of 32.8±8.3 years. Inclusion criteria were as follows: (1) remaining frozen embryos after performing fresh embryo transfer during IVF-ET or embryos at the day 3 oocyte stage that were subjected to whole embryo freezing for various reasons; (2) possession of at least one transferable embryo after thawing. Exclusion criteria included: endometrial polyps, submucosal fibroids, uterine effusion, uterine adhesions, adenomyosis, hydrosalpinx, uterine developmental abnormalities, other hereditary diseases affecting embryo implantation, and chromosomal abnormalities. Two groups were formed based on the outcomes of the individuals' post-IVF-ET pregnancies: 56 cases with clinical pregnancy in one group, and 44 cases in the non-pregnant group.

Instruments and methods

A Philips EPIQ5 color Doppler ultrasound diagnostic instrument was utilized, equipped with an intracavitary three-dimensional volumetric probe (3D9-3v) operating at a frequency of 3-9 MHz. One sonographer conducted all the scans. The women were instructed to empty their bladders and assume the lithotomy position during transvaginal ultrasonography on the day prior to embryo transfer. Under two-dimensional ultrasound mode, a longitudinal section of the uterus was selected, and the maximum distance between the myometrial and endometrial interfaces of the anterior and posterior walls was measured in the mid-uterine cavity at the perpendicular uterine cavity line, denoted as the endometrial thickness. To assess the pulsatility index (PI), resistance index (RI), and the ratio of peak systolic velocity to end-diastolic velocity (S/D), the best section for color Doppler flow was selected, and the gain was suitably set.
Subsequently, the mode was switched to three-dimensional ultrasound. The size of the sampling frame was adjusted to encompass the entire endometrium from the uterine fundus to the cervix, and the endometrial volume (V) was measured using the 3D function (Figure 1). After turning on the color Doppler mode, seven levels were manually outlined and data extracted using the GI3DQ program. Three sub-endometrial regional volumetric blood flow parameters can be automatically determined using this procedure: flow index (FI), vascularization flow index (VFI), and vascularization index (VI) (Figure 2).

On the day of the transfer of embryos, the patients fasted for more than 8 hours prior to the procedure, and 3 mL of blood was drawn from the cubital vein. Serum progesterone (P) and estradiol (E2) levels were measured using a chemiluminescent immunoassay. The testing instrument utilized was the Roche E601 immunoassay analyzer, with all reagents procured from Roche, Germany. All measurements were made accurately following the manufacturer's instructions.

