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Utility of endometrial multi-vessel blood flow ultrasound parameters in predicting pregnancy outcomes

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Abstract

Objectives: To evaluate the endometrium using multi-vessel blood flow ultrasound parameters and explore their potential utility in predicting pregnancy outcomes.
Methods: A total of 205 women were included in the study. Transvaginal three-dimensional ultrasound was used to evaluate luteal phase endometrial thickness, volume, and three-dimensional power Doppler blood flow parameters of the endometrium (vascularization index [VI], flow index [FI], and vascularization flow index [VFI]), as well as the endometrial microvascular vascularization index (VI_{MV}). Additionally, the systolic/diastolic ratio (S/D), pulsatility index (PI), and resistance index (RI) of the bilateral uterine arteries were measured. Based on pregnant outcomes, participants were divided into two groups: a clinical pregnancy group and a non-pregnancy group, and ultrasound parameters were compared between these groups.
Results: Women in the clinical pregnancy group showed significantly higher levels of endometrial FI, VI, VFI, and VI_{MV} compared to those in the non-pregnancy group ($p<0.05$). Conversely, the S/D, PI, RI, and mean levels of bilateral uterine arteries were significantly lower in the pregnancy group than in the non-pregnancy group ($p<0.05$). VI_{MV} (area under the ROC curve [AUC] 0.869; sensitivity 92.2 %, specificity 68.8 %), VFI (AUC 0.761; sensitivity 96.9 %, specificity 53.9 %), FI (AUC 0.707; sensitivity 79.7 %, specificity 46.1 %), and VI (AUC 0.637; sensitivity 90.6 %, specificity 51.8 %) all showed a significant strong positive correlation with endometrial thickness ($r>0$, $p<0.05$), indicating their role in predicting pregnancy.
Conclusions: Multi-vessel blood flow parameters reflect the endometrial blood perfusion state and are closely associated with the likelihood of achieving a successful pregnancy.

Keywords: endometrial blood flow; microvascular blood flow; pregnancy outcome; three-dimensional power doppler; ultrasound examination

Introduction

The incidence of infertility has been rising annually due to factors such as unhealthy lifestyle habits and environmental pollution [1, 2], emerging as a significant concern for couples of reproductive age worldwide. Despite ongoing advancements in assisted reproductive technology, implantation failure post-transfer has been found even with high-quality embryos. This suggests that endometrial insufficiency during the implantation window may be a critical factor contributing to unsuccessful pregnancies [3]. Endometrial thickness and blood perfusion status are considered key determinants affecting pregnancy outcomes [4]. Transvaginal three-dimensional ultrasound not only enables the measurement of parameters such as endometrial thickness and volume but also detects low-velocity, subtle blood flow signals in the endometrium. The micro-vascular flow (MV-Flow) imaging technology offers higher sensitivity, better resolution, and faster frame rates. The combination of these two methods not only allows for quantitative assessment of endometrial blood flow but also visualizes parameter values and provides real-time information on endometrial anatomical planes and blood supply [5].

In this study, our aim was to further explore and validate the potential role of ultrasound assessment techniques in predicting endometrial blood supply and receptivity, as well as their association with pregnancy outcomes, by analyzing multi-vessel blood flow parameters of the endometrium.

Materials and methods

Research participants

This study enrolled women in the preconception period who visited Wenzhou People's Hospital between June 2020 and June 2023. According to predefined inclusion and exclusion criteria, a total of 205 eligible women were included. Participants were categorized into either the pregnancy group

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(64 cases) or non-pregnancy group (141 cases) based on whether they achieved natural conception during follow-up. Pregnancy confirmation required ultrasound verification of intrauterine gestational sac presence, with subsequent follow-up observations continuing through 3 months of gestation.

Inclusion criteria:

(1) Females aged 24–40 years;
(2) Healthy women preparing for pregnancy with regular menstrual cycles (27–32 days) and normal ovulation confirmed by monitoring;
(3) Normal ovarian function;
(4) Presence of mature follicle development and confirmed normal ovulation;
(5) Endometrial thickness≥7 mm at ovulation;
(6) No use of sex hormone-related medications;
(7) No history of adverse lifestyle factors (e.g., smoking, alcohol abuse);
(8) Signed informed consent.

