ORIGINAL ARTICLE
ANIMAL STUDY

Predicting Clinical Pregnancy Rates in IVF-ET Using Transvaginal 3D Ultrasound and Serum Hormone Levels

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Abstract

Objective: To investigate the predictive value of transvaginal three-dimensional ultrasound combined with serum oestrogen and progesterone levels in enhancing the clinical pregnancy rate of in vitro fertilization-embryo transfer.

Method: The study was conducted at the Reproductive and Women-Children Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, China, from September 2020 to March 2021, and comprised patients undergoing in vitro fertilization-embryo transfer with frozen-thawed embryo cycles. The day before embryo implantation, endometrial thickness and sub-endometrial blood flow parameters were measured using two-dimensional ultrasound and then converted to three-dimensional ultrasound mode to assess endometrial volume and sub-endometrial vascularisation parameters. Serum estradiol and progesterone levels were also measured on the day of transplantation. The subjects were categorised into pregnancy and non-pregnancy groups based on their outcomes. The correlation of two-dimensional and three-dimensional ultrasound parameters was compared alongside serum estradiol and progesterone levels with respect to clinical pregnancy outcomes. Data was analysed using SPSS 22.

Results: Of the 100 women with mean age 32.8±8.3 years (range: 22-44 years), 56(56%) were in the clinical pregnancy group and 44(44%) were in the non-pregnancy group. There was no significant differences in endometrial thickness or two-dimensional ultrasound sub-endometrial blood flow parameters between the groups (p>0.05). In contrast, endometrial volume and three-dimensional sub-endometrial vascularisation indices were significantly higher in the pregnancy group (p<0.05). Combining endometrial volume with serum estradiol levels yielded a predictive accuracy with area under the curve 0.735, sensitivity 77.3% and specificity 90.7%.

Conclusion: Transvaginal three-dimensional ultrasound-derived endometrial volume and sub-endometrial vascularisation parameters, combined with serum estradiol levels, could significantly improve the prediction of clinical pregnancy success in cases of in vitro fertilization-embryo transfer.

Keywords: Transvaginal three-dimensional ultrasound, Oestrogen level, Endometrial receptivity, In vitro fertilization-embryo transfer. (JPMA 75: S-47 [Suppl. 02]; 2025) **DOI: https://doi.org/10.47391/JPMA.SRPH-08**

Introduction

In vitro fertilization-embryo transfer (IVF-ET) is a critical intervention for treating infertility.[1,2] Despite advancements in laboratory techniques that have significantly improved the quality of IVF embryos, the clinical pregnancy success rate for IVF-ET remains 20-30%. Enhancing the success rate of IVF-ET during the implantation stage continues to be a significant challenge.[3] Research has primarily focussed on the relationship between two-dimensional (2D) ultrasound parameters and IVF-ET outcomes, estradiol (E2), progesterone (P), and the P/E2 ratio. However, these assessments are relatively limited and homogeneous in scope.[4,5]

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Transvaginal three-dimensional (3D) ultrasound offers more comprehensive insights, encompassing conventional 2D ultrasound information, such as endometrial thickness and morphology, while also providing accurate evaluations of endometrial receptivity, including measurements of endometrial volume and sub-endometrial vascularisation parameters. Studies have employed 3D ultrasound with serum oestrogen (E) and P levels to assess endometrial receptivity, and to identify the optimal window for embryo transfer, thereby improving the clinical pregnancy rate of IVF-ET.

The predictive value of 3D ultrasound assessment of endometrial receptivity in preimplantation genetic diagnosis/pre-implantation genetic screening (PGD/PGS) transplantation patients has been investigated. Endometrial thickness and blood flow assessed by 3D ultrasound were found to have high predictive value for pregnancy outcomes.[6] The sensitivity and specificity of these measurements were 91.18% and 82.35%,

respectively, suggesting that 3D ultrasound can be a reliable tool for assessing endometrial receptivity and predicting successful pregnancy outcomes in patients undergoing PGD/PGS.[7]

A prospective observational study assessed the predictive value of endometrial blood flow branches on pregnancy outcomes after hormone replacement therapy-frozen embryo transfer (HRT-FET). It was concluded that endometrial blood flow perfusion during the peri-transplantation period is a good indicator of pregnancy outcomes. Power Doppler ultrasound effectively detected these blood flow changes, providing valuable information for predicting the success of HRT-FET cycles. Machine learning algorithms were applied to predict early pregnancy outcomes in FET cycles. Factors such as age, body mass index (BMI), endometrial thickness, and serum hormone levels were used to build prediction models.[8] Despite the complexity of the models, no significant difference was found among them in predicting pregnancy outcomes. This indicates the need for more effective predictors and highlights the potential of machine learning in refining predictive models for embryo transfer outcomes.

