Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of in-vitro fertilization

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An endometrial thickness of 10 mm or more has been reported to be favourable for embryo implantation. Nevertheless, many women participating in in-vitro fertilization (IVF) programmes have adequate endometrial thickness but do not achieve satisfactory implantation. With the aid of power Doppler sonography, we examined the association between intra-endometrial vascularity and reproductive outcome. For this study, we enrolled only women with endometrial thickness ≥10 mm and excluded those with apparent endometrial pathologies. Of 95 women undergoing IVF cycles, there resulted 37 intrauterine pregnancies. The women were of similar age, body mass index, peak oestradiol concentration and endometrial thickness, and a similar number of embryos were transferred. Those women with an intra-endometrial power Doppler area (EPDA) <5 mm² achieved a significantly lower pregnancy rate (23.5 versus 47.5%, \( P = 0.021 \)) and implantation rate (8.1 versus 20.2%, \( P = 0.003 \)) than those with an EPDA ≥5 mm². We conclude that, in addition to endometrial thickness, EPDA may serve as a factor indicative of endometrial receptivity. Women with adequate endometrial thickness but a small EPDA tended to have an unfavourable reproductive outcome.

Key words: implantation/in-vitro fertilization/intra-endometrial power Doppler area (EPDA)/power Doppler

Introduction

Embryo quality and endometrial receptivity are two of the parameters which determine the reproductive outcome in in-vitro fertilization (IVF) programmes. Morphological assessment of the embryo using light microscopy is a generally accepted method for the evaluation of embryo quality. However, the evaluation of endometrial receptivity remains a challenge in clinical practice. In the attempt to identify a non-invasive method of predicting endometrial receptivity during IVF procedures, sonographic imaging of endometrial patterns and thickness has been widely studied. Although some investigators have found (Dickey et al., 1992; Noyes et al., 1995) a positive correlation between endometrial echo patterns and pregnancy rates, this has been disputed (Khalifa et al., 1992; Oliveira et al., 1993). Cycles with an endometrial thickness of 10 mm or more have been reported to be favourable for embryo implantation (Check et al., 1991; Rinaldi et al., 1996), although other cut-off values have also been claimed as appropriate (Rabinowitz et al., 1986; Dickey et al., 1992; Noyes et al., 1995). However, these reports fail to explain why some women with adequate endometrial thickness do not achieve a satisfactory implantation rate.

It was first reported in 1988 that impaired perfusion of the uterine arteries may be a cause of infertility and may be related to unsuccessful in-vitro fertilization treatment (Goswamy and Steptoe, 1988). Utilizing transvaginal colour Doppler ultrasound, it has proved possible to distinguish between conception and non-conception cycles before embryo transfer, based on differences in mean uterine artery pulsatility index (PI) or resistance index (RI). Patients who became pregnant had a lower vascular impedance than those who did not (Sterzik et al., 1989; Steer et al., 1992, 1995; Coulam et al., 1994). However, recent data have challenged the predictive role of Doppler measurements in assisted reproductive technology procedures (Bassil et al., 1995; Tekay et al., 1996a; Schwartz et al., 1997). Uterine artery Doppler measurements are based on flow to the entire uterus, rather than to focal areas of the endometrium. For successful implantation of an embryo, the quality of the endometrium may be more important than the global blood flow throughout the uterus (Schwartz et al., 1997). Zaidi et al. (1995a) utilized colour Doppler imaging and found that absent subendometrial and intra-endometrial vascularization was associated with implantation failures in IVF cycles. However, the sensitivity of colour Doppler is limited and the rather weak vascular signals inside the endometrium are difficult to detect.

The aim of this study was to search for another factor that, in addition to endometrial thickness, might be indicative of endometrial receptivity. With the aid of power Doppler sonography, we established a method of measuring the intra-endometrial power Doppler area (EPDA), and examined its association with IVF outcome.

Materials and methods

In this prospective study, we enrolled women undergoing in-vitro fertilization. All of them received gonadotrophin releasing hormone agonist (GnRHa) combined with follicle stimulating hormone (FSH) and human menopausal hormone (HMG) to achieve adequate ovarian stimulation. Only cycles with endometrial thickness ≥ 10 mm were...
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Demarcated margins was then automatically calculated and expressed processing system (Encomate®; Electronic Business Machine, Taipei, 4–8 MHz transvaginal scanhead, and a signal conversion and image conversion and storage system (Encomate®; Electronic Business Machine, Taipei, Taiwan). The endometrium was imaged on the day before oocyte retrieval by transvaginal sonography. As circadian changes in uterine artery blood flow have been shown (Zaidi et al., 1995b), all colour Doppler measurements in this study were performed between 1300 and 1700 hours in a quiet room with constant light and a room temperature between 21 and 22°C. The sonographic assessments were performed by the same investigator (J-H. Yang) using the same parameters so as to eliminate any interobserver variation.