Exclusion criteria:

(1) Uterine anomalies (e.g., bicornuate uterus, septate uterus);
(2) Endometrial polyps, submucosal fibroids, or endometriosis;
(3) History of intrauterine procedures, intrauterine adhesions, or cervical stenosis;
(4) History of miscarriage, fetal demise, infertility, or related medications;
(5) Active reproductive tract infections;
(6) Thyroid dysfunction or severe endocrine disorders;
(7) Abnormal semen parameters in the male partner or unilateral/bilateral fallopian tube obstruction.

This study received approval from the Ethics Committee of the hospital (Ethics no.: KY-202410-005q). All participants provided their signed informed consent forms prior to undergoing an ultrasound examination.

Research methods

Ultrasound examinations were conducted with Samsung Medison HERA W10 diagnostic equipment (NMPA Registration No. 20192060548, 50/60 Hz) equipped with a 5–9 MHz transvaginal probe. All the selected participants began to monitor the follicles by ultrasound on the 10th to 12th day of their menstrual cycle. They were instructed to recheck the follicles every 1–2 days according to the actual growth rate of the follicles until ovulation was confirmed to be normal. After that, the couples were instructed to have sexual intercourse. Seven to nine days post-ovulation, a transvaginal three-dimensional ultrasound examination was conducted to assess uterine and endometrial conditions. All examinations were performed by the same ultrasound physician with over five years of work experience and appropriate training, using the same equipment model and ensuring optimal image clarity.

All of the following measurements were taken three times to obtain an average value:

(1) Endometrial thickness: Endometrial thickness was measured in the sagittal plane of the uterus, approximately 1 cm from the top of the uterine cavity.

(2) Uterine artery blood flow parameters: Adjust the color gain and speed range appropriately at a point 2 cm lateral to the junction of the cervix and the uterine body until the main uterine artery blood flow signal can be clearly identified. The sampling volume is 2 mm, the sampling angle is less than 30°, and five consecutive and stable blood flow spectra are collected. The systolic/diastolic ratio (S/D), pulsatility index (PI), and resistance index (RI) of both the left and right uterine arteries were measured and averaged.

(3) Endometrial volume and power Doppler blood flow assessment (Figure 1): This evaluation must be performed using a 3D power Doppler ultrasound system (recommended with an intracavitary probe≥12 MHz). Prior to operation, complete the following standardized presets: adjust the flow velocity scale to 4–8 cm/s, pulse repetition frequency (PRF) of 0.3–0.9 kHz, Doppler frequency of 5–7 MHz, and preset a 50–100 Hz wall filter. The patient should be positioned in a modified lithotomy position (hip abduction≥120°), maintain moderate bladder filling 30 min before the examination, and be instructed to perform end-expiratory breath-holding during the data acquisition window to minimize respiratory artifacts. During 3D volume sampling, ensure the insonation angle between the ultrasound beam and blood flow direction is≤30°, employ a 120°scanning angle to cover the entire endometrial layer and a 2 mm range at the myometrial junction, select a medium frame rate (18–22 Hz), and activate automatic angle correction. Quantitative analysis is conducted using the VOCAL system with a six-plane segmentation method: set a 30 dB volume threshold to calculate endometrial volume (V), derive the flow index (FI) based on color pixel intensity integration using a 0–100 scale, determine the vascularization index (VI) through blood flow distribution density percentage, and generate the standardized vascularization-flow index (VFI). Quality control protocols require triplicate measurements averaged for final reporting, with permissible error ranges of V≤5 %, VI≤10 %, and FI≤8 %, while ensuring thermal index (TI) ≤0.4 and mechanical index (MI) ≤0.7 during single-scan durations≤90 s. All parameters must be synchronized to the mid-diastolic

