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 factors1,2. 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 implantation3, and the alteration of endometrial tolerance by supraphysiologic doses of estrogen IVF-ET can be assessed by intracavitary three-dimensional (3D) ultrasound4. There have been several reports3,4 about ultrasound evaluation of endometrial receptivity to predict pregnancy rate, but few studies5 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-
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).

Patients’ pregnancy was determined by re-checking 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 χ² 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>
<thead>
<tr>
<th>Item</th>
<th>EP group (n = 27)</th>
<th>IP group (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.4 ± 4.1</td>
<td>31.5 ± 3.6</td>
<td>0.826</td>
</tr>
<tr>
<td>Years of infertility (years)</td>
<td>3.6 ± 0.5</td>
<td>3.4 ± 0.8</td>
<td>0.721</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.33 ± 2.46</td>
<td>22.12 ± 3.04</td>
<td>0.182</td>
</tr>
<tr>
<td>AMH</td>
<td>5.98 ± 3.22</td>
<td>6.02 ± 3.59</td>
<td>0.807</td>
</tr>
<tr>
<td>Secondary infertility (n, %)</td>
<td>13 (48.1)</td>
<td>19 (63.3)</td>
<td>0.924</td>
</tr>
<tr>
<td>Primary infertility (n, %)</td>
<td>14 (51.9)</td>
<td>11 (36.7)</td>
<td>0.603</td>
</tr>
</tbody>
</table>

Predictive value of endometrial receptivity evaluated by three-dimensional ultrasound in EP after IVF-ET

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

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


Table III. Intracavitary 3D ultrasound diagnosis of EP.

<table>
<thead>
<tr>
<th>Diagnostic methods</th>
<th>Tubal pregnancy</th>
<th>Horn pregnancy</th>
<th>Ovarian pregnancy</th>
<th>Cornual pregnancy with contralateral tubal pregnancy</th>
<th>Intrauterine combined tubal pregnancy</th>
<th>Intrauterine combined angular pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound diagnosis</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pathological diagnosis</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diagnostic compliance rate</td>
<td>96.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EP, Ectopic pregnancy.
tional sac echogenicity in the uterine cavity and
in the adnexal region, respectively. Intrauterine
combined with horn pregnancy shows a gesta-
tional 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 fallo-
pian tube, but also in the uterine horn, ovary,
abdominal cavity, and cervix, with tubal jugular
pregnancy being the most common. With the
development of assisted reproductive technolo-
gies, IVF-ET techniques are becoming more and
more sophisticated. Techniques such as vitrifica-
tion 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. At the same time, vitrifi-
cation of frozen oocytes has a similar clinical ef-
ciciency compared to fresh oocytes. 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. Routine
sperm examination is required prior to in vitro
fertilization to avoid negative sperm defects lead-
ing to fertilisation failure. The effect of supra-
physiologic doses of estrogen on the endometrial
microenvironment is now considered to be one of
the high-risk factors for the development of ecto-
pic pregnancy after IVF-ET. Supraphysiologic
doses of estrogen interfere with the mechanism
of embryo implantation and implantation by al-
tering endometrial morphology, thereby affecting
endometrial tolerance and thus increasing the
occurrence of EP. Earlier diagnosis and treat-
ment of ectopic pregnancy reduces the risk of
miscarriage.

Ultrasound is currently one of the most im-
portant tools to assess endometrial tolerance.
Intraluminal three-dimensional ultrasound using
color doppler technique allows monitoring the
vascular distribution and flow spectrum of uter-
ine arteries and branches and is applied for hemo-
dynamic and vascular compliance assessment of
microvessels. Spectral measurements of blood
flow were performed to obtain flow resistance
index (RI), pulsatility index (PI), and peak systo-
lic flow velocity/diastolic flow velocity (S/D),
all three parameters reflect local vascular resis-
tance 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\(^2\); type I was poor, suggesting sparse endometrial microvasculature 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 patient's 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\(^2\). 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\(^2\). 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\(^9\). 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\(^25\). 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\(^29\). 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\(^6\). 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, yet we have no data concerning 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
This study was funded by Provincial Key Clinical Specialty (Ultrasound Medicine Department) of Jiangsu Province, China (2019001308060009).

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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