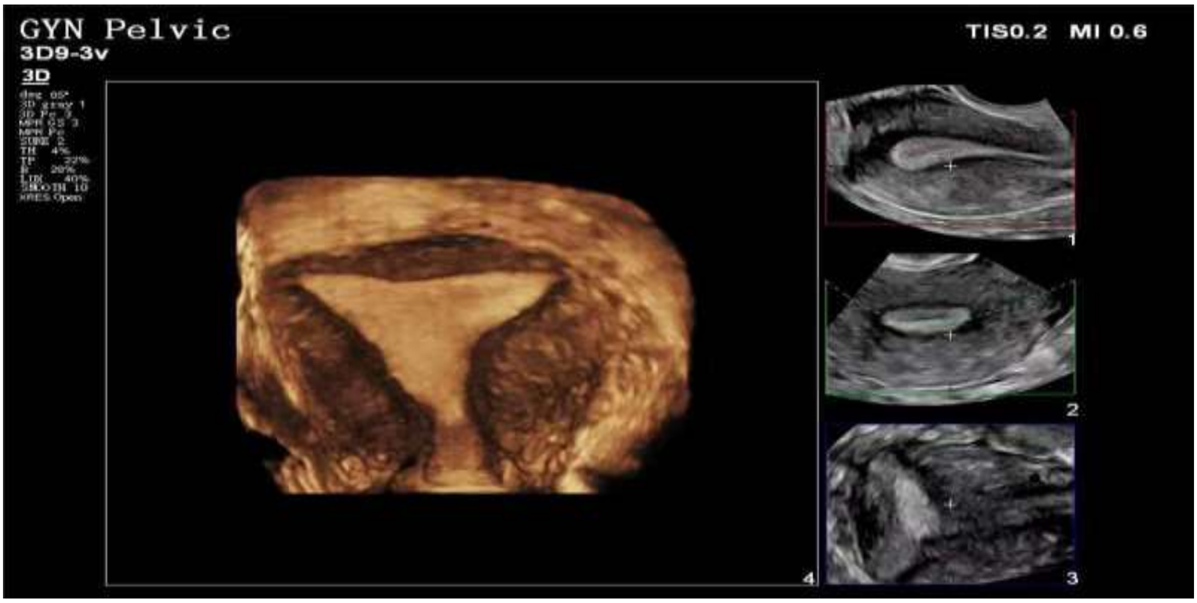

Figure 1: Transvaginal three-dimensional ultrasonography provides a thorough endometrial imaging.

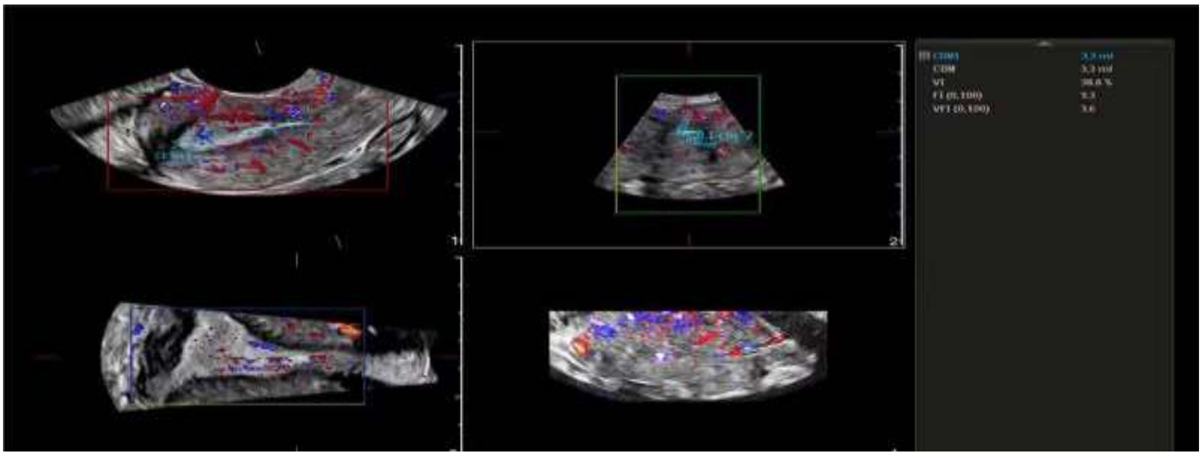

Figure 2: Using transvaginal three-dimensional ultrasonography to measure subendometrial blood flow parameters

Assessment of pregnancy results

A clinical pregnancy was confirmed if a urine pregnancy test was positive or if the blood β-HCG level was greater than 5 U/L 14 days after embryo implantation, followed by the detection of an intrauterine gestational sac and cardiac tube pulsation via vaginal ultrasound 28 days later.

If the gestational sac was not visualized and yet the HCG was high, it was considered a biochemical pregnancy.

Clinical pregnancies were included in the pregnancy group, while biochemical pregnancies and non-pregnancies were placed in the non-pregnancy group.

Statistical methods

Statistical analysis was conducted using software known as SPSS 22.0. Means with standard deviations (Mean±SD) were used to express the measurement data. The independent samples t-test was used for group comparisons. The association between the parameters of interest and clinical pregnancy outcomes was investigated using logistic regression analysis. The predictive efficacy of both single-factor and multifactorial combination testing for pregnancy outcomes was evaluated using receiver operating characteristic (ROC) curves. P-values below 0.05 were regarded as statistically significant.

Ethical considerations

The study was approved by the Ethics Committee of Chun'an Hospital of Traditional Chinese Medicine, and the ethical approval number was 2023-010. Written informed consent was obtained from all participants prior to data collection

Results

General clinical data of patients

When comparing general statistics such as age, years of infertility, body mass index (BMI), and type of infertility, there were no statistically significant differences ($P > 0.05$) between the clinical pregnancy group and the non-pregnancy group (Table 1).

