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Original Article

Decreased endometrial vascularity and receptivity in unexplained recurrent miscarriage patients during midluteal and early pregnancy phases

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ABSTRACT

Objective: To evaluate the predictive value of three-dimensional (3D)-power Doppler sonography on recurrent miscarriage.
Materials and methods: The study patients were divided into a recurrent miscarriage group (30 cases) and a normal pregnancy group (21 cases). Measurement of endometrial thickness was performed using two-dimensional transvaginal ultrasound in the midluteal phase. The endometrial volume, vascularization index (VI), flow index (FI), and vascularization-flow index (VFI) in midluteal and placenta volume, as well as the VI, FI, and VFI of early pregnancy were measured using Virtual Organ Computer-aided Analysis of 3D-power Doppler ultrasound.
Results: Endometrial thickness, endometrial volume, endometrial vascular data, VI, FI, and VFI of the midluteal phase were lower in the recurrent miscarriage group compared with the normal pregnancy group ($p < 0.05$). Placental volume, VI, and VFI during early pregnancy were lower in the miscarriage group compared with the normal pregnancy group ($p < 0.05$). There was no significant change in FI between the recurrent miscarriage and control groups during early pregnancy ($p > 0.05$). The predictive accuracy of endometrial thickness, endometrial volume, VI, FI, and VFI in the midluteal phase, and placenta volume, VI, FI, and VFI in early pregnancy as measured by the receiver operating characteristic curve to predict miscarriage before 12 gestational weeks in participants was 0.681, 0.876, 0.770, 0.720, 0.879, 0.771, 0.907, 0.592, respectively.
Conclusion: The 3D-power Doppler ultrasound is a more comprehensive and sensitive method for evaluating endometrial receptivity. Endometrial volume, VI, FI, and VFI in the midluteal phase, as well as VI in early pregnancy, can be considered as predictive factors for recurrent miscarriage.

Introduction

Recurrent miscarriage (RM) affects approximately 3% of women of reproductive age [1]. Genetic, endocrine, infectious, and anatomical factors, as well as autoantibodies, abnormal prothrombotic state, and other aspects are involved in the etiology [2–5]. Endometrial receptivity can also be explained as an endometrial state allowing embryo adhesion, invasion, and implantation [6]. Endometrial receptivity correlates with endometrial angiogenesis and the degree of vascularization [7]. Endometrial angiogenesis and vascularization form the basis for the formation of the decidual capillary network in the placenta and the foundation of embryonic growth and development. Endometrial and subendometrial hypoxia caused by inadequate blood flow induces low receptivity, which decreases embryo implantation and increases spontaneous abortion [8].

In recent years with the development of ultrasonic technology, an effective nontraumatic detection technique has been provided

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for evaluating endometrial receptivity. Several ultrasonic evaluation parameters for endometrial receptivity have been put forward, including endometrial thickness, endometrium types, endometrial volume, and endometrial and subendometrial blood flow. Three-dimensional (3D) ultrasonography using power Doppler angiography (3D-CPA) accurately measures endometrial volume and vascular parameters, including: (1) vascularization index (VI), which represents the number of blood vessels in the measured region; (2) blood flow index (FI), which represents the blood flow strength sensed over 3D-scanning time in the measured region; and (3) vascularization flow index (VFI), which represents the sum of blood flow and vascularization in the measured region. These three parameters reflect endometrial and placental blood supply sensitivity. Hafner et al [9] found that placental clearance flow could be detected through vaginal 3D-CPA at 5-weeks gestation, and that the VI and VFI positively correlated with crown-rump length and embryonic bud size, which provided the basis for the study of early pregnancy placental blood flow parameters using ultrasonic monitoring.

Most research focuses on the measurement of midluteal phase endometrial volume and vascular parameters to predict assisted reproductive success rate. Few investigations have explored predicting the risk of recurrent miscarriage using 3D ultrasound and no study has used early pregnancy placental volume and vascular parameters to evaluate the risk of recurrent miscarriage. The present study investigates endometrial volume and vascular parameters in the midluteal phase of recurrent miscarriage patients and follows patients until pregnancy, measuring first-trimester placental volume and blood flow parameters and comparing these data to normal fertile women in order to clarify the predictive value of 3D-power Doppler ultrasound for recurrent miscarriage.

Materials and methods

Patients

The experiment was conducted in the prenatal diagnosis center of The First Affiliated Hospital of GuangXi Medical University and GuangXi Women and Children Hospital (GuangXi, China). Signed informed consent was obtained from all participants and the study was approved by the Research Ethics Board of the First Affiliated Hospital of GuangXi Medical University.

