

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

Ernest Hung Yu NG, Pak Chung HO

Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong, China

Correspondence: Ernest Hung Yu NG, Department of Obstetrics and Gynecology, The University of Hong Kong, 6/F, Professorial Block, Queen Mary Hospital, Pokfulam Road, Hong Kong, China, Phone: 852-28553400, Fax: 852-28175374 e-mail: nghye@hkucc.hku.hk

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,¹ cytokine profiles in uterine flushings,² the genomic study of a timed endometrial biopsy³ or ultrasound examination of the endometrium.⁴ Ultrasonography 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.⁵ 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 intra-observer and interobserver reliability.⁶ 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.⁷

The value of endometrial thickness in predicting pregnancy remains controversial.^{8,9} A positive correlation was found between endometrial thickness and the pregnancy

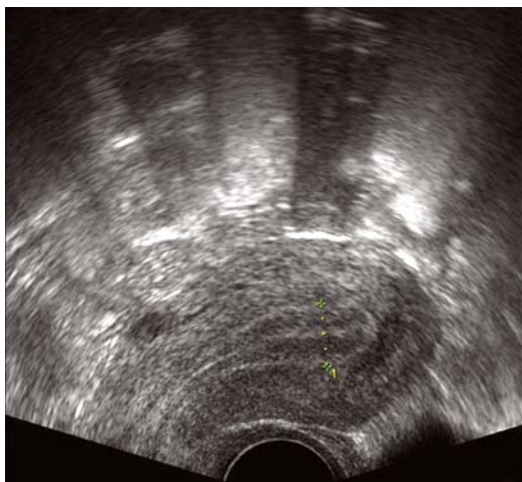


Fig. 1: Endometrial thickness

rate in earlier studies using clomiphene citrate for ovarian stimulation.¹⁰⁻¹² However, a review⁹ 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 study⁷ 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 al¹³ 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. Sundström¹⁴ and Remohi et al¹⁵ 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.⁹

On the other hand, some consider that implantation and pregnancy rates may be adversely affected by a thick endometrium. Weissman et al¹⁶ 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 al¹⁷ reported no pregnancies, if the endometrial thickness on the ET day was >15 mm and Schild et al¹⁸ found no pregnancies if the thickness on the day of oocyte retrieval (OR) was > 16 mm. However, both Dickey et al¹⁹ and Dietterich et al²⁰ 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^{10,11,21} exist. The most simplified one is proposed by Sher et al,²¹ 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 al⁹ 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.

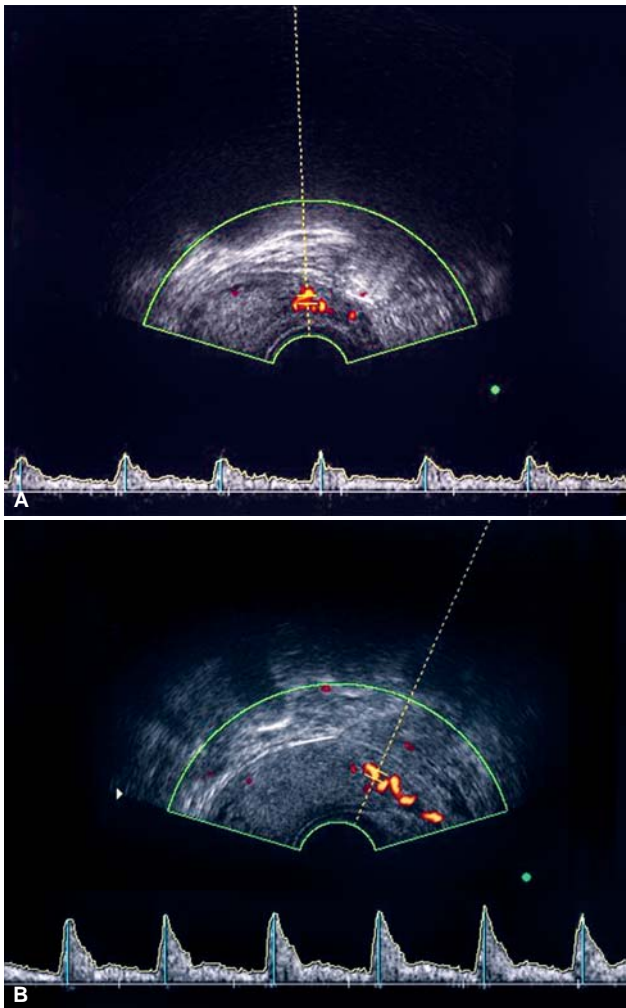
ENDOMETRIAL VOLUME

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 day of hCG administration,²² OR^{18,23,24} and ET^{17, 25} was comparable for pregnant and nonpregnant women.