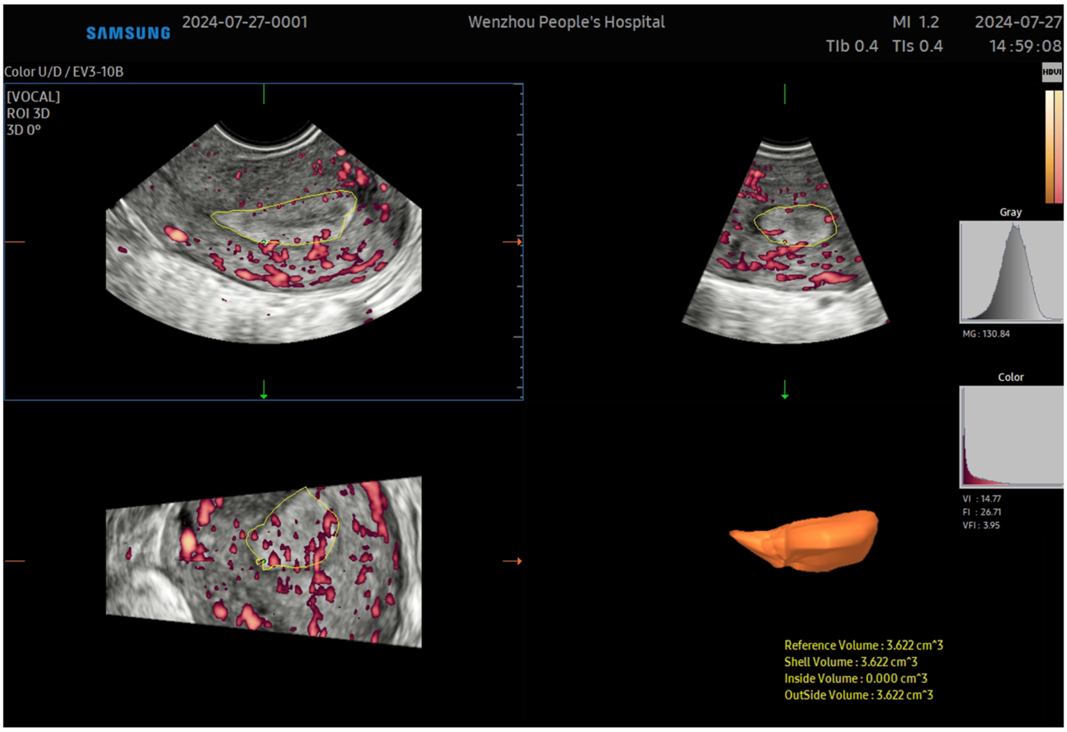

Figure 1: Endometrial volume and endometrial power Doppler blood flow.

phase of the cardiac cycle using R-wave triggering. For special cases (e.g., intrauterine adhesions, polyps), activate high-frequency harmonic imaging (H-Flow mode) and supplement with coronal plane scanning sequences.

(4) Microvascular imaging of the endometrium (Figure 2): The mid-sagittal plane was selected to ensure a clear view of the endometrium, and the MV-Flow function of the device was activated. The probe was maneuvered laterally until both uterine angles were no longer visible in the same plane, and at this point, dynamic microvascular flow images of the longitudinal section of the endometrium were captured. Based on the recorded dynamic images, manual tracing was performed every 5 to 10 frames to measure the VI of the endometrium per unit area. This process was repeated 7 to 12 times to calculate an average, thus determining the endometrial VI_{MV} per unit area.

Statistical analysis

SPSS 26.0 was used for statistical analyses. Quantitative data were tested for normality prior to further analysis. Data conforming to a normal distribution were expressed as the mean ± standard deviation ($\bar{x} \pm S$), and comparisons between groups were performed using the independent samples t-test. For data not conforming to a normal distribution, the median (interquartile range) was used, and the Mann–Whitney U test was used for intergroup comparisons. Spearman correlation analysis was employed to explore the relationships among various indicators. Receiver operating characteristic (ROC) curves were used to evaluate the predictive efficacy of each indicator for determining pregnancy results. The significance threshold was set at a p-value of<0.05 for determining statistical significance.

Results

Comparison of age and endometrial ultrasound evaluation indicators between the pregnancy and non-pregnancy groups

Independent samples t-tests were used to compare patient age, body mass index (BMI), FI, left PI, right PI, and mean PI between the pregnancy group and non-pregnancy group. There were no statistically significant differences in age and BMI between the two groups ($p>0.05$). However, FI was significantly higher in the pregnancy group than that in the non-pregnancy group, while left PI, right PI, and mean PI were significantly lower, with differences between the two groups in all these indicators being statistically significant ($p<0.05$).

