Ultrasound Assessment of Endometrial Receptivity in *in vitro* Fertilization Treatment

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**Abstract**

Ultrasonography of the endometrium is a noninvasive way to evaluate the chance of successful implantation during *in vitro* fertilization treatment. Ultrasound parameters of endometrial receptivity include endometrial thickness, endometrial pattern, endometrial volume, Doppler studies of uterine vessels and the endometrium. Endometrial thickness, pattern and volume are not predictive of pregnancy. A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation. Doppler study of uterine arteries does not reflect the actual blood flow to the endometrium. Endometrial and subendometrial vascularity can be more objectively measured with three-dimensional power Doppler ultrasound. However, the role of endometrial and subendometrial vascularity in predicting pregnancy of *in vitro* fertilization treatment remains controversial.

**Keywords:** Endometrium, endometrial vascularity, *in vitro* fertilization: pregnancy, three-dimensional ultrasound.

**INTRODUCTION**

*In vitro* fertilization-embryo transfer (IVF-ET) is an effective treatment for various causes of infertility. It involves multiple follicular development, oocyte retrieval and embryo transfer after fertilization. Despite improvement in ovarian stimulation regimens, culture media conditions and the technique of ET, there has not been a significant increase in the implantation rates of cleaving embryos, which have remained steady at 20 to 25% for a long time. Successful implantation is dependent on close interaction between the embryo and the endometrium. Receptivity of the endometrium can be evaluated by the histological dating of a timed endometrial biopsy, cytokine profiles in uterine flushings, the genomic study of a timed endometrial biopsy or ultrasound examination of the endometrium. Ultrasoundography of the endometrium is a noninvasive tool to examine the endometrium during the peri-implantation period. Ultrasound parameters of endometrial receptivity are endometrial thickness, endometrial pattern, endometrial volume and Doppler study of uterine arteries and the endometrium.

The endometrium in the follicular phase increases in thickness as a result of follicular growth and rising serum estradiol concentration. A good blood supply towards the endometrium is usually considered as an essential requirement for successful implantation. Endometrial tissue blood flow was measured in 75 infertile patients by the intrauterine laser Doppler technique between days 4 and 6 of the luteal phase of a natural cycle preceding IVF and was found to be superior to endometrial thickness, uterine Doppler flow indices and the histological dating of the endometrium in predicting endometrial receptivity. Endometrial vascularity can now be noninvasively measured by two-dimensional (2D) or three-dimensional (3D) ultrasound with color and power Doppler.

This review summarizes the role of endometrial thickness, endometrial pattern and endometrial volume, Doppler study of uterine vessels and endometrial vascularity in predicting pregnancy of IVF treatment.

**ENDOMETRIAL THICKNESS**

Endometrial thickness is the distance between the echogenic interfaces of the endometrium and the myometrium in the plane through the central longitudinal axis of the uterine body, usually at the level of the fundus (Fig. 1). It is an easily measurable ultrasound parameter with excellent intraobserver and interobserver reliability. It correlates significantly with serum estradiol concentration on the day of human chorionic gonadotrophin (hCG) administration but was not related to the age of the patients and the cause of infertility. The value of endometrial thickness in predicting pregnancy remains controversial. A positive correlation was found between endometrial thickness and the pregnancy
rate in earlier studies using clomiphene citrate for ovarian stimulation. However, a review of relevant studies involving 1,605 assisted reproduction treatment cycles with various stimulation regimens used found nearly identical range of endometrial thickness in pregnant and non-pregnant cycles. A prospective study involving more than 1,000 IVF cycles confirmed the pregnancy rate was not reduced in patients with a thin endometrium, although singleton pregnancies were more common than multiple pregnancies in those with thin endometria.

Gonen et al first observed a minimal endometrial thickness of 6 mm to achieve a pregnancy in donor insemination cycles without ovarian stimulation. Subsequently, various cut-off values between 6 to 10 mm have been proposed to discriminate between pregnant and non-pregnant cycles. Sundström and Remohi et al reported pregnancies in patients who had an endometrial thickness of 4 mm. The use of minimal endometrial thickness mainly lies in the high negative predictive value but the positive predictive value and specificity are low.