A predictive model based on endometrial receptivity evaluations by 3D ultrasound was developed. This model demonstrated high diagnostic efficiency and could be a simple and effective tool for predicting the inception of first-trimester pregnancy after IVF-ET. To assess endometrial receptivity, 3D ultrasound provides a non-invasive method to enhance the accuracy of pregnancy predictions in IVF-ET cycles. A nomogram model was constructed and validated for predicting clinical pregnancy in endometriosis patients undergoing fresh embryo transfer. Factors such as female age, American Society for Reproductive Medicine (ASRM) stage, and antral follicle count were identified as independent influencing factors. The model provides a personalised approach to predicting pregnancy outcomes, helping clinicians tailor treatments to individual patients' needs. The correlation between endometrial and sub-endometrial blood flow, measured by colour and power Doppler, and pregnancy rates during IVF treatment was examined. Specific Doppler flow indices were found to be predictive of pregnancy outcomes. This highlights the importance of assessing blood flow in the endometrium and sub-endometrium to evaluate uterine receptivity in IVF cycles. A model was developed to predict live-birth rates after embryo transfer, accounting for factors such as patient age, embryo stage, and type of transfer cycle. The model showed good predictive accuracy and could guide the number of embryos to transfer to minimise multiple gestations. This approach helps balance achieving a successful pregnancy while reducing the risk of complications associated with multiple pregnancies.[9]

Several independent factors influencing live-birth rates in fresh embryo transfer cycles for polycystic ovary syndrome (PCOS) patients were identified. These factors included female age, BMI, infertility duration, and serum hormone levels. The predictive model developed from these factors provides a valuable tool for clinicians to estimate the likelihood of live-birth in PCOS patients undergoing fresh embryo transfer.[10]

Although focussed on cerebral small vessel disease, the methodology of developing and validating a nomogram predictive model can be applied to predicting clinical pregnancy rates in IVF-ET. Integrating multiple clinical predictors into a single model enhances the accuracy and reliability of predictions, offering a comprehensive approach to patient assessment.[11]

While substantial progress has been made in using ultrasound parameters and predictive models to assess endometrial receptivity and IVF-ET outcomes, existing approaches are often limited in scope and specificity. There is a critical need to refine these models by integrating advanced 3D ultrasound metrics with hormonal profiles to improve their predictive accuracy.

The current study was planned to investigate the predictive value of transvaginal 3D ultrasound combined with serum E and P levels in enhancing the clinical pregnancy rate of in vitro fertilization-embryo transfer.

Materials and Methods

This study was conducted at the Reproductive and Women-Children Hospital, Chengdu University of Traditional Chinese Medicine (TCM), Chengdu, China, from September 2020 to March 2021, and comprised female patients who underwent frozen-thawed embryo transfer as part of their IVF-ET treatment.

Those included were subjects with either frozen embryos remaining after fresh embryo transfer during IVF-ET or embryos frozen at the day 3 oocyte stage due to various clinical or laboratory reasons, provided that at least one embryo was deemed transferable after thawing.

Those excluded were females with endometrial polyps, submucosal fibroids, uterine effusion, uterine adhesions, adenomyosis, hydrosalpinx, uterine developmental abnormalities, hereditary diseases affecting embryo implantation, and chromosomal abnormalities.

Patients were categorized into clinical pregnancy group and non-pregnancy group based on pregnancy outcome. A convenience sampling technique was used to enrol the

participants. All the participants provided written informed consent prior to enrollment.

A colour Doppler ultrasound diagnostic instrument (EPIQ5 colour Doppler ultrasound, Philips Healthcare, The Netherlands) with an intracavitary 3D volumetric probe was utilised for all ultrasound assessments that were done by a single trained sonographer. On the day before embryo transfer, the participants underwent transvaginal ultrasonography after emptying their bladders.