However, Mercè et al²⁶ 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.



Figs 2A and B: Left (A) and right (B) uterine blood flow measured by 2D Doppler ultrasound

Studies²⁷⁻³⁰ have shown that good uterine blood flow is correlated with conception following IVF as shown by low PI or RI. Steer et al²⁸ classified PI measured on the day of

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 3.3,²⁹ 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 1: Endometrial vascularity measured by 2D color doppler

Study	IVF cycles	USS parameters	USS day	Results
Zaidi et al ³³	96 cycles using a long protocol	Spiral PI and PSV Presence of endometrial and subendometrial vascularity	hCG	No difference in subendometrial PI and PSV between pregnant and nonpregnant cycles Absent subendometrial flow associated with no pregnancy
Battaglia et al ³⁵	60 cycles	Uterine and spiral PI Presence of endometrial vascularity	OR	Uterine and spiral PI lower in pregnant than nonpregnant cycles Absent subendometrial vascularity associated with no pregnancy
Chien et al ³⁸	623 cycles using ultrashort and ultralong protocols	Uterine and spiral PI and RI Presence of endometrial and subendometrial (< 10 mm) vascularity	ET	Significantly lower implantation and pregnancy rates in patients without endometrial/subendometrial vascularity Presence of subendometrial vascularity 5.9 times to become pregnant than those with absent vascularity

USS—Ultrasound; OR—oocyte retrieval; ET—embryo transfer, PI—pulsatility index, PSV—peak systolic velocity

Table 2: Endometrial vascularity measured by 2D power Doppler

Study	IVF cycles	USS parameter	USS day	Results
Yang et al ³⁶	95 cycles using long and short protocols endometrium ≥ 10 mm	Intraendometrial power Doppler area (EDPA) < 5 mm ² ; > 5 mm ²	OR	Higher EDPA in pregnant cycles Lower implantation and pregnancy rates when EDPA < 5 mm ²
Yuval et al ³⁴	156 cycles using a long protocol	PI and RI	OR and ET	No difference in any USS parameters between pregnant and nonpregnant cycles
Contart et al ³⁷	185 cycles using a long protocol	Fundal region along transverse plan; Grades I, II, III & IV according to visualization of power Doppler in the quadrants	hCG	Implantation and pregnancy rates similar in all grades of endometrial vascularity
Schild et al ¹⁸	135 cycles using a long protocol; first cycle only	PI and PSV of vessels in endometrium and subendometrial area (< 5 mm)	OR	No difference in spiral artery PI and PSV between pregnant and nonpregnant cycles Nondetectable spiral vascularity was not associated with a lower implantation rate
Maugey-Laulom et al ³⁹	144 cycles using a long protocol	Presence of endometrial and subendometrial vascularity	ET	Absent endometrial and subendometrial vascularity associated with a lower pregnancy rate

USS—ultrasound; OR—oocyte retrieval; ET—embryo transfer; PI—pulsatility index; PSV—peak systolic velocity