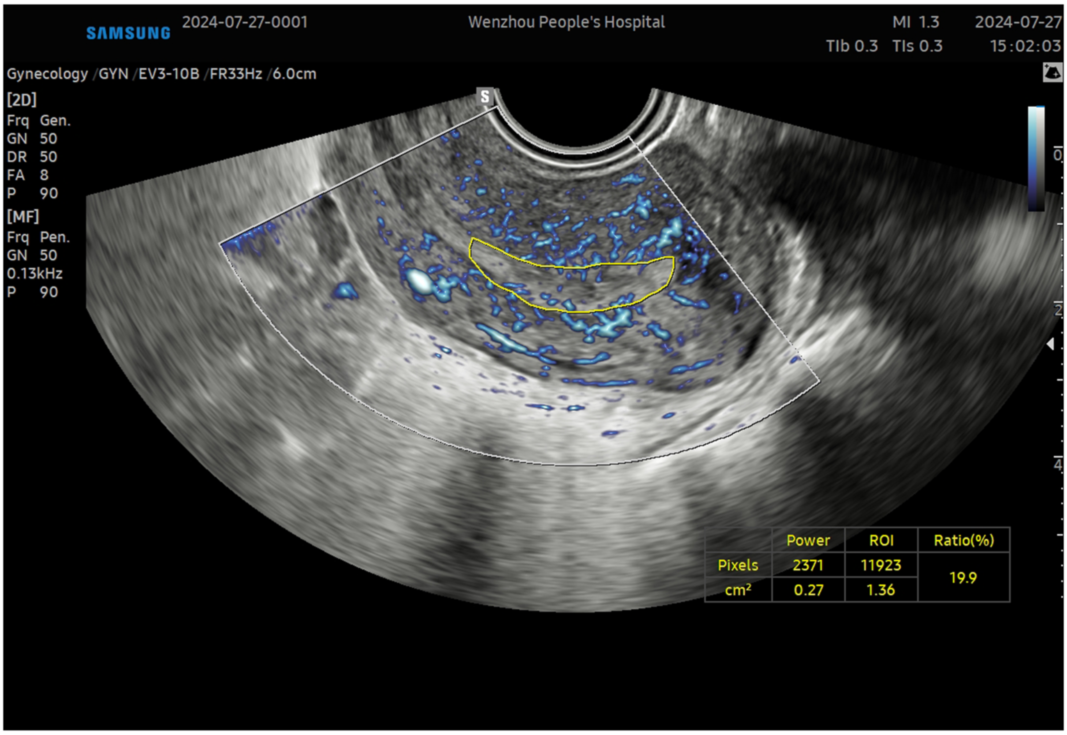

Figure 2: Endometrial microvascular imaging.

The results of the Mann–Whitney U tests for endometrial thickness, endometrial volume, VI, VFI, VI_{MV}, left S/D, right S/D, mean S/D, left RI, right RI, and mean RI between the pregnancy group and non-pregnancy group showed no statistically significant differences in endometrial thickness and endometrial volume between the two groups ($p>0.05$). However, VI, VFI, and VI_{MV} of women in the pregnancy group were significantly higher than those in the non-pregnancy group, while left S/D, right S/D, mean S/D, left RI, right RI, and mean RI were significantly lower than those in the non-pregnancy group, with statistically significant differences ($p<0.05$) (Table 1).

Correlation analysis of positive endometrial ultrasound evaluation indicators

Correlation between VI, VFI, FI, and VI_{MV}

Spearman correlation analysis indicated significant positive correlations ($r>0$, $p<0.05$) among VI, VFI, FI, and VI_{MV} (Table 2).

Correlation of VI, VFI, FI, and VI_{MV} with mean uterine artery parameters

Results of the Spearman correlation analysis revealed significant negative correlations ($r<0$, $p<0.05$) between VFI and VI_{MV} and the mean S/D, mean PI, as well as mean RI of the uterine artery. However, the correlations between VI, FI, and mean S/D, mean PI, and mean RI were not statistically significant ($p>0.05$) (Table 3).

Correlation of VI, VFI, FI, and VI_{MV} with endometrial thickness

There were significant positive correlations ($r>0$, $p<0.05$) between VI, VFI, FI, VI_{MV}, and endometrial thickness as indicated by the Spearman correlation analysis (Table 4).