On the other hand, some consider that implantation and pregnancy rates may be adversely affected by a thick endometrium. Weissman et al found a significant lower implantation and pregnancy rate in patients when an endometrial thickness on the day of hCG administration was > 14 mm. Kupesic et al reported no pregnancies, if the endometrial thickness on the ET day was > 15 mm and Schild et al found no pregnancies if the thickness on the day of oocyte retrieval (OR) was > 16 mm. However, both Dickey et al and Dieterich et al demonstrated no adverse effects of a thickened endometrium on implantation and pregnancy rates as these rates were similar in patients with endometrial thickness of ≤ 14 mm and > 14 mm on the day of hCG administration.

ENDOMETRIAL PATTERN
The type of relative echogenicity of the endometrium and the adjacent myometrium is defined as endometrial pattern, which is usually evaluated on the day of hCG administration. Several classifications exist. The most simplified one is proposed by Sher et al, which consists of multilayered and nonmultilayered. A multilayered endometrium has a typical triple-line pattern and reflects receptive endometrium whereas a nonmultilayered pattern has homogenous hyperechogenic or isoechogenic endometrium compared with adjacent myometrium and was frequently associated with nonpregnant cycles.

Friedler et al concluded that the multilayered pattern had a negative predictive value of 85.7%, a positive predictive value of 33.1%, a sensitivity of 95% and a specificity of 13.7% for conception after reviewing 3258 natural, stimulated and hormonal replacement transfer cycles. Similar to the endometrial thickness, the positive predictive value and the specificity of endometrial pattern are quite low.

Patients with localized echogenic areas in the endometrium may have endometrial polyps and should be further investigated.

ENDOMETRIAL VOLUME
Endometrial volume cannot be obtained in 2D ultrasound but can now be reliably determined by the recent 3D ultrasound, which allows acquisition and storage of volume data of volume calculation of pelvic organs. Although, it has been shown that the endometrium must attain at least 2.0 to 2.5 ml to achieve a pregnancy, endometrial volume measured on the day of hCG administration and ET was comparable for pregnant and nonpregnant women.

However, Mercè et al showed that endometrial volume measured on the day of hCG administration was statistically significantly higher in the pregnant group. The area under receiver operating characteristic (ROC) curve was statistically significant for endometrial volume only when no grade 1 embryos or only one were transferred.

DOPPLER STUDY OF UTERINE VESSELS
Doppler study of uterine vessels reflecting downstream impedance to flow is assumed in many studies to reflect the blood flow towards the endometrium. It is usually expressed as the pulsatility index (PI) and the resistance index (RI) (Figs 2A and B). PI is calculated as the peak systolic velocity (PSV) minus end-diastolic velocity divided by the mean whereas RI is the ratio of PSV minus end-diastolic velocity divided by PSV.
ET as low, medium and high in the ranges of 0 to 1.99, 2.00 to 2.99 and ≥ 3.00 respectively and reported a 35% implantation failure, when PI was > 3.0. Using a PI upper limit of 3.028 or 3.329 the uterine Doppler flow indices have a high negative predictive value and sensitivity (in the ranges of 88 to 100% and 96 to 100% respectively) and a relatively higher range of positive predictive value and specificity (44-56% and 13-35% respectively) when compared with endometrial thickness and pattern.9

Doppler study of uterine vessels may not reflect the actual blood flow to the endometrium because the major compartment of the uterus is the myometrium and there is collateral circulation between uterine and ovarian vessels. This is reflected in our study,31 which could not demonstrate any correlation between uterine blood flow assessed by 2D color Doppler and endometrial and subendometrial vascularity measured by 3D power Doppler in both stimulated and natural cycles. Endometrial and subendometrial 3D power Doppler flow indices were similar among patients with averaged uterine PI < 2.0, 2.0 to 2.99 and ≥ 3.0. Therefore, it is more logical to directly assess the endometrial vascularity.