From January 2013 to January 2014, 122 women were enrolled in the study and 51 women remained until study completion. The experimental design was a case control study. The women were divided into two groups: the recurrent miscarriage (study) group and the normal pregnancy (control) group. Inclusion criteria for the study group were: (1) the pregnancy was ending before 12 gestational weeks; (2) patients had experienced two or more spontaneous abortions; (3) parents both had no chromosomal abnormalities; (4) patients had no abnormality of the uterus; (5) patients had no history of toxic material exposure; and (6) patients had a healthy medical history outside of miscarriage. Inclusion criteria for the control group were: (1) the pregnancy lasted until 12 gestational weeks; (2) patients had no history of spontaneous abortion; (3) patients had no abnormality of the uterus; (4) patients had no history of toxic material exposure; and (5) patients had a healthy medical history. Case baseline assessment included age.

Ultrasound data acquisition

Using a Voluson E8 Ultrasound (GE Medical Systems Kretztechnik GmbH, Zipf, Austria), a ~5–9-MHz transvaginal volumetric probe, and Virtual Organ Computer-aided Analysis (VOCAL) software (GE Medical Systems Kretztechnik GmbH), all patients received a transvaginal ultrasound scan performed by a single investigator unaware of participant status.

The entire endometrial section was displayed in uterine longitudinal sections using two-dimensional (2D) Doppler ultrasonography. Taking the maximum distance of the interface between the diameter of anterior and posterior uterine myometrium, then perpendicular across the midline of the endometrium as endometrial thickness, three continuous measurements were taken and averaged.

Using the optional multiplatform model and a volume angle of 120°, the volume of the sampling frame size was adjusted to ensure that the endometrium was completely oriented within the sampling frame. Volume scanning was then started and three planar volumetric measurements obtained and stored for analysis. The best reference plane was selected to display the endometrial cavity from the uterus fundus to the cervical. The VOCAL software application was used to manually sketch and set the volume of data extraction for every aspect angle within 30°, then trace the outline of the endometrium in each section. The system automatically calculated and displayed the volumetric results after completion of outlining, then applied 3D histogram-analysis software to calculate the energy in order to obtain endometrial volume flow parameters, VI, FI, and VFI.

Intrauterine early pregnancy can be diagnosed using B-scan ultrasonography, showing a “double ring sign” gestational sac or yolk sac. At the beginning of 6–8 weeks in early pregnancy, the placenta can be identified using B-scan ultrasonography. Placenta essence can be explored using a stronger uniform light spot than echogenicity of the surrounding muscle tissue, which is mostly “crescent-shaped.” All patients received an early pregnancy ultrasound examination using the methods described above and the placental volume, endometrial volume flow parameters VI, FI, and VFI were measured.

Recurrent miscarriage patients who met the inclusion criteria were recruited. Their ovulation was monitored using transvaginal ultrasound for the first 10 days of menstruation until ovulation was confirmed. At 5–7 days after ovulation, midluteal-phase (embryo in the womb, endometrial implantation window) endometrial thickness was measured using 2D Doppler ultrasonography, and 3D-power Doppler ultrasound was used to measure the endometrial volume, VI, FI, and VFI. At 14–16 days after ovulation (pregnant 4 weeks), patients who confirmed pregnancy with serum β-Human chorionic gonadotropin (β-HCG) $\geq$ 100 mIU/mL and progesterone $\geq$ 20 ng/mL had their cycles continuously monitored before measuring placental volume and VI, FI, and VFI at 6–8 weeks of pregnancy. These patients were included in the study group if spontaneous abortion occurred before 12 weeks of pregnancy.

The women who met the inclusion criteria for the control group underwent the same monitoring protocol as the study group. These women were followed up to 12 weeks and then included in the control group if spontaneous abortion had not occurred before 12 gestational weeks.

Statistical analysis

Using SPSS version 18.0 software (SPSS, Inc., Armonk, NY, USA) for statistical analysis, measurement data were expressed as mean ± standard deviation. The independent samples *t*-test was applied to compare data. A p value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were applied to compare the predicted performance of different indicators.

Results

Characteristics of the participants with RM and normal control are presented in Table 1. There were no differences in age, measurement time of midluteal phase, or ultrasound measurement for early pregnancy ($p > 0.05$).

Assessment of the endometrial thickness, volume, and uterine blood flow parameter index of luteal phase in participants are presented in Table 2. The measurement of endometrial thickness and volume, VI, FI, and VFI are shown in Figures 1A and 1B. Endometrial thickness in RM was thinner than that observed in the normal control group ($p < 0.05$). There were significantly lower endometrial volume, VI, FI, and VFI measurements in the RM group relative to the control group ($p < 0.05$).