The sonographic equipment consisted of a colour Doppler unit (HDI 1000; Advanced Technology Laboratories, Bothell, WA, USA), a 4–8 MHz transvaginal scanner, and a signal conversion and image processing system (Encomate®; Electronic Business Machine, Taipei, Taiwan). The endometrium was imaged on the day before oocyte retrieval by transvaginal sonography. As circadian changes in uterine artery blood flow have been shown (Zaidi et al., 1995b), all colour Doppler measurements in this study were performed between 1300 and 1700 hours in a quiet room with constant light and a room temperature between 21 and 22°C. The sonographic assessments were performed by the same investigator (J-H. Yang) using the same parameters so as to eliminate any interobserver variation.

The settings for power Doppler sonography were standardized for the highest sensitivity in the absence of apparent noise using a high-pass filter at 50 Hz, pulsed repetition frequency at 750 Hz, and moderate long persistence. Colour versus echo write priority was set at a high level to facilitate the display of colour signals. Under these conditions, the lowest possible measurable velocity was below 5 cm/s. A power Doppler sonography study with slow sweep technique was performed and the intra-endometrial vascularity was obtained by longitudinal scanning of the uterus. At least three pictures were captured and the images were converted and stored as digital images in red-green-blue (RGB) format for later analysis of vascularity with Encomate®. After the examination, the previously captured images were retrieved and displayed on the monitor. The endometrium–myometrium interface was demarcated manually on the computer with a cursor. The area (mm²) of the vascular signals within the demarcated margins was then automatically calculated and expressed as the EPDA (Figure 1). The scan plane with the largest vascularity area after analysis was documented.

The key step in calculating the EPDA was to identify the colour pixels. Theoretically, in an RGB colour system, if R, G and B are not all equal for a pixel, this pixel may be considered a colour pixel. We adopted the luminance-in-phase-quadrature (YIQ) colour model for colour pixel identification. The YIQ model is widely used in commercial colour television broadcasting. In the YIQ colour model, the luminance and colour information are decoupled. Y represents the luminance of the colour, which is proportional to the amount of light perceived by our eyes. I and Q jointly give the colour information. The square root of (I² + Q²), defined as L, stands for the level of hue and saturation of colour under different luminances. Each pixel may be converted from the RGB colour system to the YIQ model through a linear transformation as follows.

\[
\begin{align*}
Y &= 0.299R + 0.587G + 0.114B \\
I &= 0.596R - 0.275G - 0.321B \\
Q &= 0.212R - 0.523G + 0.311B
\end{align*}
\]

From this linear transformation, one can see that for an ideal grey-scale pixel (that is, R = G = B), L is equal to zero. However, to account for possible analogue-to-digital conversion errors and quantification errors, a preset threshold was established in this study such that a pixel could be considered a colour pixel if its L was greater than or equal to this preset value.

With this procedure, an independent comparison of 20 cases in our settings revealed acceptably high intra-observer correlation between two different measurements (intraclass correlation coefficient 0.91).

**Power Doppler assessment of intra-endometrial vascularity**

The sonographic equipment consisted of a colour Doppler unit (HDI 1000; Advanced Technology Laboratories, Bothell, WA, USA), a 4–8 MHz transvaginal scanhead, and a signal conversion and image processing system (Encomate®; Electronic Business Machine, Taipei, Taiwan). The endometrium was imaged on the day before oocyte retrieval by transvaginal sonography. As circadian changes in uterine artery blood flow have been shown (Zaidi et al., 1995b), all colour Doppler measurements in this study were performed between 1300 and 1700 hours in a quiet room with constant light and a room temperature between 21 and 22°C. The sonographic assessments were performed by the same investigator (J-H. Yang) using the same parameters so as to eliminate any interobserver variation.