**ENDOMETRIAL VASCULARITY MEASURED BY 2D DOPPLER ULTRASOUND**

Endometrial blood vessels come from the radial artery, which divides after passing through the myometrial-endometrial junction to form the basal arteries that supply the basal portion of the endometrium, and the spiral arteries that continue up towards the endometrium. Kupesic and Kurjak32 first reported endometrial vascularity determined by transvaginal color Doppler study during the peri-ovulatory period in patients undergoing donor insemination. However, the results were not correlated with the outcome of the treatment. Subsequently, endometrial and subendometrial vascularity measured by color (Table 1) and power

<table>
<thead>
<tr>
<th>Study</th>
<th>IVF cycles</th>
<th>USS parameters</th>
<th>USS day</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaidi et al33</td>
<td>96 cycles using a long protocol</td>
<td>Spiral PI and PSV</td>
<td>hCG</td>
<td>No difference in subendometrial PI and PSV between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td>Battaglia et al35</td>
<td>60 cycles</td>
<td>Presence of endometrial and subendometrial vascularity</td>
<td>OR</td>
<td>Absent subendometrial flow associated with no pregnancy</td>
</tr>
<tr>
<td>Battaglia et al35</td>
<td>60 cycles</td>
<td>Uterine and spiral PI</td>
<td>OR</td>
<td>Uterine and spiral PI lower in pregnant than nonpregnant cycles</td>
</tr>
<tr>
<td>Battaglia et al35</td>
<td>60 cycles</td>
<td>Presence of endometrial vascularity</td>
<td>OR</td>
<td>Absent subendometrial vascularity associated with no pregnancy</td>
</tr>
<tr>
<td>Battaglia et al35</td>
<td>60 cycles</td>
<td>Uterine and spiral PI</td>
<td>OR</td>
<td>Significantly lower implantation and pregnancy rates in patients without endometrial/subendometrial vascularity</td>
</tr>
<tr>
<td>Battaglia et al35</td>
<td>60 cycles</td>
<td>PI and RI</td>
<td>ET</td>
<td>Presence of subendometrial vascularity 5.9 times to become pregnant than those with absent vascularity</td>
</tr>
</tbody>
</table>

USS—Ultrasound; OR—oocyte retrieval; ET—embryo transfer, PI—pulsatility index, PSV—peak systolic velocity
Doppler (Table 2) were examined during IVF treatment. The 2D Doppler flow indices of spiral arteries are not predictive of pregnancy, although Battaglia et al. and Kupesic et al. found significantly lower spiral artery PI in pregnant cycles than nonpregnant cycles. Yang et al. used a computer software to measure the area and intensity of color signals present in the endometrium in a longitudinal axis, i.e. intraendometrial power Doppler area (EDPA). Significantly higher EDPA were found in pregnant cycles than nonpregnant cycles. Patients with EDPA < 5 mm² had significantly lower pregnancy rate (23.5% vs 47.5%; P = 0.021) and implantation rate (8.1% vs 20.2%; P = 0.003) than those with ≥ 5 mm². Contart et al. graded endometrial vascularity by the visualization of power Doppler in the quadrants in the fundal region of the transverse plane but could not demonstrate any predictive value of such grading system.

Absent endometrial and subendometrial vascularity on 2D Doppler ultrasound may be associated with no pregnancy or a much reduced pregnancy rate.

