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Predictive value of endometrial receptivity evaluated by three-dimensional ultrasound in ectopic pregnancy after *in vitro* fertilization-embryo transfer

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Abstract. – OBJECTIVE: The aim of this study was to use three-dimensional (3D) ultrasound to detect ultrasound parameters related to the uterine artery and endometrium, evaluate endometrial receptivity, and investigate the predictive value of each parameter for ectopic pregnancy (EP) after *in vitro* fertilization-embryo transfer (IVF-ET).

PATIENTS AND METHODS: Fifty-seven cases of pregnancy following IVF-ET in our institution were collected and split into EP and intrauterine pregnancy (IP) groups based on the location of pregnancy, with 27 cases in EP and 30 cases in IP. Endometrial thickness, type, volume, endometrial blood flow parameters, and uterine artery blood flow parameters were all measured one day before transplantation in both groups, and the differences between the two groups were examined.

RESULTS: There were differences in endometrial blood flow typing between the two groups, with type III endometrium accounting for the highest proportion in both; the uterine spiral artery pulsatility index PI was significantly higher in the EP group than in the IP group; there were no statistical differences in uterine volume, uterine artery resistance index mRI, or uterine artery resistance index S/D between the two groups; there were no statistical differences in uterine volume, or uterine artery.

CONCLUSIONS: Intracavitary 3D ultrasound can assess endometrial tolerance and may predict pregnancy outcome after IVF-ET.

Key Words:
Three-dimensional ultrasound, Endometrial tolerance, Embryo transfer, Ectopic pregnancy.

Introduction

Pregnancy and infertility are common problems among couples of childbearing ages who increasingly need to use assisted reproductive technology (ART). Numerous factors may lead to infertility. Such as oligospermia, sperm abnormalities, cervical factors, cystic fibrosis and psychological factors[1,2]. *In vitro* fertilization-embryo transfer (IVF-ET) is one of the most effective ways to treat infertility.

Good endometrial blood supply is essential for embryo implantation[3], and the alteration of endometrial tolerance by supraphysiologic doses of estrogen IVF-ET can be assessed by intracavitary three-dimensional (3D) ultrasound[4]. There have been several reports[3,4] about ultrasound evaluation of endometrial receptivity to predict pregnancy rate, but few studies[5] on endometrial receptivity to predict EP (ectopic pregnancy) and IP (intrauterine pregnancy) have been discovered. In this paper, we aim to investigate the prediction of EP in early IVF-ET by monitoring uterine and endometrial ultrasound parameters with intracavitary 3D ultrasound.

Patients and Methods

Patients

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Approval No. 121-2022), and writ-

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ten informed consent was obtained from each participant. Twenty-seven cases with surgically confirmed EP and 30 cases with IP after IVF-ET at our fertility center from January 2019 to December 2019 were selected, all of which were assessed by ultrasound for endometrial tolerance on the day before transplantation. Inclusion criteria: patients were aged ≥18 years and had non-spontaneous pregnancies. Exclusion criteria: patients with connective tissue disease and elevated β-HCG not due to pregnancy.

Examination Methods

Using a GE Voluson E8 color diagnostic ultrasound machine (Kretztechnik, Zipf, Austria) with a RIC-9-D intracavitary probe at 10 MHz, intracavitary 3D ultrasound was performed in all patients by the same senior physician on the day before transplantation. Ultrasound parameters measured included: endometrial thickness, staging, volume, uterine spiral artery flow parameters (resistance index RI, pulsatility index PI, S/D), and uterine artery flow parameters (mRI)[6].

Patients' pregnancy was determined by rechecking serum β-HCG at day 14th after transplantation. Patients with β-HCG > 5.6 mIU/ml underwent intracavitary 3D ultrasonography at 5 weeks after transplantation, and those with gestational sac and yolk sac or germ bud visible in the uterine cavity were considered IP, including double gestational sac and single gestational sac; those with masses detected in the adnexal area on ultrasonography and confirmed by surgery were considered EP.

Statistical Analysis

SPSS 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and quantitative data were expressed as mean ± SD when they conformed to a normal distribution, and *t*-tests were taken for comparison of differences between two groups. Count data were expressed as number of cases and percentage, and χ^2 test was used for comparison between groups. Differences were considered statistically significant when $p<0.05$.