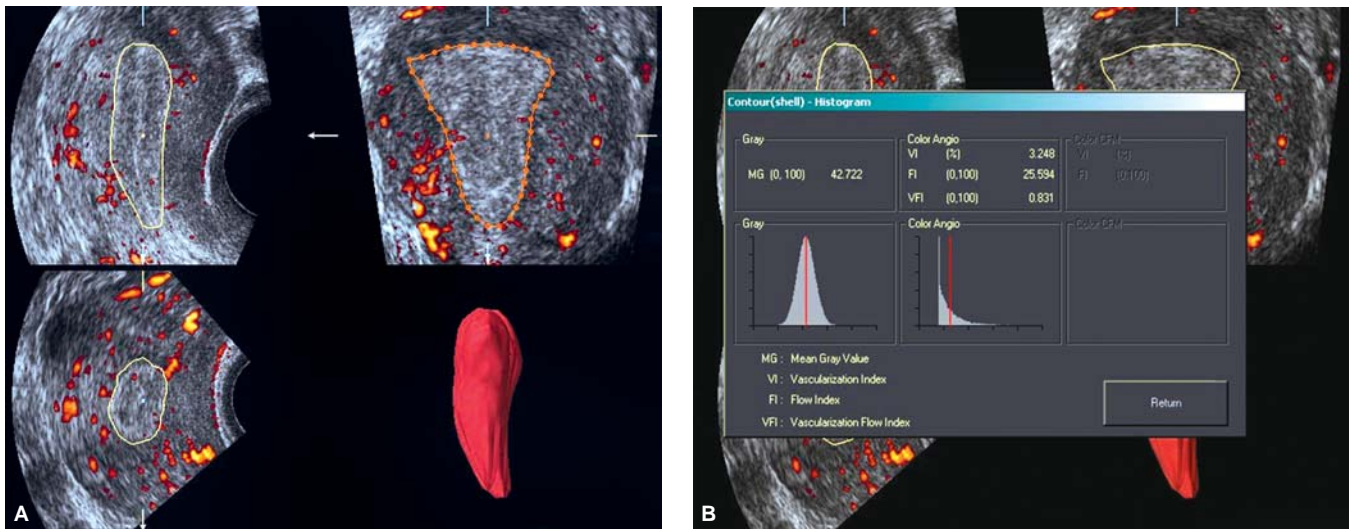
Doppler (Table 2) were examined during IVF treatment. The 2D Doppler flow indices of spiral arteries are not predictive of pregnancy,^{18, 33-34} although Battaglia et al³⁵ and Kupesic et al¹⁷ found significantly lower spiral artery PI in pregnant cycles than nonpregnant cycles.

Yang et al³⁶ 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 al³⁷ 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.

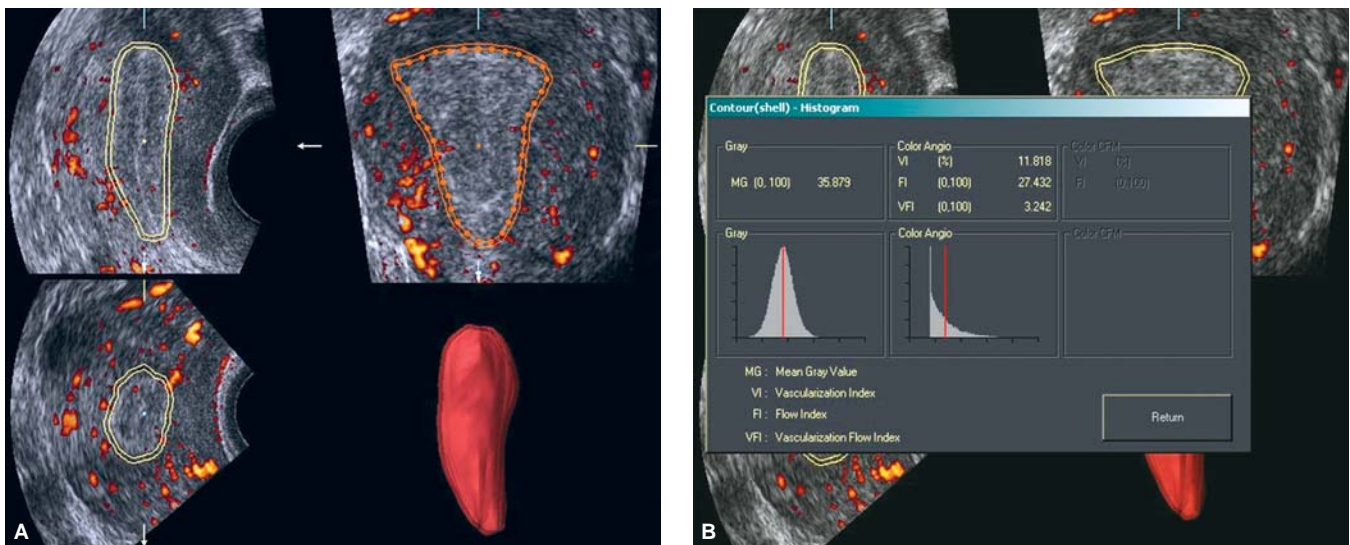
Absent endometrial and subendometrial vascularity on 2D Doppler ultrasound may be associated with no pregnancy^{33, 35} or a much reduced pregnancy rate.^{38, 39}