ENDOMETRIAL VASCULARITY MEASURED BY 3D DOPPLER ULTRASOUND

In combination with a 3D ultrasound, power Doppler provides a unique tool with which to examine the vascularity of the endometrial and subendometrial regions. The built-in VOCAL® (Virtual Organ Computer-aided Analysis) imaging program for the 3D power Doppler histogram can be used in the analysis to measure the endometrial volume and indices of blood flow within the endometrium (Figs 3A and B). Vascularization index (VI), which measures the ratio of the number of color voxels to the number of all the voxels, is thought to represent the presence of blood vessels (vascularity) in the endometrium, and this was expressed as

### Table 2: Endometrial vascularity measured by 2D power Doppler

<table>
<thead>
<tr>
<th>Study</th>
<th>IVF cycles</th>
<th>USS parameter</th>
<th>USS day</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al.¹⁶</td>
<td>95 cycles using long and short protocols endometrium ≥ 10 mm</td>
<td>Intraendometrial power Doppler area (EDPA) &lt; 5 mm²; &gt; = 5 mm²</td>
<td>OR</td>
<td>Higher EDPA in pregnant cycles Lower implantation and pregnancy rates when EDPA &lt; 5 mm²</td>
</tr>
<tr>
<td>Yuval et al.³⁴</td>
<td>156 cycles using a long protocol</td>
<td>PI and RI</td>
<td>OR and ET</td>
<td>No difference in any USS parameters between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td>Contart et al.²⁷</td>
<td>185 cycles using a long protocol</td>
<td>Fundal region along transverse plan; Grades I, II, III &amp; IV according to visualization of power Doppler in the quadrants</td>
<td>hCG</td>
<td>Implantation and pregnancy rates similar in all grades of endometrial vascularity</td>
</tr>
<tr>
<td>Schild et al.¹⁸</td>
<td>135 cycles using a long protocol; first cycle only</td>
<td>PI and PSV of vessels in endometrium and subendometrial area (&lt; 5 mm)</td>
<td>OR</td>
<td>No difference in spiral artery PI and PSV between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td>Maugey-Laulom et al.⁹</td>
<td>144 cycles using a long protocol</td>
<td>Presence of endometrial and subendometrial vascularity</td>
<td>ET</td>
<td>Absent endometrial and subendometrial vascularity associated with a lower pregnancy rate</td>
</tr>
</tbody>
</table>

USS—ultrasound; OR—oocyte retrieval; ET—embryo transfer; PI—pulsatility index; PSV—peak systolic velocity
a percentage (%) of the endometrial volume. Flow index (FI), the mean power Doppler signal intensity inside the endometrium, is thought to express the average intensity of flow. Vascularization flow index (VFI) is a combination of vascularity and flow intensity. The subendometrial region can be examined through the application of “shell-imaging” (Figs 4A and B). The intraobserver and interobserver reliability of endometrial and subendometrial blood flows by 3D Doppler is high with intra-class correlation > 0.9.41,42

There are several studies addressing the role of endometrial or subendometrial vascularity measured by 3D Doppler in IVF treatment (Table 3). The first study was reported by Schild et al.,43 who measured the subendometrial vascularity after pituitary down-regulation but prior to ovarian stimulation. Subendometrial 3D Doppler flow indices were significantly lower in pregnant cycles than non-pregnant ones. Logistic regression analysis found that the subendometrial FI was the strongest predictive factor for the pregnancy outcome among other 3D Doppler flow indices. The authors suggested that a lesser degree of intrauterine vascularization and perfusion at the beginning of ovarian stimulation indicated a more favorable endometrial milieu. Another possibility is that lower subendometrial 3D Doppler flow indices may indicate a better functional down-regulation of the endometrium following the use of GnRH agonist, which increases the
Table 3: Endometrial vascularity measured by 3D power Doppler ultrasound