Comparing the 2D ultrasound parameters of clinical pregnant and non-pregnant groups

Endometrial thickness and 2D ultrasonography blood flow parameters (RI, PI, and S/D) did not differ statistically significantly between the clinical pregnancy and non-pregnant groups ($P > 0.05$ for all) (Table 2).

Comparison of three-dimensional ultrasound parameters between clinical pregnancy and non-Pregnant groups

The clinical pregnancy group had significantly larger endometrial volume and three-dimensional ultrasonography vascularization parameters (VI, FI, and VFI) than the non-pregnant group ($P < 0.05$ for all) (Table 3).

Comparison of clinical pregnancy and non-pregnant groups' pre-transplantation estrogen and progesterone levels

The clinical pregnancy group had considerably greater serum levels of estradiol (E2) than the non-pregnant group ($P < 0.05$).However, Table 4 demonstrates that the clinical pregnancy and non-pregnant groups did not differ statistically significantly in serum progesterone (P) levels ($P > 0.05$).

Multifactorial logistic regression analysis

In the logistic regression study, pregnancy was the dependent variable, while the independent factors were serum estrogen level, endometrial volume, VI, FI, and VFI. The results indicated that endometrial volume and estrogen levels were significantly correlated with pregnancy outcomes ($P < 0.05$).

Predictive efficacy of endometrial volume, estrogen level alone, and combined tests

Endometrial volume, estrogen level, and their combination were evaluated as predictors of clinical pregnancy. After ROC curves were generated, the area under the curve (AUC) was calculated.
The combined prediction of endometrial volume and estrogen level yielded an AUC of 0.735, with a sensitivity of 77.3% and a specificity of 90.7%. At 75.2% sensitivity and 83.3% specificity, the AUC for endometrial volume alone was 0.704. The sensitivity and specificity were 70.5% and 68.7%, respectively, while the AUC for the estrogen level alone was 0.636. The best sensitivity and specificity for forecasting clinical pregnancy rates were found when three-dimensional ultrasonography indices and estrogen levels were combined (Figure 3).

Discussion

The IVF-ET technique, also known as in vitro fertilization[4], is a highly effective treatment for infertility wherein the fertilization of oocytes and sperm occurs ex vivo. The resulting embryos are cultured until they reach either the cleavage or blastocyst stages and are subsequently transferred into the uterine cavity to facilitate implantation and development into a fetus. Maternal age, the receptivity of the endometrial implantation environment, the timing of the implantation window, the density of estrogen and progesterone receptors, and subendometrial blood perfusion are some of the factors that affect the success rate of clinical pregnancy through IVF-ET. Among these, the adequacy of the intrauterine implantation environment is a critical determinant.

Table 1: Comparison of two groups' general information ($\bar{x} \pm s$)

Group	Number of cases	Age (years)	Years of infertility (years)	BMI (kg/m^2)	Type of infertility	
					Primary infertility	Secondary infertility
Pregnancy group	56	29±4.1	2.9±0.2	23.1±3.0	33	23
Non- Pregnancy group	44	30±3.7	3.1±0.3	23.3±2.5	20	24
t		1.79	1.80	1.79	2.34	1.98
P		0.10	0.12	0.23	0.67	0.54

Table 2: Two groups' comparison of 2D ultrasound parameters ($\bar{x} \pm s$)

Group	Number of cases	Endothelial thickness (mm)	RI	PI	S/D
Pregnancy group	56	10.72±3.05	0.65±0.18	1.47±0.51	3.69±1.44
Non- Pregnancy group	44	9.23±2.60	0.74±0.33	1.49±0.53	4.54±6.13
T		3.15	2.09	0.26	0.89
P		0.16	0.23	0.79	0.37

Note: S/D, or the ratio of peak systolic velocity to end-diastolic velocity; RI, or resistance index; PI, or pulsatility index

Table 3: Comparison of the 3D ultrasonography blood flow characteristics in two groups($\bar{x} \pm s$)