Results

General Information of Patients in EP and IP Groups

A total of 57 study subjects were included in this study, and the statistics of both groups were not statistically significant in terms of age, years of infertility, type of infertility, body mass index (BMI), anti-Mullerian factor (AMH), and number of embryos transferred (Table I).

General Data of Patients in the EP Group

All 27 patients were excluded from hydrocele, including 14 cases (51.9%) with bilateral tubal patency, 2 cases (7.4%) with bilateral post-tubectomy, 5 cases (18.5%) with patency contralateral to one tubal resection, 3 cases (11.1%) with bilateral proximal tubal obstruction, and 3 cases (11.1%) with patency contralateral to one tubal proximal obstruction. The 27 cases in the surgically confirmed EP group, including 1 horn pregnancy (3.7%), 1 ovarian pregnancy (3.7%), 1 horn pregnancy combined with contralateral tubal pregnancy (3.7%), 3 intrauterine combined with ectopic pregnancy (11.1%), and 21 tubal pregnancies (77.8%).

Comparison of Ultrasound Parameters Between EP and IP Groups

There was no difference in endometrial thickness in the EP group compared with the control group. No differences in endometrial typing were found between the two groups, with type C endo-

Table I. Comparison of general information between EP group and IP group.

Item	EP group (n = 27)	IP group (n = 30)	*p*
Age (years)	30.4 ± 4.1	31.5 ± 3.6	0.826
Years of infertility (years)	3.6 ± 0.5	3.4 ± 0.8	0.721
BMI (kg/m^2)	21.33 ± 2.46	22.12 ± 3.04	0.182
AMH	5.98 ± 3.22	6.02 ± 3.59	0.807
Secondary infertility (n, %)	13 (48.1)	19 (63.3)	0.924
Primary infertility (n, %)	14 (51.9)	11 (36.7)	
Number of embryos transferred	1.5	1.4	0.603

EP, Ectopic pregnancy. IP, Intrauterine pregnancy. BMI, Body mass index. AMH, Anti Mullerian hormone.

Table II. Comparison of ultrasound parameters between EP group and IP group.

Parameters	EP group (n = 27)	IP group (n = 30)	p
Inner membrane thickness	9.60 ± 2.14	10.57 ± 2.16	0.094
Endothelial typing			
Type A	2 (7.4%)	1 (3.3%)	0.702
Type B	2 (7.4%)	3 (10%)	
Type C	23 (85.2%)	26 (86.7)	
Endometrial blood flow typing			
Type I	7 (25.9%)	0 (0.00%)	0.035
Type II	3 (11.1%)	5 (16.7%)	
Type III	17 (63.0%)	25 (83.3%)	
Uterine spiral artery RI	0.471 ± 0.069	0.439 ± 0.059	0.082
Uterine spiral artery PI	0.726 ± 0.166	0.639 ± 0.122	0.034
Uterine spiral artery S/D	1.642 ± 0.237	1.573 ± 0.202	0.097
Uterine cavity volume	4.044 ± 1.403	4.718 ± 1.86	0.131
Uterine artery mRI	0.824 ± 0.060	0.817 ± 0.052	0.610
Uterine artery mPI	2.25 ± 0.488	2.145 ± 0.437	0.379
Uterine artery mS/D	12.959 ± 4.513	11.958 ± 3.05	0.327

EP, Ectopic pregnancy. IP, Intrauterine pregnancy.

metrium accounting for the greatest proportion of patients in both groups. Type III endometrium accounted for the highest proportion of endometrial blood flow typing in both groups. The uterine spiral artery pulsatility index PI was significantly higher in the EP group than in the normal intrauterine pregnancy group. There were no differences in uterine spiral artery resistance index RI and S/D between the two groups. Uterine volume, resistance index mRI, mPI, mS /D were not significantly changed in the EP group compared with the IP group, as shown in Table II.