<table>
<thead>
<tr>
<th>Study</th>
<th>IVF cycles</th>
<th>Inclusion/exclusion criteria</th>
<th>USS day</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schild et al</td>
<td>75 cycles</td>
<td>Inclusion criteria:</td>
<td>Before</td>
<td>Subendometrial VI, FI and VFI lower in pregnant than nonpregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>down-regulation confirmed</td>
<td>stimulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IET 2 days after OR</td>
<td>(endometrium &lt; 5 mm; no ovarian cyst of &gt; 2.5 cm; serum estradiol &lt; 60 pg/ml)</td>
<td></td>
<td>Subendometrial FI is the strongest predictive factor for IVF in logistic regression analysis</td>
</tr>
<tr>
<td>Kupesic et al</td>
<td>89 cycles</td>
<td>Inclusion criteria:</td>
<td>ET (hCG +7)</td>
<td>Higher subendometrial FI in pregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>down-regulation confirmed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blastocyst transfer 5 days after OR</td>
<td>serum FSH &lt; 10IU/L; no fibroid, ovarian cysts and ovarian endometriosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al</td>
<td>54 cycles</td>
<td>Inclusion criteria:</td>
<td>hCG</td>
<td>Higher subendometrial VFI in pregnant cycles</td>
</tr>
<tr>
<td></td>
<td>(details of ovarian stimulation and ET not given)</td>
<td>first cycle; age &lt; 38 year; normal uterine cavity; serum FSH &lt; 15 IU/L; ≥ 2 good quality embryos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorn et al</td>
<td>42 cycles</td>
<td>Exclusion criteria:</td>
<td>OR</td>
<td>No difference in subendometrial VI, FI and VFI between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>polycystic ovary syndrome; endometrium &lt; 6 mm; gynaecological surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Järvelä et al</td>
<td>35 cycles</td>
<td>Exclusion criteria:</td>
<td>After stimulation and OR</td>
<td>No difference in endometrial and subendometrial VI between pregnant and nonpregnant cycles on both days</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>uterine fibroids; endometriosis; single ovary; previous operation on uterus or salpingectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET 2 days after OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ng et al</td>
<td>451 cycles</td>
<td>Inclusion criteria:</td>
<td>OR</td>
<td>Lower endometrial VI and VFI in pregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>first cycle; normal uterine cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ET 2 days after OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ng et al</td>
<td>193 cycles</td>
<td>Inclusion criteria:</td>
<td>LH+1</td>
<td>No difference in endometrial and subendometrial 3D Doppler flow indices between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>first cycle; normal uterine cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercè et al</td>
<td>80 cycles</td>
<td>Inclusion criteria:</td>
<td>hCG</td>
<td>Higher endometrial VI, FI and VFI in pregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>first cycle; normal uterine cavity; serum FSH &lt; 10 IU/L; regular cycles; nonsmokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ng et al</td>
<td>293 cycles</td>
<td>Inclusion criteria:</td>
<td>OR and ET</td>
<td>No difference in endometrial and subendometrial 3D Doppler flow indices on the 2 days and changes in these indices between pregnant and nonpregnant cycles</td>
</tr>
<tr>
<td></td>
<td>using a long protocol</td>
<td>first cycle; normal uterine cavity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

USS—ultrasound, OR—oocyte retrieval; ET—embryo transfer; VI—vascularization index; FI—flow index; VFI—vascularization flow index
chances of successful implantation. Unfortunately, there are no further studies to substantiate findings of this interesting study.

Kupesic et al\textsuperscript{17} performed 3D ultrasound examination on the day of blastocyst transfer and found that subendometrial FI was significantly higher in pregnant cycles. Wu et al\textsuperscript{44} measured subendometrial vascularity on the day of hCG and demonstrated that subendometrial VFI was significantly higher in the pregnant group. Subendometrial VFI was superior to subendometrial VI, subendometrial FI and endometrial volume in predicting the successful outcome in the ROC curve analysis and the best predictive rate was achieved by a subendometrial VFI cutoff value of $> 0.24$.

On the day of OR, Dorn et al\textsuperscript{45} compared the subendometrial vascularity before and after an intravenous administration of Levovist, a contrast agent. All subendometrial 3D Doppler flow indices after the administration of Levovist were significantly higher than those without Levovist. However, all subendometrial 3D Doppler flow indices with and without the contrast agent were comparable between pregnant and nonpregnant cycles. Järvelä et al\textsuperscript{23} determined endometrial and subendometrial VI after gonadotrophin stimulation but before hCG administration and again the day of OR. No differences were found between pregnant and nonpregnant groups in endometrial thickness, volume, endometrial and subendometrial VI on either day examined.