Serum E2 and P levels were measured using an immunoassay analyser (E601, Roche Diagnostics, Germany) as per to the manufacturer's instructions. On the day of embryo transfer, the patients fasted for at least 8 hours, and 3mL of venous blood was collected for hormone measurements.

Clinical pregnancy was confirmed if a urine pregnancy test was positive or if blood beta human chorionic gonadotropin (β-HCG) levels exceeded 5U/L 14 days post-embryo transfer, followed by the detection of an intrauterine gestational sac with cardiac pulsation via ultrasound 28 days later.

Data was analysed using SPSS 22. Data was expressed as frequencies and percentages or as means±standard deviation, as appropriate. Independent samples *t*-test was used for group comparisons, and logistic regression was used to analyse the correlation between ultrasound and hormonal parameters with clinical pregnancy outcomes. ROC curves evaluated the predictive value of these parameters. P<0.05 was considered statistically significant.

Results

Of the 100 women with mean age 32.8±8.3 years (range: 22-44 years), 56(56%) were in the clinical pregnancy group with mean age 29±4.1 years, and 44(44%) were in the non-pregnancy group having mean age 30±3.7 years (*p*=0.12). There was no significant difference between the groups with respect to baseline parameters (Table 1).

In terms of 2D ultrasound parameters, there was no significant difference between the groups with respect to endometrial thickness, resistance index (RI), pulsatility index (PI) and systolic/diastolic (S/D) ratio (Table 2).

Table-1: Intergroup comparison of general characteristics.

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-test		1.79	1.80	1.79	2.34	1.98
p-value		0.12	0.12	0.23	0.67	0.54

BMI: Body mass index.

Figure 1 demonstrates a complete endometrial image acquired via transvaginal three-dimensional (3D) ultrasound, while Figure 2 illustrates the acquisition process using transvaginal 3D ultrasound technology.

However, 3D ultrasound parameters, including endometrial volume (V), vascularisation index (VI), flow index (FI) and vascularisation flow index (VFI), were significantly higher

Table-2: Intergroup comparison of two-dimensional (2D) ultrasound parameters.

Group	Number of cases	Endothelial thickness (mm)	RI	PI	S/D velocity
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-test		3.15	2.09	0.26	0.89
p-value		0.16	0.23	0.79	0.37

RI: Resistance index, PI: Pulsatility index, S/D: Ratio of peak systolic velocity to end-diastolic velocity.

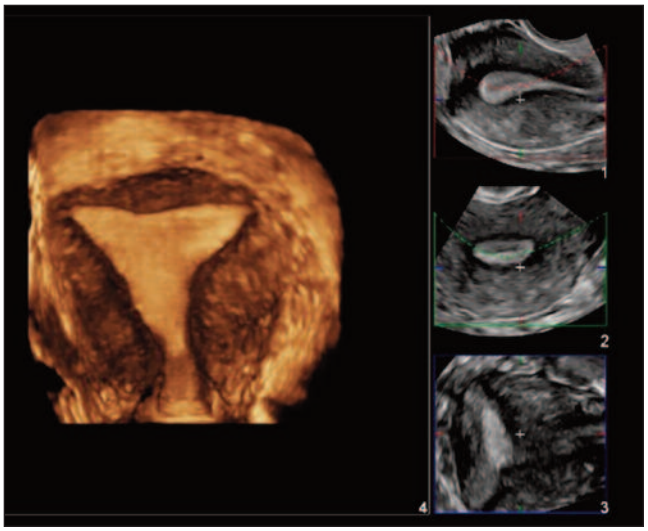

Figure-1: Transvaginal three-dimensional (3D) ultrasound showing a complete endometrial image.