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**Measurement of uterine haemodynamics**

The methodology for measurements of uterine haemodynamics has been described previously (Chen et al., 1998). Briefly, the scanhead was directed to image the ascending branches of the bilateral uterine arteries lateral to the cervix. The values of uterine artery peak systolic velocity (PSV) and PI were measured by pulsed Doppler spectral analysis. The PI was calculated according to the formula: (S–D)/A, where S was the maximal systolic velocity, D was the end-diastolic velocity and A was the average of the maximal Doppler shifted frequencies over one cardiac cycle. The PSV and PI for both uterine arteries were obtained, and the mean values were calculated.

**Statistical analysis**

The data were analysed using the Mann–Whitney test, the two-sample t-test, the χ² test and the Fisher’s exact test with the aid of the Statistical Program for Social Sciences (SPSS) version 7.0 (SPSS Inc., Chicago, IL, USA) for IBM personal computer. A P value < 0.05 was considered statistically significant.
Implantation (%) 51/253 (20.2) 10/124 (8.1) 0.003
Pregnancies (%) 29 (47.5) 8 (23.5) 0.021
No. of oocytes retrieved 10.3 ± 6.5
Peak oestradiol (pg/ml) 2243
No. of embryos transferred 4.1
Menstrual day of ultrasound 13.7

### Results

In this study, 95 women underwent IVF cycles. The aetiologies of infertility were tubal factor in 28 cycles, ovarian factor in six, male factor in 21, endometriosis in six, combined factor in 23, and unexplained infertility in 11. Of 43 pregnancies achieved, while three were biochemical and three were tubal, 37 were intrauterine pregnancies. The EPDA of the 95 IVF cycles ranged from 0.4–39.5 mm², with a median area of 37 mm² (interquartile range 4.2–11.5). Women who achieved intrauterine pregnancies had a significantly higher EPDA than those who did not [8.8 mm² (5.1–16) versus 5.8 mm² (4–9.3), P < 0.02]. The lowest EPDA in a cycle resulting in pregnancy was 1.1 mm².

When EPDA in relation to pregnancy and implantation rates was analysed, the pregnancy and implantation rates were found to be higher for women with a higher EPDA (Figure 2). The implantation rates in women with an EPDA between 5 and 8 (n = 24), between 8 and 11 (n = 12), and >11 mm² (n = 25) were all significantly higher than those with an EPDA <5 mm² (n = 34) (25.9, 17.6 and 17.4% respectively, versus 8.1%, P < 0.05). Therefore, we defined an EPDA of 5 mm² as the threshold, and an area <5 mm² was regarded as poor vascularization. Table I shows that there were no significant differences in age and body mass index (BMI) or in peak oestradiol levels and the numbers of oocytes between women having an EPDA ≥5 mm² and those having an EPDA <5 mm². After the transfer of a similar number of embryos, women with an EPDA ≥5 mm² achieved significantly higher pregnancy and implantation rates than those with an EPDA <5 mm². There were no significant differences in multiple pregnancy rates or in the rates of spontaneous abortion. When fetal viability (>20 weeks) was analysed, the association between EPDA and implantation rates was confirmed (17.8 versus 5.6%, P = 0.001).

Table II indicates that despite similarities between endometrial thicknesses, mean uterine artery PI and PSV, intra-endometrial vascularization exhibited significant differences.

### Discussion

Adequate endometrial receptivity is required for successful implantation in both natural and IVF cycles. Assessment of endometrial thickness using grey-scale ultrasound is by far the most important method of predicting implantation (Rabinowitz et al., 1986; Check et al., 1991; Noyes et al., 1995; Rinaldi et al., 1996). However, it was reported that there was an extensive overlap in the ranges of endometrial thickness present in pregnant and non-pregnant cycles (Schwartz et al., 1997). We also observed that many women with favourable endometrial thickness had unsuccessful cycles, suggesting something other than endometrial thickness affected endometrial receptivity.

Impaired perfusion of uterine arteries was reported as a possible cause of unsuccessful implantation (Goswamy and Steptoe, 1988). A significant difference in uterine artery perfusion was found between those patients who became pregnant following IVF and those who did not (Sterzik et al., 1989; Coulam et al., 1994; Levi-Setti et al., 1995), although this has been disputed (Bassil et al., 1995; Zaidi et al., 1996; Schwartz et al., 1997). A major problem in comparing conflicting results following uterine artery perfusion may be heterogeneities in the stimulation protocols, study populations, parameters studied, and the timing of Doppler sonography investigation (Tekay et al., 1996b). Nevertheless, in agreement with Schwartz et al. (1997), we believe that the major reason was that uterine artery Doppler measurements were based on flow to the entire uterus, not to focal areas of the endometrium. For an embryo to implant, the quality of the endometrium may be more important than the global blood flow throughout the uterus. In this study, we tried to evaluate intra-endometrial vascularization.
vascularity using power Doppler sonography, to analyse the flow that is truly representative of the endometrial condition.