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



Figs 3A and B: Endometrial volume and blood flow measured by 3D Doppler ultrasound



Figs 4A and B: Subendometrial volume and blood flow measured by 3D Doppler ultrasound

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.⁴⁰ 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

Study	IVF cycles	Inclusion/exclusion criteria	USS day	Results
Schild et al ⁴³	75 cycles using a long protocol IET 2 days after OR	Inclusion criteria - down-regulation confirmed (endometrium < 5 mm; no ovarian cyst of > 2.5 cm; serum estradiol < 60 pg/ml)	Before stimulation	Subendometrial VI, FI and VFI lower in pregnant than nonpregnant cycles Subendometrial FI is the strongest predictive factor for IVF in logistic regression analysis
Kupesic et al ¹⁷	89 cycles using a long protocol Blastocyst transfer 5 days after OR	Inclusion criteria - serum FSH < 10IU/L - no fibroid, ovarian cysts and ovarian endometriosis	ET (hCG +7)	Higher subendometrial FI in pregnant cycles
Wu et al ⁴⁴	54 cycles (details of ovarian stimulation and ET not given)	Inclusion criteria - first cycle - age < 38 year - normal uterine cavity - serum FSH < 15 IU/L - ≥ 2 good quality embryos	hCG	Higher subendometrial VFI in pregnant cycles
Dorn et al ⁴⁵	42 cycles using a long protocol	Exclusion criteria - polycystic ovary syndrome - endometrium < 6 mm - gynaecological surgery	OR	No difference in subendometrial VI, FI and VFI between pregnant and nonpregnant cycles
Järvelä et al ²⁴	35 cycles using a long protocol ET 2 days after OR	Exclusion criteria - uterine fibroids - endometriosis - single ovary - previous operation on uterus or salpingectomy	After stimulation and OR	No difference in endometrial and subendometrial VI between pregnant and nonpregnant cycles on both days
Ng et al ²⁴	451 cycles using a long protocol ET 2 days after OR	Inclusion criteria - first cycle - normal uterine cavity	OR	Lower endometrial VI and VFI in pregnant cycles
Ng et al ⁴⁷	193 cycles	Inclusion criteria - FET cycles - normal uterine cavity	LH+1	No difference in endometrial and subendometrial 3D Doppler flow indices between pregnant and nonpregnant cycles
Mercè et al ²⁶	80 cycles using a long protocol	Inclusion criteria - first cycle - normal uterine cavity - serum FSH < 10 IU/L - regular cycles - nonsmokers	hCG	Higher endometrial VI, FI and VFI in pregnant cycles
Ng et al ⁵⁵	293 cycles using a long protocol ET 2 days after OR	Inclusion criteria - first cycle - normal uterine cavity	OR and ET	No difference in endometrial and subendometrial 3D Doppler flow indices on the 2 days and changes in these indices between pregnant and nonpregnant cycles

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 al¹⁷ performed 3D ultrasound examination on the day of blastocyst transfer and found that subendometrial FI was significantly higher in pregnant cycles. Wu et al⁴⁴ 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 al⁴⁵ 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.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 study²⁴ 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 ≤ 35 years with endometrial thickness > 8 mm, transfer of ≥ 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.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.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,⁴⁸ endometrial and subendometrial vascularity was significantly higher in pregnant patients with livebirth following stimulated IVF and FET treatment.

More recently, Mercè et al²⁶ 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 al¹⁷ and Wu et al⁴⁴ found significantly higher subendometrial vascularity in pregnant cycles whereas Mercè et al²⁶ found significantly higher endometrial vascularity in pregnant cycles. On the other hand, Dorn et al⁴⁵ and Järvelä et al²³ could not demonstrate any differences in endometrial and subendometrial 3D Doppler indices between pregnant and nonpregnant cycles. Our findings²⁴ 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.^{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 al¹⁷ 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 al⁴⁴ 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 ≥ 2 good quality embryos transferred. The details of ovarian stimulation and day of ET were not described in this study. Dorn et al⁴⁵ recruited patients who had no evidence of polycystic ovary syndrome and whose endometrial thickness ≥ 6 mm. Järvelä et al²³ 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 al²⁹ studied patients who had serum basal FSH concentration < 10 IU/L and were non-smokers.