Group	Number of cases	Endothelial volume (ml)	VI (%)	FI (0, 100)	VFI (0, 100)
Pregnancy group	56	3.59±1.55	16.29±10.5	7.39±4.73	1.90±3.66
Non- Pregnancy group	44	2.90±1.38	9.54±7.83	5.57±1.76	0.64±0.63
t		2.316	2.468	2.427	2.248
P		0.023	0.009	0.010	0.014

Note: VI, vascularization index; FI, blood flow index; VFI, vascularization flow index

Table 4: Pre-transplant Estrogen and Progesterone ($\bar{x} \pm s$)

Group	Number of cases	E2 (pg/ml)	P (ng/ml)
Pregnancy group	56	274.18±37.17	16.82±10.19
Non- Pregnancy group	44	173.20±12.51	16.09±7.79
T		2.32	0.38
P		0.012	0.699

Note: E2, estradiol; P, progesterone

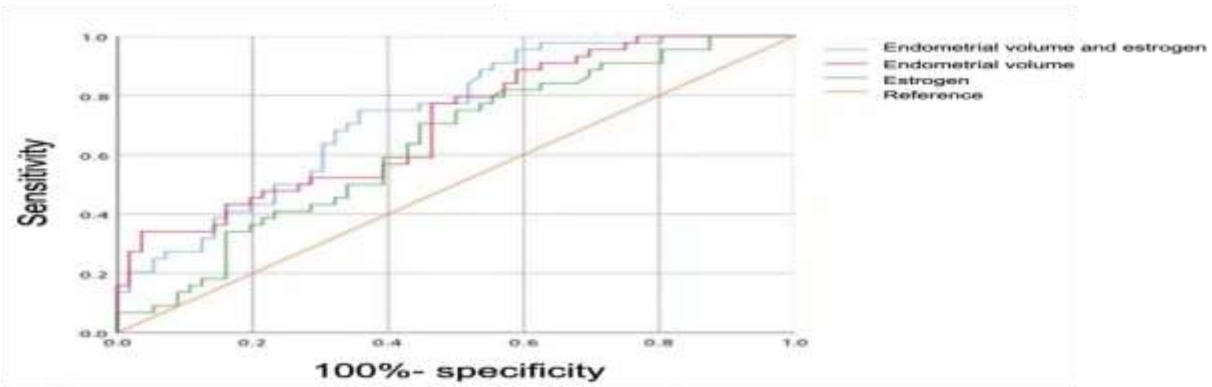

Figure 3: ROC curves of endometrial volume, estrogen levels, and their combination in predicting pregnancy rate.

The ability of the embryo to connect, penetrate, and implant is known as endometrial receptivity, and it describes the endometrium's ability to accept the implantation of the embryo.

Transvaginal color Doppler ultrasound, a non-invasive imaging modality, is currently the most widely utilized technique for assessing endometrial receptivity during the implantation window. Blood flow in the uterine and subendometrial arteries, as well as endometrial thickness and shape, are important evaluation parameters, even in the absence of a defined assessment technique.

The proliferative, secretory, and menstrual stages of the menstrual cycle are correlated with the cyclical changes in endometrial histology. The endometrium is separated morphologically into two layers: the basal layer and the functional layer. In response to increasing estrogen levels, the functional layer—the location of embryo implantation—continually thickens and undergoes morphological changes. To monitor endometrial changes and ascertain the best time for embryo transfer, two-dimensional ultrasonography is utilized. During IVF-ET cycles, gonadotropin-assisted regulated ovarian stimulation starts when the endometrial thickness is less than 5 mm. For embryo implantation, endometrial thickness of ≥8 mm with a trilinear or multilayered shape is considered appropriate. Conversely, when the endometrial thickness ranges between 5-8 mm, endometrial receptivity diminishes, significantly reducing the likelihood of pregnancy.