Power Doppler sonography has the advantages of less direction dependence, higher sensitivity, and better contrast of vascular contour, making it the favoured tool in studies of tissue and tumour vascularity (Taylor et al., 1996). Compared to colour Doppler, power Doppler has been claimed to be more sensitive to low flow states (Jain et al., 1991). In addition to qualitative studies, computer-assisted quantitative assessment of power Doppler vascular signals has been applied in studies of breast tumours (Huber et al., 1994; Kedar et al., 1995) and cervical lymphadenopathies (Wu et al., 1998). This technique could discriminate clearly between benign and malignant tissues because of their different densities of vascularity. Utilizing the power Doppler sonography, we were able to obtain an in-vivo quantification of macroscopic vascularity in the endometrium, an area containing rather weak vascular signals that are difficult to detect with conventional colour Doppler.

Endometrial thickness of 10 mm or more has been generally regarded as a favourable factor for implantation (Check et al., 1991; Rinaldi et al., 1996). Nevertheless, even with an adequate endometrial thickness, many embryos are unable to implant. In order to delineate the factor affecting endometrial receptivity for women in this category, we enrolled only those who had an endometrial thickness ≥10 mm. The data available so far suggest that the EPDA is an important factor in determining the probability of implantation. Women with a higher EPDA had a better chance of achieving successful implantation than those with a lower EPDA (Figure 2).

An EPDA cut-off value of 5 mm$^2$ was utilized in this study. In a population of women of similar age, BMI, peak oestradiol and embryo number, those with poor endometrial vascularity (EPDA <5 mm$^2$) demonstrated significantly lower pregnancy and implantation rates than those who had adequate vascularity. Furthermore, the EPDA appeared to be a unique parameter that was independent of endometrial thickness and uterine artery perfusion (Table II). The results confirmed that uterine artery Doppler measurements are not representative of endometrial receptivity since they are based on flow to the entire uterus.

Nevertheless, the predictive role of EPDA in embryo implantation is limited. Approximately one out of four women in this category achieved an intrauterine pregnancy despite an unfavourable EPDA. Hence, endometrial blood flow may not be the sole factor affecting endometrial receptivity. Other factors such as endometrial echo pattern, leukaemia inhibitory factor (LIF) and transforming growth factor β (TGFβ) might also play important roles.

In an IVF programme, the appropriate number of embryos to be transferred remains a dilemma in that the transfer of a higher number results in not only a higher pregnancy rate but also in an increase in multiple pregnancies. In our study, after transferring similar numbers of embryos, the multiple pregnancy rate was approximately four times higher in women with an EPDA ≥5 mm$^2$ than in those with an EPDA <5 mm$^2$. Therefore, the number of transferred embryos should be determined on the basis of the quantity of intra-endometrial vascularity as well as the endometrial thickness, so as to improve the reproductive outcome for those with poorly vascularized endometrium, and to reduce the potential risk of multiple pregnancies for those containing adequate intra-endometrial vascularity. For women with a small EPDA, cryopreservation might be suggested if a good freezing programme is available. A prospective study is necessary to verify this assumption.

Various regimens such as low dose aspirin, heparin and nitroglycerin have been found to improve endometrial receptivity due to their promoting effects on uterine perfusion. We found that, in the same patient, the EPDA values between the study cycle and the subsequent one were similar, suggesting that endometrial vascularity may be a persistent character. The measurement of EPDA might serve as an alternative method to re-evaluate the specific effects of therapy on endometrium. Further large-scale studies are needed.

As in any clinical study, this study has its limitation. The EPDA may vary according to the colour Doppler unit and image processing system used. The cut-off value of 5 mm$^2$ is based on our own experience, and other IVF centres should establish their own criteria. Nevertheless, our results may provide guidelines so that other criteria can be determined.

To our knowledge, this is the first study evaluating vascularity that is specific for endometrium. We conclude that, in addition to endometrial thickness, EPDA may be a valuable predictor of endometrial receptivity. Women with adequate endometrial thickness but a small EPDA appear to have an unfavourable reproductive outcome.

Acknowledgements

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References


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