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 al²⁶ examined the endometrial vascularity only while Kupesic et al,¹⁷ Wu et al²³ and Dorn et al⁴⁵ studied the subendometrial region only. The subendometrial region is considered to be within 1,²⁴ 5^{17,44} or 10 mm²³ 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.⁴⁹

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 al⁵⁰ 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 substantial fall and a secondary slow luteal phase rise that was maintained until the onset of menstruation. More recently, Raine-Fenning et al⁵¹ 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 hypoxia⁵² 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 study⁵⁵ 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 ≥ 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.

REFERENCES

1. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril* 1950;1:3-25.
2. Lédée-Bataille N, Laprée-Delage G, Taupin JL, Dubanchet S, Frydman R, Chaouat G. Concentration of leukaemia inhibitory factor (LIF) in uterine flushing fluid is highly predictive of embryo implantation. *Hum Reprod* 2002;17:213-18.
3. Horcajadas JA, Pellicer A, Simón C. Wide genomic analysis of human endometrial receptivity: New times, new opportunities *Hum Reprod Update* 2007;13:77-86.
4. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. The role of endometrial blood flow in the prediction of pregnancy during in vitro fertilization treatment. *Eur J Obstet Gynecol Reprod Biol* 2007;135:8-16.
5. Jinno M, Ozaki T, Iwashita M, Nakamura Y, Kudo A, Hirano H. Measurement of endometrial tissue blood flow: A novel way to assess uterine receptivity for implantation. *Fertil Steril* 2001;76:1168-74.
6. Spandorfer SD, Arrendondo-Soberon F, Loret de Mola JR, Feinberg RF. Reliability of intraobserver and interobserver