Subendometrial blood flow is another critical parameter of endometrial receptivity. Endometrial spiral arteries, a more accurate indicator of endometrial receptivity than uterine arteries, proliferate when estrogen and progesterone levels are elevated. Visible sub-endometrial blood flow on the day of embryo transfer is linked to a higher clinical pregnancy rate than when it is absent[5]. Sub-endometrial blood flow is quantitatively evaluated using two-dimensional color Doppler ultrasound using metrics including the systolic/diastolic ratio (S/D), pulsatility index (PI), and resistance index (RI). These indices reflect vascular resistance; lower resistance indicates well-perfused endometrial blood flow and greater endometrial receptivity. An RI ≥ 0.9, PI ≥ 3.0, or absence of end-diastolic flow suggests reduced endometrial receptivity. However, the normal ranges for RI, PI, and S/D values are broad.

In this study, a clinical pregnancy group and a non-pregnant group were compared and their endometrial thickness, RI, PI, and S/D were examined. Our results indicate that two-dimensional ultrasonography indices have limited prognostic value for clinical pregnancy outcomes because there were no statistically significant differences in these indices between the two groups. These results are consistent with findings from other studies[6,7].

Three-dimensional ultrasound offers superior spatial visualization capabilities compared to two-dimensional ultrasound, enabling detailed imaging of the uterus. This technique can determine the volume of an irregular endometrial cavity and evaluate the thickness of the bilateral uterine horns and the endocervical lining. The basal layer of the endometrium exhibits excellent acoustic contrast with the myometrium, particularly during the implantation window, facilitating the acquisition of three-dimensional endometrial volume images. With a notable drop in embryo implantation rates noted when the volume is less than 2 ml, the majority of research has shown that an endometrial volume ≥2 ml is a key threshold for predicting endometrial receptivity [8,9].

Three-dimensional ultrasound parameters for evaluating subendometrial blood flow differ from those used in two-dimensional ultrasound. The subendometrial vascularization index (VI), flow index (FI), and vascularized flow index (VFI) are three-dimensional ultrasound measurements in color Doppler mode that provide accurate indicators of the blood perfusion in the subendometrium.VI measures the quantity of vessels in the area of interest as a percentage. The intensity of blood flow in the area during the 3D scan period is represented by FI, which is computed using the average intensity of the observed color signal. The vascularized blood flow index (VFI), which is determined by dividing the vascularized blood flow index by the color and intensity data for every frame of the gathered image, represents the combined measure of blood flow and vascularization in the region of interest. For successful embryo implantation, subendometrial blood perfusion and a vascularized microenvironment are crucial. Because three-dimensional ultrasound records a greater range of blood flow data than two-dimensional ultrasound, it can provide a more accurate assessment of the number of vessels and blood flow in the measured area. This thorough analysis improves the determination of endometrial receptivity[10,11].

In the current study, there was a statistically significant difference between the endometrial volume, VI, FI, and VFI of the clinical pregnancy group and the non-pregnant group.

These findings suggest that three-dimensional ultrasound has certain advantages over two-dimensional ultrasound in evaluating endometrial receptivity, particularly concerning endometrial volume. The most important factor affecting the clinical pregnancy rate, according to the logistic regression analysis, is endometrial volume. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are two gonadotropins that cause granulosa cells and follicular theca cells to create estrogen. Estrogen binds to endometrial estrogen receptors, promoting endometrial repair and proliferation, which manifests as the proliferation and thickening of endometrial glands. Therefore, serum estrogen levels can indirectly reflect endometrial growth. When progesterone binds to its progesterone receptors, the endometrium transitions from the proliferative to the secretory phase, which is known as the "implantation window. "Both estrogen and progesterone provide essential conditions for embryo implantation and growth during IVF-ET[12].

In this study, serum estrogen (E2) levels were higher in the pregnant group than in the non-pregnant group, while progesterone levels did not differ substantially between the groups. This implies that endometrial proliferation may be influenced by estrogen levels, which in turn may impact the outcome of pregnancy. Pregnancy outcomes are much better when E2 levels are ≥150 pg/ml as opposed to ≥150 pg/ml, according to related research [13]. In a similar vein, increasing clinical pregnancy rates in frozen-thawed embryo transfer cycles requires the use of estrogen replacement therapy to maintain normal physiological levels of estrogen. Higher estrogen levels, along with synchronized proliferation of endometrial glands and stroma, can partially predict pregnancy outcomes.