ROC curve analysis of VI, VFI, FI, and VI_{MV} in predicting pregnancy

ROC curve analysis (Figure 3) showed that the area under the ROC curve (AUC) values for endometrial power Doppler ultrasound parameters in predicting pregnancy were 0.707 for VI, 0.637 for FI, 0.761 for VFI, and 0.869 for VI_{MV}, with all p-Values<0.05, indicating that these indicators had statistically significant pregnancy predictive capacity. The analysis revealed a pregnancy prediction threshold of 2.509 for VI, with a sensitivity of 90.6 % and a specificity of 51.8 %. For FI, the pregnancy prediction threshold was 21.579, with a sensitivity of 79.7 % and a specificity of 46.1 %. The threshold for predicting pregnancy for VFI was 0.728, with a sensitivity of

Table 1: Comparison of age and endometrial ultrasound evaluation parameters between the pregnancy and non-pregnancy groups.

Indicators	Pregnancy group (n=64)	Non-pregnancy group (n=141)	t/Z	p-Value
Age	31.77 ± 4.04	31.315.06	0.697	0.487
BMI	21.87 ± 1.56	21.83 ± 1.54	0.835	0.401
Endometrial thickness	8.00 (7.00, 9.00)	8.00 (6.50, 10.00)	−1.462	0.144
Endometrial volume	3.03 (2.54, 3.92)	3.11 (2.28, 4.21)	−0.376	0.707
Three-dimensional power Doppler blood flow parameters				
VI	3.39 (2.94, 3.80)	2.43 (1.49, 3.70)	−4.740	<0.001
FI	24.63 ± 3.99	22.19 ± 4.92	3.482	0.001
VFI	1.16 (0.89, 1.62)	0.67 (0.22, 1.05)	−5.984	<0.001
Endometrial VI_{mv}	11.15 (9.95, 12.5)	8.50 (7.20, 9.65)	−8.451	<0.001
Uterine artery				
Left S/D	5.24 (4.35, 6.73)	6.22 (5.08, 8.5)	−3.659	<0.001
Right S/D	5.46 (4.19, 6.97)	6.39 (5.22, 8.04)	−3.382	0.001
Mean S/D	5.20 (4.60, 6.62)	6.42 (5.27, 8.20)	−4.215	<0.001
Left PI	2.14 ± 0.51	2.38 ± 0.62	2.939	0.004
Right PI	2.05 ± 0.49	2.41 ± 0.61	4.180	<0.001
Mean PI	2.09 ± 0.42	2.40 ± 0.55	4.314	<0.001
Left RI	0.81 (0.77, 0.85)	0.84 (0.80, 0.88)	−3.740	<0.001
Right RI	0.82 (0.76, 0.85)	0.84 (0.81, 0.88)	−3.968	<0.001
Mean RI	0.80 (0.77, 0.85)	0.85 (0.81, 0.89)	−4.261	<0.001

BMI, body mass index; VI, vascularization index; FI, flow index; VFI, vascularization flow index; VI_{mv}, velocity index of microvascular; S/D, systolic/diastolic rate; PI, pulsatility index; RI, resistance index.

Table 2: Correlations between VI, VFI, FI, and VI_{MV}

Indicators	VI_{MV}	
	r	p-Value
VI	0.694	<0.001
FI	0.339	<0.001
VFI	0.531	<0.001

VI, vascularization index; FI, flow index; VFI, vascularization flow index; VImv, velocity index of microvascular.

Table 4: Correlation between VI, VFI, FI, VI_{MV}, mean uterine artery values, and endometrial thickness.

Indicators	Endometrial thickness	
	r	p-Value
VI	0.341	<0.001
FI	0.158	0.024
VFI	0.218	0.002
VI_{mv}	0.710	<0.001

VI, vascularization index; FI, flow index; VFI, vascularization flow index; VI_{mv}, velocity Index of microvascular.

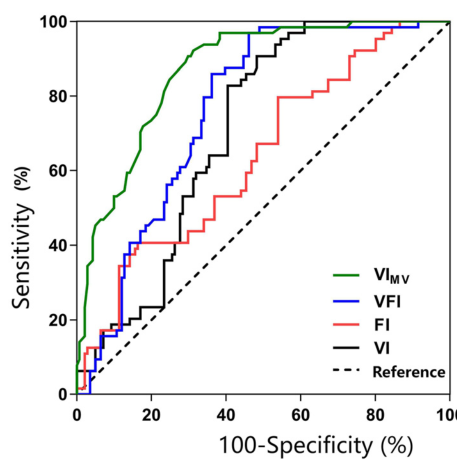

Figure 3: ROC curves of various parameters in predicting pregnancy outcomes.