Our study\textsuperscript{24} involved 451 transfer cycles and the 3D ultrasound examination was performed on the day of OR. Patients in the pregnant group had significantly lower uterine RI, endometrial VI and VFI than those in the nonpregnant group. Endometrial thickness, endometrial volume, endometrial pattern, uterine PI, endometrial FI and subendometrial VI, FI and VFI were similar between the nonpregnant and pregnant groups. The number of embryos replaced and endometrial VI were the only two predictive factors for pregnancy in a logistic multiple regression analysis. ROC curve analysis revealed that the area under the curve was around 0.5 for all ultrasound parameters for endometrial receptivity. In a subgroup analysis of patients with good prognosis defined as patients aged $\leq 35$ years with endometrial thickness $> 8$ mm, transfer of $\geq 2$ good quality embryos and the availability of frozen embryo(s), there were no significant differences between the nonpregnant and pregnant groups in all endometrial and subendometrial 3D Doppler flow indices.

Endometrial vascularity was negatively affected by serum estradiol concentration on the day of hCG.\textsuperscript{46} The age of women, their smoking habits, their types of infertility and parity, and causes of infertility had no effect on all endometrial and subendometrial 3D Doppler flow indices. We have also studied the role of the endometrial and subendometrial vascularity in a natural cycle by 3D Doppler ultrasound in the prediction of pregnancy during frozen-thawed transfer cycles.\textsuperscript{47} Again, endometrial thickness, endometrial volume, endometrial pattern, endometrial and subendometrial 3D Doppler flow indices were comparable between the nonpregnant and pregnant groups. In the follow-up study,\textsuperscript{48} endometrial and subendometrial vascularity was significantly higher in pregnant patients with livebirth following stimulated IVF and FET treatment.

More recently, Mercè et al\textsuperscript{26} found that endometrial 3D power Doppler flow indices were statistically significantly higher in the pregnant group. The area under ROC curve was statistically significant for endometrial VI, FI and VFI when no grade 1 embryos or only one were transferred but not when two or three grade 1 embryos were transferred.

**DIFFERENCES AMONG THE ABOVE STUDIES**

Kupesic et al\textsuperscript{17} and Wu et al\textsuperscript{44} found significantly higher subendometrial vascularity in pregnant cycles whereas Mercè et al\textsuperscript{26} found significantly higher endometrial vascularity in pregnant cycles. On the other hand, Dorn et al\textsuperscript{45} and Järvelä et al\textsuperscript{23} could not demonstrate any differences in endometrial and subendometrial 3D Doppler indices between pregnant and nonpregnant cycles. Our findings\textsuperscript{24} were even contradictory to that of others. We published the largest study while a much small number of subjects ranging from 35 to 89 were evaluated by others.\textsuperscript{17, 23, 26, 44-45} These studies were clearly different in patients’ characteristics, the day of ultrasound examination and the selection of the subendometrial region.

Kupesic et al\textsuperscript{17} recruited patients undergoing repeated IVF attempts following a long protocol of pituitary downregulation, who had serum basal FSH concentration $< 10$ IU/L, no uterine fibroids, ovarian cysts or ovarian endometriomas. One to two good quality blastocysts were replaced five days after OR. Wu et al\textsuperscript{44} examined patients in their first IVF cycle who were aged $< 38$ years with basal FSH concentration $< 15$ IU/L and had normal uterine cavity on scanning and $\geq 2$ good quality embryos transferred. The details of ovarian stimulation and day of ET were not described in this study. Dorn et al\textsuperscript{45} recruited patients who had no evidence of polycystic ovary syndrome and whose endometrial thickness $\geq 6$ mm. Järvelä et al\textsuperscript{21} excluded women with uterine fibroids, known endometriosis or a single ovary and those who had undergone a previous operation on the uterus or salpingectomy. All our patients recruited were in their first IVF cycle and had two to three
embryos replaced at the early cleavage stage two days after OR following a standard protocol of ovarian stimulation. Patients with an abnormal uterine cavity on 3D scanning were excluded.\(^{24}\) Mercè et al\(^{29}\) studied patients who had serum basal FSH concentration < 10 IU/L and were nonsmokers.