Assessment of the placental volume and the uterine blood flow parameter index in early pregnancy is presented in Table 3. The measurement of early pregnancy placental volume, VI, FI, and VFI are shown in Figures 1C and 1D. There were significantly lower placental volume, VI, FI, and VFI measurements in the RM group relative to the control group ($p < 0.05$). There were no significant differences in FI between RM and control groups ($p > 0.05$).

Predictive accuracy of the different parameter indices are presented in Figures 2 and 3. The predictive accuracies of endometrial thickness, endometrial volume, VI, FI, and VFI in the midluteal phase as measured by the ROC curve to predict miscarriage before the 12-week gestational period were 0.681, 0.876, 0.770, 0.720, and 0.879, respectively. The predictive accuracies of the placenta volume, VI, FI, and VFI in early pregnancy as measured by the ROC curve to predict miscarriage before the 12-week gestational period were 0.771, 0.907, 0.592, and 0.771, respectively.

Discussion

Quality of gametes, embryos, and good endometrial receptivity are the bases of pregnancy that can be continued in reproductive age. Endometrial receptivity has been widely recognized through extensive research in recent years. Pinopode [10], leukemia inhibitory factor [10,11], integrin $\alpha\gamma\beta3$ [12], and vascular endothelial growth factor [12] were used to evaluate endometrial receptivity because these parameters were moderate expressions favoring pregnancy. However, to obtain the above data, endometrial biopsies within the embryo implantation window are required, which is a traumatic detection method with a time lag. Therefore, given the importance of noninvasive, real-time assessment, 3D-power Doppler ultrasound assessment of endometrial receptivity was used for the progressive development of this case.

Endometrial thickness and morphology were the main indicators for assessment of endometrial receptivity using 2D ultrasound assessment in the early stage. Maintaining adequate endometrial thickness is a prerequisite for embryo implantation. Endometrial thickness of 8–12 mm is considered optimal and an excessively thin or thick endometrium is not conducive to pregnancy [13]. Some researchers have suggested suspending or stopping the implementation of assisted reproductive technology for patients with endometrium <7 mm thick [14]. According to the endometrial- and substrate-relative echo state [15], endometrial morphology is divided into A-type (typical three-line), B-type (three-wire intrinsic weak), or C-type (homogeneous echogenic type). A-type appears within the follicular phase and ovulation, B-type occurs in the transition period between ovulation and the luteal phase, and C-type is often seen in the luteal phase. Pregnancy rates are generally higher when the endometrial ultrasound morphology is A- or B-type compared with C-type in the ovulation phase [16]. However, some believe that there is no relationship between pregnancy, miscarriage rate, and endometrial morphology [17]. In our study, the average midluteal phase endometrium thickness was 0.73 ± 0.1 cm in the recurrent miscarriage group, which was less than the normal control group (0.83 ± 0.03 cm). However, the value under the ROC curve area was 0.681, indicating that the accuracy of the predicted value for early spontaneous abortion was low.

Using 3D-power Doppler ultrasound measurements for endometrial volume and vascularization flow parameters VI, FI, and VFI in midluteal phase to predict miscarriage, Zohav et al [18] applied 3D ultrasound to 60 patients after the 15th to 17th day of transplant. They found that the miscarriage rate was higher when endometrial volume was <2 mL compared with those >2 mL. Kovachev et al [19] also found similar results. Both results proved that endometrial volume is a meaningful predictor of miscarriage. Schild et al [7] considered that the minimum endometrial volume and endometrial thickness for pregnancy were 6.9 mm and 1.59 mL, respectively. Miscarriage rates increase in those patients with endometrial volume <1.59 mL. Ng et al [20] showed that the indices associated with endometrium and subendometrium vascularization were lower in the miscarriage group compared with the normal pregnancy group. This research investigated the endometrial volume and endometrial vascular parameters in the midluteal phase of patients in a recurrent miscarriage or normal pregnancy group. The results between groups were similar to previous studies mentioned above. Compared with the control, the miscarriage group displayed lower endometrial volume, VI, FI, and VFI, suggesting that endometrial receptivity is lower in patients experiencing recurrent miscarriages. The endometrial volume, VI, FI, and VFI areas under the curve were between 0.7 and 0.9, which demonstrates that these parameter indices have better predictive value than endometrium thickness.