- sonographic endometrial stripe thickness measurements. *Fertil Steril* 1998;70:152-54.
7. De Geyter C, Schnitter M, De Geyter M, Nieschlag E, Holzgreve W, Schneider HPG. Prospective evaluation of the ultrasound appearance of the endometrium in a cohort of 1,186 infertile women. *Fertil Steril* 2000;73:106-13.
 8. Turnbull LW, Lesny P, Killick SR. Assessment of uterine receptivity prior to embryo transfer: A review of currently available imaging modalities. *Hum Reprod Update* 1995;1:505-14.
 9. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: A critical review. *Hum Reprod Update* 1996;2:323-35.
 10. Smith B, Porter R, Ahuja K, Craft I. Ultrasonic assessment of endometrial changes in stimulated cycles in an in vitro fertilization and embryo transfer program. *J In Vitro Fertil Embryo Transf* 1984;1:233-38.
 11. Glissant A, de Mouzon J, Frydman R. Ultrasound study of the endometrium during in vitro fertilization cycles. *Fertil Steril* 1985;44:786-90.
 12. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). *J In Vitro Fertil Embryo Transf* 1990;7:146-52.
 13. Gonen Y, Calderon M, Drenfeld M, Abramovici H. The impact of sonographic assessment of the endometrium and meticulous hormonal monitoring during natural cycles in patients with failed donor artificial insemination. *J Ultrasound Obstet Gynecol* 1991;1:122-26.
 14. Sundström P. Establishment of a successful pregnancy following in vitro fertilization with an endometrial thickness of no more than 4 mm. *Hum Reprod* 1998;13:1550-52.
 15. Remohi J, Ardiles G, Garcia-Velasco JA, Gaitan P, Simon C, Pellicer A. Endometrial thickness and serum estradiol concentrations as predictors of outcome in oocyte donation. *Hum Reprod* 1997;12:2271-75.
 16. Weissman A, Gottlieb L, Casper R. The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an in vitro fertilization program. *Fertil Steril* 1999;71:147-49.
 17. Kupesic S, Bekavac I, Bjelos D, Kurjak A. Assessment of endometrial receptivity by transvaginal color Doppler and three-dimensional power Doppler ultrasonography in patients undergoing in vitro fertilization procedures. *J Ultrasound Med* 2001;20:125-34.
 18. Schild RL, Knoblock C, Dorn C, Fimmers R, van der Ven H, Hansmann M. Endometrial receptivity in an in vitro fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. *Fertil Steril* 2001;75:361-66.
 19. Dickey RP, Olar TT, Taylor SN, Rye PH. Endometrial pattern and thickness associated with pregnancy outcome after assisted reproduction technologies. *Hum Reprod* 1992;7:418-21.
 20. Dietherich C, Check JH, Choe JK, Nazari A, Lurie D. Increased endometrial thickness on the day of human chorionic gonadotrophin injection does not adversely affect pregnancy or implantation rates following in vitro fertilization-embryo transfer. *Fertil Steril* 2002;77:781-86.
 21. Sher G, Herbert C, Maassarani, G, Jacobs MH. Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing in-vitro fertilization and embryo transfer (IVF/ET). *Hum Reprod* 1991;6:232-37.
 22. Yaman C, Ebner T, Sommergruber M, Polz W, Tews G. Role of three-dimensional ultrasonographic measurement of endometrium volume as a predictor of pregnancy outcome in an IVF-ET program. A preliminary study. *Fertil Steril* 2000;74:797-801.
 23. Järvelä IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Evaluation of endometrial receptivity during in-vitro fertilization using three-dimensional power Doppler ultrasound. *Ultrasound Obstet Gynecol* 2005;26:765-69.
 24. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. The role of endometrial and subendometrial blood flow measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during in vitro fertilization treatment. *Hum Reprod* 2006;21:164-70.
 25. Raga R, Bonilla-Musoles F, Casan EM, Klein O, Bonilla F. Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: Clues to endometrial receptivity. *Hum Reprod* 1999;14:2851-54.
 26. Mercè LT, Barco MJ, Bau S, Troyano J. Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to in vitro fertilization/embryo transfer outcome? *Fertil Steril* 2008;1:111-17.
 27. Sterzik K, Grab D, Sasse V, Hutter W, Rosenbusch B, Terinde R. Doppler sonographic findings and their correlation with implantation in an in vitro fertilization program. *Fertil Steril* 1989;52:825-28.
 28. Steer CV, Campbell S, Tan SL, Crayford T, Mills C, Mason BA, Collins WP. The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. *Fertil Steril* 1992;57:372-76.
 29. Coulam CB, Bustillo M, Soenksen DM, Britten S. Ultrasonographic predictors of implantation after assisted reproduction. *Fertil Steril* 1994;62:1004-10.
 30. Serafini P, Batzofin J, Nelson J, Olive D. Sonographic uterine predictors of pregnancy in women undergoing ovulation induction for assisted reproductive treatments. *Fertil Steril* 1994;62:815-22.
 31. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. Relationship between uterine blood flow and endometrial and subendometrial blood flow during stimulated and natural cycles. *Fertil Steril* 2006;85:721-27.
 32. Kupesic S, Kurjak A. Uterine and ovarian perfusion during periovulatory period assessed by transvaginal color Doppler. *Fertil Steril* 1993;60:439-43.
 33. Zaidi J, Campbell S, Pittrof FR, Tan SL. Endometrial thickness morphology, vascular penetration and velocimetry in predicting implantation in an IVF program. *Ultrasound Obstet Gynecol* 1995;6:191-98.
 34. Yuval Y, Lipitz S, Dor J, Achiron R. The relationship between endometrial thickness, and blood flow and pregnancy rates in in-vitro fertilization. *Hum Reprod* 1999;14:1067-71.
 35. Battaglia C, Artini PG, Giulini S, Salvatori M, Maxia N, Petraglia F, Volpe A. Colour Doppler changes and thromboxane production after ovarian stimulation with gonadotrophin-releasing hormone agonist. *Hum Reprod* 1997;12:2477-82.