Ultrasound examination was performed on the day of hCG,\(^{26,44}\) OR\(^{23-24,45}\) and blastocyst transfer.\(^{17}\) There is still no consensus when the ultrasound examination for assessing endometrial receptivity in IVF treatment should be done. The day of the ultrasound examination in these studies was chosen for logistic reasons and did not take into consideration the physiological changes of endometrial blood flow throughout the menstrual cycle.\(^{47-48}\)

Mercè et al\(^{29}\) examined the endometrial vascularity only while Kupesic et al,\(^{17}\) Wu et al\(^{23}\) and Dorn et al\(^{45}\) studied the subendometrial region only. The subendometrial region is considered to be within 1,\(^{24}\) 5\(^{17,44}\) or 10 mm\(^{23}\) of the originally defined myometrial-endometrial contour. Dorn et al,\(^{45}\) did not give the details of the subendometrial shell. We reported endometrial and subendometrial vascularity separately and the subendometrial region was defined as a shell within 1 mm of the myometrial-endometrial interface. Only the myometrium immediately underlying the endometrium exhibits a cyclic pattern of steroid receptors expression as that of the endometrium.\(^{39}\)

**CHANGES OF ENDOMETRIAL VASCULARITY IN THE LUTEAL PHASE**

Ultrasound examination was performed only once in the above studies. However, endometrial blood flow changes throughout the menstrual cycle.\(^{50,51}\) Fraser et al\(^{50}\) determined endometrial blood flow through the menstrual cycle in non-pregnant women with the use of the clearance of radiolabelled xenon133 following its instillation into the uterine cavity. There was a significant elevation in the middle to late follicular phase, followed by a secondary slow luteal phase rise that was maintained until the onset of menstruation. More recently, Raine-Fenning et al\(^{51}\) showed that endometrial and subendometrial vascularity by 3D ultrasound increased during the proliferative phase, peaking around 3 days prior to ovulation before decreasing to a nadir 5 days postovulation.

Hypoxia in the endometrium may play a beneficial role for implantation as the expression of vascular endothelial growth factor is upregulated by hypoxia\(^{52}\) and relatively low oxygen tension was present around the blastocyst during the time of implantation.\(^{53}\) The degree of change in endometrial perfusion from the late follicular phase through to the early luteal phase may be a more important determinant of endometrial receptivity.\(^{54}\)

We recently published another study\(^{55}\) evaluating endometrial and subendometrial vascularity on the days of hCG and ET and the percentage change in endometrial and subendometrial vascularity between these two days in the prediction of pregnancy during IVF treatment. Patients in non-pregnant and pregnant groups had comparable endometrial thickness, endometrial volume and 3D Doppler flow indices of endometrial and subendometrial regions measured on either day. Percentage changes in endometrial and subendometrial 3D Doppler flow indices were also similar. Again, none of the ultrasound parameters was predictive of pregnancy in a multiple logistic regression analysis and the ROC curve analysis.

**CONCLUSION**

Ultrasound examination of the endometrium provides a non-invasive method to assess endometrial receptivity during IVF treatment. The use of minimal endometrial thickness mainly lies in the high negative predictive value but the positive predictive value and specificity are low. Endometrial thickness \(\geq 14\) mm appears to have no adverse effect on implantation and pregnancy rates. Endometrial volume is not predictive of pregnancy, although the endometrium may need to attain at least 2.0 to 2.5 ml to achieve a pregnancy during IVF treatment.

Doppler study of uterine vessels is a poor reflection of endometrial and subendometrial vascularity as demonstrated by 3D power Doppler ultrasound. Doppler flow study of spiral arteries is again not predictive of pregnancy. The role of endometrial and subendometrial vascularity assessed by 3D power Doppler ultrasound in predicting pregnancy is still controversial and more studies are warranted.

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