Chorionic villosum invades to the basal decidua and forms the original placenta in the 6th week of pregnancy. Ultrasound has typically shown placental imaging, indicating a diffuse punctuate echogenic area at 7–10 weeks [21]. Morel et al [22] confirmed that there was high correlation between placental blood flow *in vivo* and perfusion parameters. Using 3D-CPA, Merce et al [23] monitored placental blood flow parameters in 46 cases of spontaneous abortion and 100 normal controls in early pregnancy, which confirmed that VI, FI, and VFI could be detected at 6 weeks and that these indices were lower in 50% of women experiencing miscarriage. This research also found that miscarriage would occur soon after abnormal increases in uteroplacental circulation resistance index, VI, FI, and VFI. In this study and compared with midluteal phase data, the placenta vascularization parameter, VI, and VFI increased significantly during early pregnancy. Placenta volume, VI, and VFI were lower in the recurrent miscarriage group compared with the normal control. Regarding FI, there was no significant difference between the two groups. Using these indices to predict ongoing pregnancy lasting until 12 gestational weeks, the placental volume, VI, FI, and VFI areas under the curve were 0.771, 0.907, 0.592, and 0.771, respectively, indicating that VI can be used as a predictive factor for recurrent miscarriage.

Although the data suggest some interesting results, disadvantages in the research were unavoidable. The fluctuations of

Table 1
Comparison of baseline characteristics between the RM and control groups.

Groups	Cases (n)	Age (y)	Days after ovulation (d)	Measurement time of pregnancy (wk)
RM	30	30.96 ± 13.20	6.24 ± 0.47	7.12 ± 0.13
Control	21	30.18 ± 7.26	6.76 ± 0.56	7.17 ± 0.24
p		1.608	1.126	0.978

RM = recurrent miscarriage.

Table 2
Comparison of endometrial thickness, volume, and uterine blood-flow parameter indices in the luteal phase between the RM and control groups.

Groups	Cases (n)	Midluteal phase				
		Endometrial thickness (cm)	Endometrial volume (mL)	VI	FI	VFI
RM	30	0.73 ± 0.12	2.22 ± 1.53	1.75 ± 0.82	27.04 ± 11.97	0.60 ± 0.39
Control	21	0.83 ± 0.03	8.08 ± 6.77	2.56 ± 0.63	31.34 ± 17.84	2.17 ± 1.89
p		0.006	0.001	0.001	0.003	0.001

FI = flow index; RM = recurrent miscarriage; VI = vascularization index; VFI = vascularization-flow index.