However, in estrogen replacement therapy cycles, there is no normal follicular growth; instead, exogenous estrogen stimulates endometrial growth, and progesterone is used to induce endometrial transformation. Consequently, estrogen levels are influenced by exogenous factors, making the prediction of pregnancy outcomes based solely on high and low estrogen levels somewhat limited.

In this study, the pregnant group's endometrial volume, VI, FI, and VFI were higher than those of the non-pregnant group.

This is likely due to an increase in estrogen receptors (ER) in individuals with better endometrial receptivity, leading to more substantial endometrial proliferation and increased volume, as well as improved blood perfusion through the branches of the spiral arteries. As a result, these people had higher three-dimensional ultrasound parameters like VI, FI, and VFI. The endometrium is the target organ of estrogen and progesterone, regulated through binding to estrogen receptors (ER) on the endometrial tissue, thereby reflecting endometrial receptivity. Uniform distribution of ER typically results in uniform endometrial thickening and increased subendometrial perfusion in response to rising estrogen levels. However, if the expression of ER in the endometrium is pathologically altered, overly sensitive, or unevenly distributed, endometrial reactivity may become desynchronized with estrogen levels. In such cases, elevated estrogen does not necessarily lead to endometrial thickening or increased blood flow, rendering accurate prediction of embryo transfer outcomes challenging[14].

Three-dimensional ultrasound indices are not affected by localized ER distribution within the endometrium. Instead, three-dimensional ultrasound, which measures endometrial volume, offers a real-time, non-invasive, and understandable depiction of the overall amount of endometrial tissue. This reduces the reliance on dynamic estrogen measurements for evaluating endometrial receptivity. Previous studies have primarily focused on either single ultrasound indices or serum estrogen levels to assess endometrial receptivity. In contrast, our study evaluated endometrial volume and estrogen levels together, finding a predictive efficacy of 0.735, with a sensitivity of 77.3% and specificity of 90.7%. This combined approach proved superior to the measurement of either endometrial volume or estrogen levels alone.

Study strengths and limitations

This study presents several notable strengths. First, the multimodal approach combining transvaginal

3D ultrasonography with serum hormone analysis provides a comprehensive evaluation of endometrial receptivity, addressing the limitations of conventional 2D ultrasound assessments. The inclusion of quantitative vascularization indices (e.g., vascularization flow index, sub-endometrial vascularization index) offers objective measures of endometrial perfusion, which are strongly associated with implantation success. Additionally, the standardized protocol for hormone measurement on the day of embryo transfer minimizes inter-assay variability, enhancing the reliability of the hormonal data. The study's prospective design and well-defined inclusion criteria further strengthen its internal validity.

Despite these strengths, several limitations should be acknowledged. The sample size of 100 patients, while adequate for preliminary analysis, may limit the generalizability of the findings, particularly for subgroups with specific infertility etiologies. Furthermore, the study did not account for potential confounding factors such as embryo quality or genetic abnormalities, which could influence pregnancy outcomes. Future multicenter studies with larger cohorts and longitudinal follow-up are warranted to validate these findings and refine the predictive model.

Conclusion

When evaluating endometrial volume and blood flow, transvaginal three-dimensional ultrasound outperforms two-dimensional ultrasound. It provides a more comprehensive and accurate reflection of endometrial receptivity. When combined with estrogen level measurements, it enhances the ability to determine the optimal timing for IVF-ET, thereby improving clinical pregnancy rates.

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Authors’ contributions

Yanxia Yu: Conceptualized and designed the study, interpreted the data, and wrote the manuscript. Jia Xing: Conducted the statistical analysis, assisted in data collection, and contributed to manuscript revisions. Huiqin Xiao: Performed the transvaginal ultrasound procedures, collected clinical data, and contributed to the interpretation of results. Lijuan Jiang: Assisted in data analysis, provided critical feedback on the manuscript, and helped with manuscript editing.

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Conflicting interests

The authors declare no competing interests.

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