96.9 % and a specificity of 53.9 %. VI_{MV} had a threshold of 9.150, with a sensitivity of 92.2 % and a specificity of 68.8 % (Table 5).

Discussion

An optimal uterine cavity environment is crucial for successful embryo implantation, with endometrial receptivity serving as a key indicator of the health of this environment. During the early stages of implantation, angiogenesis-related factors in the endometrium become highly active,

Table 3: Correlations between VI, VFI, FI, VI_{MV}, and mean uterine artery values.

Indicators	Mean S/D		Mean PI		Mean RI	
	r	p-Value	r	p-Value	r	p-Value
VI	−0.112	0.110	−0.087	0.215	−0.082	0.240
FI	−0.103	0.143	−0.082	0.243	−0.120	0.087
VFI	−0.189	0.007	−0.162	0.020	−0.194	0.005
VI_{mv}	−0.176	0.011	−0.149	0.033	−0.142	0.043

VI, vascularization index; FI, flow index; VFI, vascularization flow index; VI_{mv}, velocity index of microvascular; S/D, systolic/diastolic rate; PI, pulsatility index; RI, resistance index.

Table 5: ROC curve analysis of various indicators predicting pregnancy outcomes.

Parameters	VI	FI	VFI	VI_{mv}
AUC	0.707	0.637	0.761	0.869
95 % CI	0.638–0.776	0.557–0.717	0.696–0.826	0.820–0.917
p	<0.001	0.002	<0.001	<0.001
Threshold	2.509	21.579	0.728	9.150
Sensitivity	90.6	79.7	96.9	92.2
Specificity	51.8	46.1	53.9	68.8

AUC, area under the ROC, curve. 95 % CI, confidence interval; VI, vascularization index; FI, flow index; VFI, vascularization flow index; VI_{mv}, velocity index of microvascular.

and newly formed blood vessels exhibit dynamic changes, supplying abundant blood flow that creates favorable conditions and supports implantation and subsequent development of the embryo [6]. Therefore, the blood supply to the endometrium can reflect the perfusion status of the implantation site and is considered a simple, effective, and non-invasive method for assessing endometrial receptivity, as well as an effective indicator for predicting pregnancy outcomes [7].

The primary blood supply to the endometrium is derived from the spiral arteries of the endometrium, which directly reflect the blood perfusion status within the microenvironment of the embryo implantation area. However, some spiral arteries located beneath the endometrium are relatively small, and while they influence overall endometrial blood flow, their low flow velocity makes it difficult to image them with traditional two-dimensional Doppler ultrasound. As a result, this technique fails to capture the true blood perfusion status of the endometrium comprehensively [8]. Giuseppe Rizzo et al. [9] investigated the effects of maternal smoking on fetal development by utilizing three-dimensional power Doppler imaging to measure placental volume and vascularization indices. Concurrently, Yu Zhen et al. [10] applied microvascular flow imaging (MFI) technology to assess endometrial microcirculation in women with recurrent miscarriage, achieving notable diagnostic outcomes in this field. In this study, we used three-dimensional power Doppler ultrasound technology and microvascular imaging methods to effectively visualize the course and connections of microvessels while accurately quantifying and assessing blood flow in the endometrial region. This method offers greater objectivity and accuracy compared to previous assessment approaches such as endometrial blood flow grading [11, 12].

In this study, we compared three-dimensional power Doppler and micro-blood flow ultrasound parameters of endometrial blood supply between the pregnancy group and non-pregnancy group. The results showed that women in the pregnancy group had significantly higher values for VI, FI, VFI, and VI_{MV} compared to the non-pregnancy group. These findings are consistent with previous research [13, 14], indicating a strong association between the ultrasound characteristics of endometrial blood flow parameters and pregnancy outcomes. Adequate blood perfusion indicates a rich vascular network and enhanced endometrial receptivity, both of which are conducive to favorable pregnancy results.

Moreover, the significant positive correlations observed among VI, FI, VFI, and VI_{MV} suggest that these parameters have similar effectiveness in assessing endometrial blood supply. The ROC curve analysis revealed AUC values were 0.707, 0.637, 0.761, and 0.869 for VI, FI, VFI, and VI_{MV}, respectively, demonstrating. high sensitivity and specificity in predicting pregnancy outcomes. Among them, VI_{MV} emerged as a more powerful predictor of pregnancy success.