36. Yang JH, Wu MY, Chen CD, Jiang MC, Ho HN, Yang YS. Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of in-vitro fertilization. *Hum Reprod* 1999;14:1606-10.
37. Contart P, Baruffi RL, Coelho J, Mauri AL, Petersen C, Franco Junior JG. Power Doppler endometrial evaluation as a method for the prognosis of embryo implantation in an ICSI program. *J Assist Reprod Genet* 2000;17:329-34.
38. Chien LW, Au HK, Chen PL, Xiao J, Tseng CR. Assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2002;78:245-51.
39. Maugey-Laulon B, Commenges-Ducos M, Jullien V, Papaxanthos-Roche A, Scotet V, Commenges D. Endometrial vascularity and ongoing pregnancy after IVF. *Eur J Obstet Gynecol Reprod Biol* 2002;104:137-43.
40. Pairleitner H, Steiner H, Hasenoehrl G, Staudach. A Three-dimensional power Doppler sonography: Imaging and quantifying blood flow and vascularization. *Ultrasound Obstet Gynecol* 1999;14:139-43.
41. Raine-Fenning NJ, Campbell BK, Clewes JS, Kendall NR, Johnson IR. The reliability of virtual organ computer-aided analysis (VOCAL) for the semiquantification of ovarian, endometrial and subendometrial perfusion. *Ultrasound Obstet Gynecol* 2003;22:633-39.
42. Raine-Fenning NJ, Campbell BK, Clewes JS, Kendall NR, Johnson IR. The interobserver reliability of three-dimensional power Doppler data acquisition within the female pelvis. *Ultrasound Obstet Gynecol* 2004;23:501-08.
43. Schild RL, Holthaus S, d'Alquen J, Fimmers R, Dorn C, van Der Ven H, Hansmann M. Quantitative assessment of subendometrial blood flow by three-dimensional-ultrasound is an important predictive factor of implantation in an in-vitro fertilization programme. *Hum Reprod* 2000;15:89-94.
44. Wu HM, Chiang CH, Huang HY, Chao AS, Wang HS, Soong YK. Detection of the subendometrial vascularization flow index by three-dimensional ultrasound may be useful for predicting the pregnancy rate for patients undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2003;79:507-11.
45. Dorn C, Reinsberg J, Willeke C, Wendt A, van der Ven H, Schild RL. Three-dimensional power Doppler ultrasound of the subendometrial blood flow under the administration of a contrast agent (Levovist). *Arch Gynecol Obstet* 2004;270:94-98.
46. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. Factors affecting endometrial and subendometrial blood flow measured by three-dimensional power Doppler ultrasound during in vitro fertilization treatment. *Hum Reprod* 2006;21:1062-69.
47. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. The role of endometrial and subendometrial vascularity measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during frozen-thawed embryo transfer cycles. *Hum Reprod* 2006;21:1612-17.
48. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. Endometrial and subendometrial vascularity is higher in pregnant patients with live birth following ART than in those who suffer a miscarriage. *Hum Reprod* 2007;22:1134-41.
49. Noe M, Kunz G, Herberitz M, Mall G, Leyendecker G. The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: Characterization of the endometrial-subendometrial unit. *Hum Reprod* 1999;14:190-97.
50. Fraser IS, McCarron G, Hutton B, Macey D. Endometrial blood flow measured by xenon 133 clearance in women with normal menstrual cycles and dysfunctional uterine bleeding. *Am J Obstet Gynecol* 1987;156:158-66.
51. Raine-Fenning NJ, Campbell BK, Kendall NR, Clewes JS, Johnson IR. Quantifying the changes in endometrial vascularity throughout the normal menstrual cycle with three-dimensional power Doppler angiography. *Hum Reprod* 2004;19:330-38.
52. Sharkey AM, Day K, McPherson A, et al. Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. *J Clin Endocrinol Metab* 2000;85:402-09.
53. Graham CH, Postovit LM, Park H, Canning MT, Fitzpatrick TE. Adriana and Luisa Castellucci award lecture 1999: Role of oxygen in the regulation of trophoblast gene expression and invasion. *Placenta* 2000;21:443-50.
54. Raine-Fenning NJ, Campbell BK, Kendall NR, Clewes JS, Johnson IR. Endometrial and subendometrial perfusion are impaired in women with unexplained subfertility. *Hum Reprod* 2004;19:2605-14.
55. Ng EHY, Chan CCW, Tang OS, WSB Yeung, Ho PC. Changes in endometrial and subendometrial blood flows in IVF. *Reproductive BioMedicine Online* 2009;18:269-75.