Basic Diagnosis of EP by Intracavitary 3D Ultrasound

Among the 27 surgically confirmed EP cases, intracavitary 3D ultrasound diagnosed 21 tubal pregnancies, 1 angular pregnancy, 1 ovarian pregnancy, 1 angular pregnancy combined with contralateral tubal pregnancy, 2 intrauterine combined with ectopic combined pregnancies, and 1 intrauterine combined with ectopic combined pregnancy was missed, with a diagnostic compliance rate of 96.3% (Table III).

Ultrasonographic Manifestations of Different Types of EP

Intrauterine pregnancy presents with an echogenic sac in the uterine cavity with a yolk sac and/or germ within the sac and with (or without) primitive cardiac pulsations. A tubal pregnancy presents with a mass in the adnexal region with or without a gestational sac-like echogenicity, with a visible yolk sac and/or germ within the sac and with (or without) primitive cardiac vascular pulsations, and with colored blood flow signals in its periphery and interior on CDFI. A horn pregnancy presents with a gestational sac located in one of the uterine horns. Intrauterine combined with tubal complex pregnancy presents with ges-

Table III. Intracavitary 3D ultrasound diagnosis of EP.

Diagnosticmethods	Tubal pregnancy	Horn pregnancy	Ovarian pregnancy	Cornual pregnancy with contralateral tubal pregnancy	Intrauterine combined tubal pregnancy	Intrauterine combined angular pregnancy
Ultrasound diagnosis	21	1	1	1	1	1
Pathological diagnosis	21	1	1	1	2	1
Diagnostic compliance rate	96.3%					

EP, Ectopic pregnancy.

tational sac echogenicity in the uterine cavity and in the adnexal region, respectively. Intrauterine combined with horn pregnancy shows a gestational sac echo in the uterine cavity and in the horn of the uterus (Figure 1 A-D).

Discussion

EP is the implantation of a fertilized egg outside the uterine cavity, mostly in the fallopian tube, but also in the uterine horn, ovary, abdominal cavity, and cervix, with tubal jugular pregnancy being the most common[7,8]. With the development of assisted reproductive technologies, IVF-ET techniques are becoming more and more sophisticated. Techniques such as vitrification of oocytes, freezing of embryos, make IVF-ET easier and have no clear impact on the risk of neurodevelopmental disease onset and cognitive ability in newborns[9,10]. At the same time, vitrification of frozen oocytes has a similar clinical efficiency compared to fresh oocytes[11,12]. However, the IVF-ET technique has been associated with an increased incidence of ectopic pregnancies in conjunction with higher pregnancy rates, with ectopic pregnancies reported to occur after IVF-ET at approximately 2-11%, which is 2-4 times higher than in natural pregnancies[13,14]. Routine sperm examination is required prior to *in vitro* fertilization to avoid negative sperm defects leading to fertilisation failure[15]. The effect of supraphysiologic doses of estrogen on the endometrial microenvironment is now considered to be one of the high-risk factors for the development of ectopic pregnancy after IVF-ET[16]. Supraphysiologic doses of estrogen interfere with the mechanism of embryo implantation and implantation by altering endometrial morphology, thereby affecting endometrial tolerance and thus increasing the occurrence of EP[17,18]. Earlier diagnosis and treatment of ectopic pregnancy reduces the risk of miscarriage[19].

Ultrasound is currently one of the most important tools to assess endometrial tolerance. Intraluminal three-dimensional ultrasound using color doppler technique allows monitoring the vascular distribution and flow spectrum of uterine arteries and branches and is applied for hemodynamic and vascular compliance assessment of microvessels[20]. Spectral measurements of blood flow were performed to obtain flow resistance index (RI), pulsatility index (PI), and peak systolic flow velocity/diastolic flow velocity (S/D), all three parameters reflect local vascular resistance and perfusion, PI reflects the magnitude of