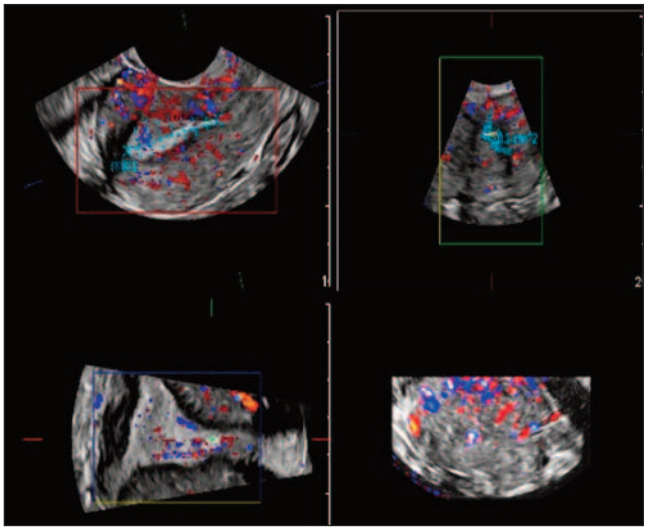

Figure-2: Transvaginal three-dimensional (3D) ultrasound acquisition.

in the clinical pregnancy group compared to the non-pregnancy group (Table 3).

Regarding pre-transfer serum hormone levels, E2 levels were significantly higher in the pregnancy group compared to the non-pregnancy group (p=0.012). There was no significant difference in serum P levels between the groups (p=0.699) (Table 4).

Logistic regression analysis comprised E2 levels, endometrial volume, VI, FI and VFI as independent variables, and clinical pregnancy as the dependent variable. Endometrial volume and E2 levels were significantly correlated with pregnancy outcomes (p<0.05).

ROC curve analysis assessing the predictive value of combining endometrial volume and E2 levels for clinical pregnancy showed that area under the curve (AUC) for the combined predictors was 0.735, with sensitivity 77.3% and specificity 90.7%. For endometrial volume alone, the AUC was 0.704 with sensitivity 75.2% and specificity 83.3%, while for E2 levels alone, the AUC was 0.636 with sensitivity 70.5% and specificity 68.7%. Combining the 3D ultrasound indices with E2 levels yielded the highest sensitivity and specificity for predicting clinical pregnancy outcomes (Figure 3).

Table-3: Intergroup comparison of three-dimensional (3D) ultrasound blood flow parameters.

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-test		2.316	2.468	2.427	2.248
p-value		0.023	0.009	0.010	0.014

VI: Vascularisation index, FI: Blood flow index, VFI: Vascularisation flow index.

Table-4: Pre-transplant estrogen and progesterone levels.

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-test		2.32	0.38
p-value		0.012	0.699

E2: Estradiol, P: Progesterone

Discussion

The IVF-ET technique, also known as in vitro fertilization,[12] 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 foetus. The success rate of clinical pregnancy through IVF-ET is influenced by various factors, including but not limited to maternal age, the receptivity of the endometrial implantation environment, the timing of the implantation window, the density of E and P receptors, and sub-endometrial blood perfusion. Among these, the adequacy of the intrauterine implantation environment is a critical determinant.

Endometrial receptivity refers to the endometrium's capacity to accept embryo implantation, characterised by the ability of the embryo to attach, penetrate and implant. Transvaginal colour Doppler ultrasound, a non-invasive imaging modality, is currently the most widely utilised technique for assessing endometrial receptivity during the implantation window. Despite the absence of a