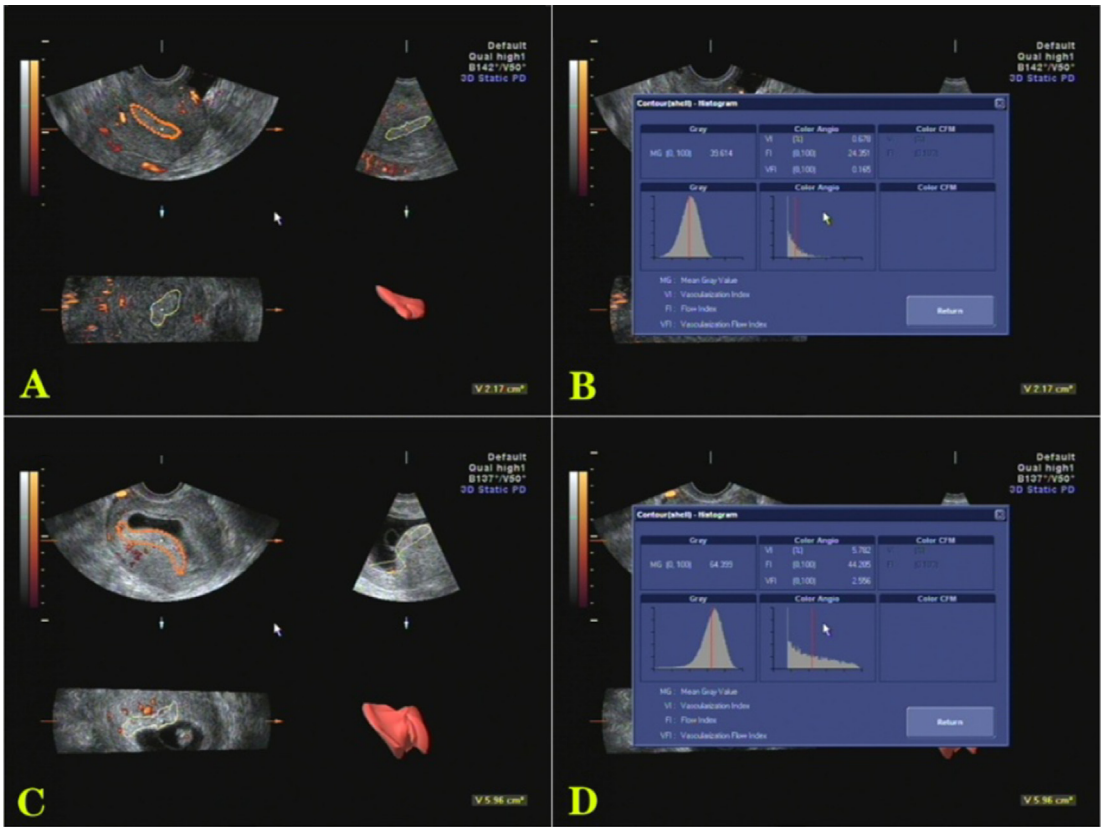

Figure 1. Parameter measurements using three-dimensional ultrasonograpy. Endometrial thickness, volume (A), and uterine blood flow parameter inde (B) of luteal phase. The placental volume (C) and the uterine blood flow parameter index (D) in early pregnancy.

Table 3
Comparison of placenta volume and uterine blood-flow parameter indices in early pregnancy between the RM and control groups.

Groups	Cases (n)	Early pregnancy			
		Placenta volume (mL)	VI	FI	VFI
RM	30	3.12 ± 2.25	6.18 ± 4.31	31.73 ± 20.75	2.72 ± 1.49
Control	21	4.42 ± 1.78	12.74 ± 7.40	35.49 ± 22.49	3.72 ± 1.16
p		0.001	0.001	0.058	0.003

FI = flow index; RM = recurrent miscarriage; VI = vascularization index; VFI = vascularization-flow index.

some data were large. This may have been caused by the small number of cases in this study or the difficulty in exposing the endometrium sagittal section using uterine-position distortion. In this study, we only focused on blood-vascular parameter measurements, which may have simplified the data. Results would be more comprehensive when combined with embryo length measurements.

In conclusion, there is lower endometrial receptivity in unexplained recurrent miscarriage patients. Measurement of

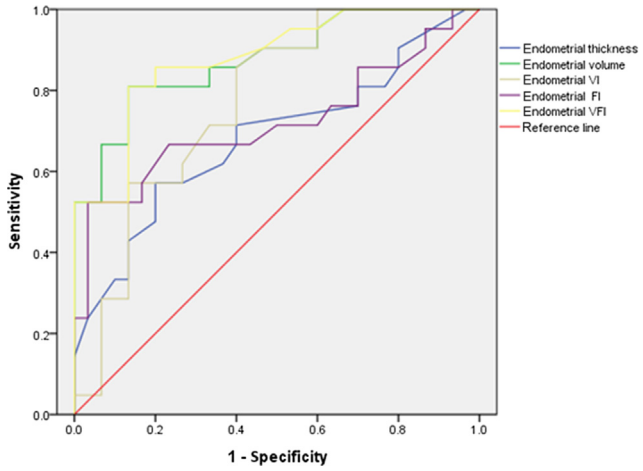

Figure 2. ROC curve of endometrial thickness, endometrial volume, VI, FI, and VFI in the midluteal phase. FI = flow index; ROC = receiver operating characteristic; VFI = vascularization-flow index; VI = vascularization index.

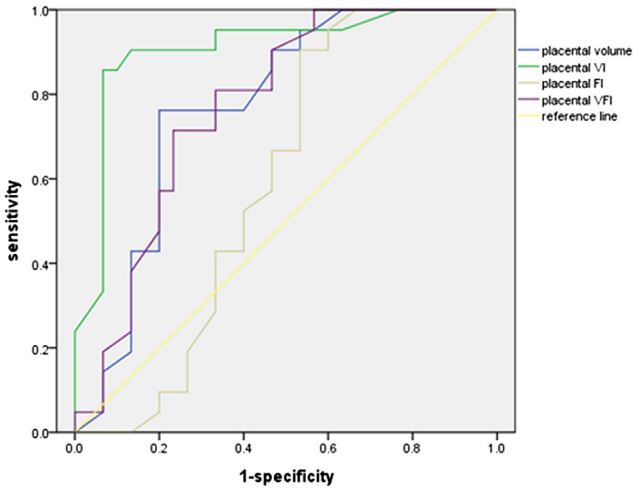

Figure 3. ROC curve of placenta volume in early pregnancy, VI, FI, and VFI in early pregnancy. FI = flow index; ROC = receiver operating characteristic; VFI = vascularization-flow index; VI = vascularization index.

endometrial volume, VI, FI, and VFI in the midluteal phase and placental volume, VI, and VFI in early pregnancy can be considered economical and noninvasive screening methods for prediction of spontaneous abortion.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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