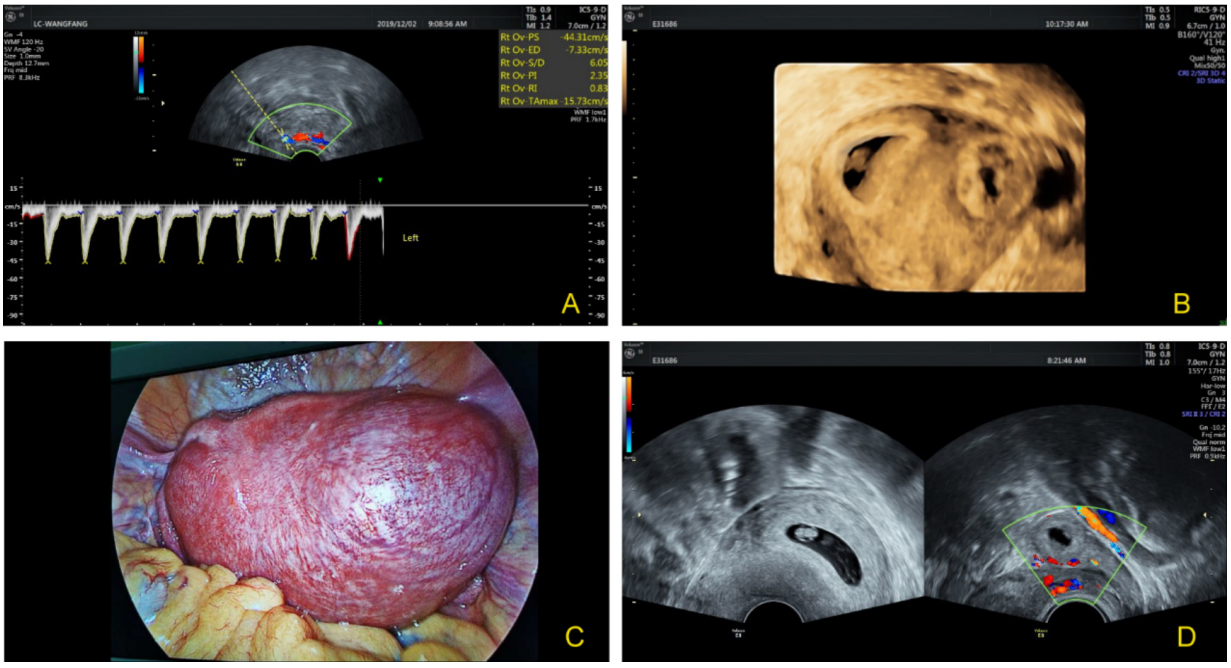

Figure 1. Ultrasonics examples. **A,** Uterine artery blood flow parameters. **B,** Three-dimensional ultrasound shows intrauterine combined with angular pregnancy. **C,** laparoscopic intrauterine combined with angular pregnancy. **D,** Ultrasound shows intrauterine combined tubal pregnancy.

flow resistance, and a high PI value indicates low mean flow velocity and end-diastolic flow velocity, i.e., high vascular resistance, while mean flow velocity also represents the blood flow waveform, therefore, PI better reflects the flow resistance and compliance of the vascular bed. This study showed that the PI of the spiral uterine artery in the normal pregnancy group was significantly lower than that in the ectopic pregnancy group, and the difference between the two groups was statistically significant, indicating that low resistance endometrial microflow is more favorable for embryo implantation and reduces the risk of EP. The distribution of endometrial spiral arteries was classified into 3 types using the Applebaum method[21]; type I was poor, suggesting sparse endometrial microvascularity and less perfusion; type III was better, with vessels entering the endometrium directly, providing abundant blood supply and nutrients for embryo implantation. The results of this study showed that the largest proportion of patients with type III blood flow typing was transplanted. Although we choose endometrial typing type III for embryo transfer endometrial preparation as much as possible, for patients with special conditions such as endometritis, pale endometrium, post-operative uterine adhesions, and repeated transfer failures, individualized treatment will be performed according to the patients' conditions for transplantation as appropriate, and retrospective Rombauts concluded that thin endometrium is an independent risk factor for the occurrence of EP by comparing endometrial thickness groups of 8,120 patients[22]. The mean endometrial thickness in this study was 9.60±2.14 mm in the EP group and 10.57±2.16 mm in the normal intrauterine pregnancy group, which is consistent with the findings regarding endometrial thickness <10 mm as an independent risk factor for EP after IVF-ET[23]. In patients with endometrial thickness below 10 mm on the day of embryo transfer, they need to be alerted to the occurrence of EP during the following ultrasound follow-up in early pregnancy.