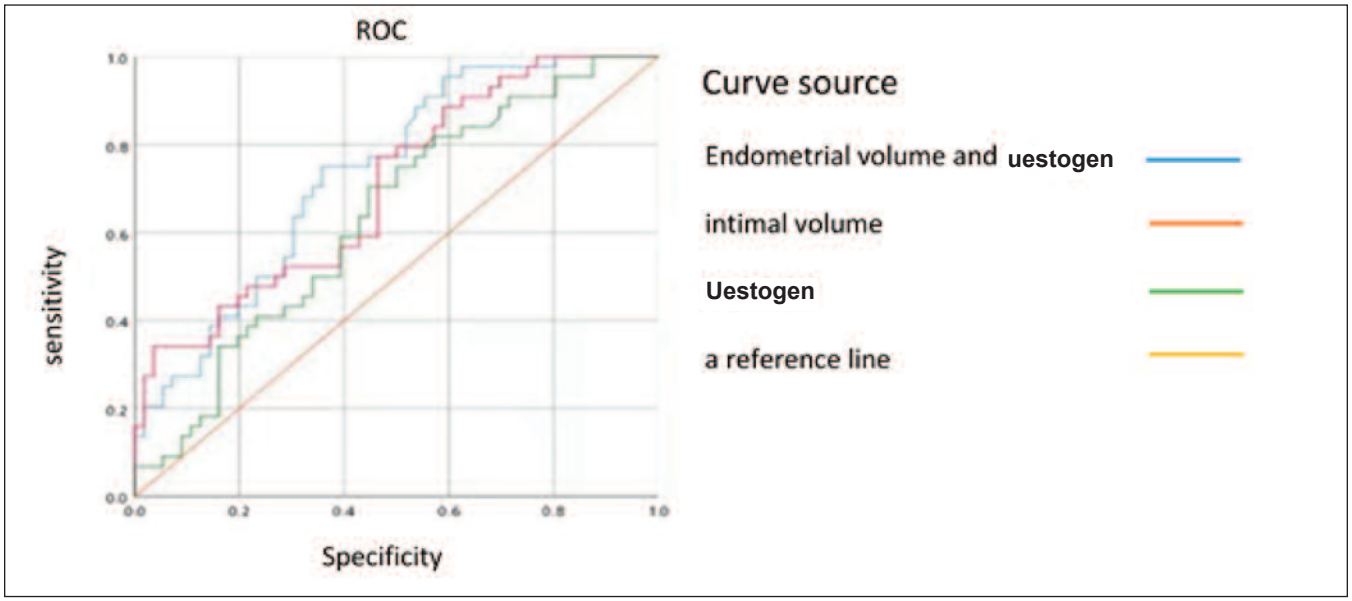

Figure-3: Receiver operating characteristic (ROC) curves of endometrial volume, oestrogen levels, and their combination in predicting pregnancy rate.

standardised assessment protocol, key evaluation parameters include endometrial thickness, morphology and blood flow in the uterine and sub-endometrial arteries.

The histology of the endometrium undergoes cyclical changes corresponding to the menstrual cycle's proliferative, secretory and menstrual phases. Morphologically, the endometrium is divided into functional and basal layers.[13] The functional layer, which serves as the site for embryo implantation, experiences continuous thickening and morphological alterations in response to rising E levels. A2D ultrasound is employed to observe endometrial changes and to determine the optimal timing for embryo transfer. During IVF-ET cycles, controlled ovarian stimulation with gonadotropins is initiated when the endometrial thickness is ≤5mm. An endometrial thickness of ≥8mm, with a trilinear or multilayered morphology, is deemed suitable for embryo implantation. Conversely, when the endometrial thickness ranges 5-8 mm, endometrial receptivity diminishes, significantly reducing the likelihood of pregnancy.[14,15]

Subendometrial blood flow is another critical parameter of endometrial receptivity. Elevated levels of E and P promote the proliferation of endometrial spiral arteries, which serve as a more precise measure of endometrial receptivity than uterine arteries. The presence of detectable sub-endometrial blood flow on the day of embryo transfer correlates with a higher clinical pregnancy rate compared to the absence of such blood flow.[16] A 2D colour Doppler ultrasound quantitatively assesses sub-endometrial blood flow using indices, such as RI, PI, and S/D. 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.

The current study compared and analysed endometrial thickness, RI, PI and S/D between a clinical pregnancy group and a non-pregnant group. The findings revealed no significant differences in these indices between the groups, suggesting that 2D ultrasound indices had limited predictive value for clinical pregnancy success. These results are consistent with earlier findings.[17,18]

A 3D ultrasound offers superior spatial visualisation capabilities compared to 2D ultrasound, enabling detailed imaging of the uterus. This modality can measure the thickness of the endocervical lining and the bilateral uterine horns, and calculate the volume of an irregular endometrial cavity. The basal layer of the endometrium exhibits excellent acoustic contrast with the myometrium, particularly during the implantation window, facilitating the acquisition of 3D endometrial volume images. Most studies have indicated that an endometrial volume ≥2ml is a critical threshold for predicting endometrial receptivity, with a significant decrease in embryo implantation rates observed when the volume is <2ml.[19,20]

Further, 3D ultrasound parameters for evaluating sub-endometrial blood flow differ from those used in 2D ultrasound. In colour Doppler mode, 3D ultrasound can measure the sub-endometrial VI, FI and VFI, which provide accurate reflections of sub-endometrial blood perfusion. VI, expressed as a percentage, quantifies the number of vessels in the region of interest. FI, calculated using the average intensity of the detected colour signal, represents the intensity of blood flow in the region during the 3D scan time. VFI, the ratio of the vascularised blood flow index to the colour and intensity data for each frame of the acquired image, represents the combined measure of blood flow and vascularisation in the region of interest.