In addition, compared to three-dimensional power Doppler technology, MV-flow technology presents fewer operational challenges, making ultrasound examinations more convenient in clinical practice. This two-dimensional ultrasound modality allows for clearer and more intuitive visualization of endometrial microvascular flow, yielding more detailed images of vascular structures and enhancing sensitivity in detecting blood flow [15].

The spiral arteries of the endometrium are the final branches of the uterine artery, meaning that the blood flow characteristics of the uterine artery may indirectly affect the blood supply to these spiral arteries. In this study, further analysis of uterine artery blood flow in groups with different pregnancy outcomes revealed that the S/D, RI, and PI values of the bilateral uterine arteries were significantly lower among women in the pregnancy group than those in the non-pregnancy group. This suggests that decreased resistance in uterine artery blood flow may lead to increased blood perfusion in the endometrial spiral arteries, potentially facilitating a more favorable environment for the smooth progression of pregnancy.

However, some studies have indicated that the relationship between uterine artery blood flow and pregnancy outcomes is strongly predictive of endometrial receptivity to embryos [16]. To address this, in this study, we performed a correlation analysis between subendometrial blood flow parameters – VI, VFI, FI, and VI_{MV} – and uterine artery characteristics. Significant negative correlations were observed between subendometrial blood flow VFI, VI_{MV}, and uterine artery mean S/D, mean PI, and mean RI ($r<0$, $p<0.05$), aligning with the preliminary findings from this project [17]. These results suggest that lower-resistance uterine arteries can deliver richer blood flow to the endometrium, creating a more conducive microenvironment for embryo implantation.

Endometrial thickness and total endometrial volume are often used as ultrasound parameters to evaluate endometrial receptivity. However, there are differing opinions on their effectiveness in predicting pregnancy outcomes. It has been highlighted in some studies that changes in normal endometrial thickness may affect embryo implantation and development [18]. Conversely, some scholars posit that endometrial thickness alone cannot fully reflect the actual endometrial volume [19], and that its changes do not seem to significantly impact pregnancy success rates [20]. Although endometrial volume – which correlates with endometrial thickness and may offer a more comprehensive measure – has been suggested as a more objective indicator, its role in predicting endometrial receptivity still requires further investigation [17, 21].

To minimize the potential confounding influence of these factors on study outcomes, we selected and ensured that there were no significant differences in endometrial thickness and volume between the pregnancy and non-pregnancy groups, focusing instead on the comparative analysis of endometrial blood supply. Additional analyses revealed significant positive correlations between endometrial VI, VFI, FI, as well as VI_{MV}, and endometrial thickness ($r>0$, $p<0.05$). This finding further confirms that endometrial growth is reliant on nutritional support from adequate endometrial blood supply, which in turn promotes endometrial growth and enhances conditions for embryo implantation, ultimately resulting in higher implantation and pregnancy rates.

Conclusions

In conclusion, we found that the use of three-dimensional power Doppler and microvascular flow technologies to obtain multiple endometrial blood flow parameters played a crucial role in evaluating endometrial blood supply in women. In particular, the endometrial VI_{MV}, derived from MV-Flow technology, provides a quantitative assessment of microvascular perfusion status per unit area of the endometrium in women in the pre-pregnancy phase, serving as a new effective ultrasound marker for evaluating endometrial receptivity. Moving forward, we will continue our investigations with larger samples and integrate these findings with fundamental biological research to help identify optimal ultrasound parameters for assessing endometrial receptivity.

Research ethics: This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Wenzhou Peoples' Hospital (KY-202410-005q).

Informed consent: Consent for publication was obtained from every individual whose data are included in this manuscript.

Author contributions: Conceptualization: Cai-Cha Yu, Yin-Qqing Huang. Data curation: Yan Jiao,Cai-Cha Yu, Jia-Jia Wang, Xiao-Si Zhao. Data analysis: Cai-Cha Yu, Yan Jiao. Statistical analysis: Cai-Cha Yu, Yin-Qing Huang, Jia-Jia Wang, Xiao-Si Zhao. Funding acquisition: Cai-Cha Yu. Roles/ Writing – original draft: Cai-Cha Yu. Writing – review & editing: Cai-Cha Yu, Yin-Qing Huang. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Conflict of interest: The authors state no conflict of interest.

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Data availability: The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author (Yin-Qing Huang) on reasonable request.

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