EP occurring in the fallopian tube may present as a mixed or solid mass in the adnexal region with or without gestational sac echogenicity, with or without primitive fetal ventricular pulsation, and CDFI. blood flow signal is seen (or not seen) in the periphery and within it. Horn pregnancy is a site-specific ectopic pregnancy in which the embryo is implanted in the horn of the uterus at the junction of the uterus and the opening of the fallopian tube, which accounts for 2-3% of ectopic pregnancies and approximately 1/76,000 of all pregnancies[18]. According to the Chinese expert consensus on the management of horn pregnancy[24], horn pregnancy is classified as type I when the gestational sac is located in one horn of the uterus, mostly in the uterine cavity and surrounded by meconium, and a small portion is surrounded by the myometrium of the horn of the uterus and the thickness of the myometrium is >5 mm at the thinnest point. Type II when the gestational sac is located in one horn of the uterus, a small portion is located in the uterine cavity and surrounded by meconium, and a large portion is surrounded by the myometrium of the horn of the uterus and the thickness of the myometrium is >5 mm at the thinnest point. The unique sagittal imaging of intracavitary 3D ultrasound can show the relationship between the gestational sac and the uterine cavity and the tubal junction, which has unique advantages in identifying the horn of uterus pregnancy from the interstitial tubal area and in the staging of horn of uterus pregnancy, and is essential for the choice of treatment modality, preoperative assessment of the patient and postoperative fertility management[25-27]. In this study, one case of horn pregnancy and one case of horn pregnancy combined with contralateral tubal pregnancy, both type II horn pregnancy, were treated laparoscopically. HP is a pregnancy that occurs in two or more implantation sites simultaneously, and can occur in intrauterine combined with ectopic, bilateral tubal pregnancy and tubal combined with ovarian pregnancy, among which intrauterine combined with ectopic pregnancy is the most common[28]. HP is rare in natural pregnancy, and the incidence is about 1:10, HP is rare in natural pregnancies, with an incidence of about 1:10,000-1:50,000 in the pregnancy population[29], but when it occurs, it is extremely dangerous. In recent years, the incidence of HP has increased yearly to 2.1-9.4% as a result of ultra-ovulatory treatment protocols[30]. Despite the high rate of diagnostic compliance with intracavitary 3D ultrasound, the possibility of compound pregnancy in patients is ignored after the first examination confirms the intrauterine gestational sac or the first examination is shorter than after transplantation, as well as after the discovery of intrauterine gestational sac-like echogenicity, resulting in untimely detection and hemorrhage due to rupture of the patient's sac. In this study, four cases of compound pregnancy and one case of intrauterine combined tubal compound pregnancy were missed in a patient with only one embryo trans-

ferred and a gestational sac echo was found in the uterine cavity at the first ultrasound examination 5 weeks after transfer. We were overconfident that HP could not occur in patients with single embryo transfer and had insufficient knowledge about the occurrence of HP in IVF-ET patients and ignored the possibility of pregnancy at intercourse during the patient's ovulation.

Limitations

Some limitations still exist in our study. Psychological variables are important in infertility[31], yet we have no data concering psychological evaluation between two groups. The sample size of this study was relatively small and not suitable for multifactorial regression analysis. The next step should be to organize a multicenter, collect larger samples for retrospective analysis, and also design prospective studies to further validate the above results.

Conclusions

Endometrial blood flow typing and Uterine spiral artery PI data collected by intracavitary 3D ultrasound could be used to assess endometrial tolerance and predict the occurrence of ectopic pregnancy after IVF-ET.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

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Ethics Approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University [Approval No. 121(2022)]. All methods were performed in accordance with the relevant guidelines and regulations in practice.

Informed Consent

Written informed consent was obtained from each participant.

Availability of Data and Materials

The data that supports the findings of this study is available upon reasonable request.

Authors' Contribution

LP and YZ involved in conceiving the idea of the study, participated in its design, data analysis and interpretation, writing the manuscript and managing the overall progress of the study. WZ, CM, QK, YZ and ZP involved in conceiving the study, data analysis and in revising the manuscript. The final manuscript was read and approved by all the authors.

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