Sub-endometrial blood perfusion and a vascularised microenvironment are crucial for successful embryo implantation. A 3D ultrasound captures more blood flow information than 2D ultrasound, providing a more accurate assessment of vessel number and blood flow in the measured area. This comprehensive evaluation enhances the assessment of endometrial receptivity.[21-23]

In the present study, significant differences were observed in endometrial volume, VI, FI and VFI between the clinical pregnancy group and the non-pregnant group. These findings suggest that 3D ultrasound had certain advantages over 2D ultrasound in evaluating endometrial receptivity, particularly concerning endometrial volume. Logistic regression analysis further indicated that endometrial volume was the most critical factor influencing the clinical pregnancy rate.

Oestrogen is synthesised by both follicular theca cells and granulosa cells under the influence of two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). It binds to endometrial E receptors, promoting endometrial repair and proliferation, which manifests as the proliferation and thickening of endometrial glands. Therefore, serum E levels can indirectly reflect endometrial growth. P binds to endometrial progesterone receptors, transitioning the endometrium from the proliferative to the secretory phase, and inducing the "implantation window." Both E and P provide essential conditions for embryo implantation and growth during IVF-ET.[24]

In the current study, serum E2 levels were higher in the pregnancy group than in the non-pregnancy group,

whereas P levels showed no significant difference. This suggests that E levels may influence endometrial proliferation, subsequently affecting pregnancy outcomes. Related studies[25] have concluded that pregnancy outcomes are significantly better with E2 levels ≥150 pg/ml than with <150 pg/ml. Similarly, in frozen-thawed embryo transfer cycles, maintaining normal physiological E levels through E replacement therapy (ERT) is crucial for improving clinical pregnancy rates. Higher E levels and synchronised proliferation of endometrial glands and stroma can partially predict pregnancy outcomes.

However, in ERT cycles, there is no average follicular growth; exogenous E stimulates endometrial growth, and P is used to induce endometrial transformation. Consequently, E levels are influenced by exogenous factors, making the prediction of pregnancy outcomes based solely on high and low E levels somewhat limited.

In the current study, endometrial volume, VI, FI and VFI were higher in the pregnancy group compared to the non-pregnancy group. This is likely due to increased E receptors (ERs) in individuals with better endometrial receptivity, leading to a more substantial endometrial proliferation, increased volume and improved blood perfusion through the branches of the spiral arteries. Consequently, 3D ultrasound parameters, such as VI, FI and VFI, were more significant in these individuals.

The endometrium is the target organ of E and P, regulated through binding to ERs on the endometrial tissue, thereby reflecting endometrial receptivity.

Uniform distribution of ER typically results in uniform endometrial thickening and increased sub-endometrial perfusion in response to rising E levels. However, if the expression of ER in the endometrium is pathologically altered, overly sensitive, or unevenly distributed, endometrial reactivity may become desynchronised with E levels. In such cases, elevated E level does not necessarily lead to endometrial thickening or increased blood flow, rendering accurate prediction of embryo transfer outcomes challenging.[26]

The 3D ultrasound indices are not affected by localised ER distribution within the endometrium. Instead, as measured by 3D ultrasound, the endometrial volume reflects the overall quantity of endometrial tissue in a real-time, intuitive and non-invasive manner. This reduces the reliance on dynamic E measurements for evaluating endometrial receptivity.

Previous studies have primarily focussed on either single ultrasound index or serum E levels to assess endometrial receptivity.[27-29] In contrast, the current study evaluated endometrial volume and E levels together. This combined approach, a strength of the current study, proved superior to measuring endometrial volume or E level alone.

The current study has limitations as the sample size was not calculated, which may have affected the power of the study and the generalisability of the findings.

Conclusion

Transvaginal 3D ultrasound surpassed 2D ultrasound in assessing endometrial volume and blood flow, providing a more comprehensive and accurate reflection of endometrial receptivity. When combined with E levels, it enhanced the ability to determine the optimal timing for IVF-ET, thereby improving clinical pregnancy rates.

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Conflict of Interest: None.

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