The role of endometrial and subendometrial blood flows measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during IVF treatment

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BACKGROUND: A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation. We aimed to evaluate the role of endometrial and subendometrial blood flows in the prediction of pregnancy during IVF treatment. METHODS: Patients undergoing the first IVF cycle were recruited. A three-dimensional (3D) ultrasound examination with power Doppler was performed on the day of oocyte retrieval to determine endometrial thickness, endometrial pattern, pulsatility index (PI) and resistance index (RI) of uterine vessels, endometrial volume, vascularization index (VI), flow index (FI) and vascularization flow index (VFI) of endometrial and subendometrial regions. RESULTS: Uterine RI, endometrial VI and VFI were significantly lower in the pregnant group than the non-pregnant group. There was a non-significant trend of higher implantation and pregnancy rates in patients with absent endometrial or subendometrial blood flow. The number of embryos replaced and endometrial VI were the only two predictive factors for pregnancy. Receiver operator characteristic curve analysis revealed that the area under the curve was ~0.5 for all ultrasound parameters for endometrial receptivity. CONCLUSION: Endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound were not good predictors of pregnancy if they were measured at one time-point during IVF treatment.

Key words: endometrial and subendometrial blood flows/IVF/three-dimensional power Doppler

Introduction

The endometrium undergoes adequate proliferation and differentiation in the follicular phase, which is followed by timely secretory changes during the luteal phase. Successful implantation is dependent on a close dialogue between the blastocyst and the receptive endometrium. Endometrial receptivity can be evaluated by histological evaluation of an endometrial biopsy (Noyes et al., 1950), endometrial proteins in uterine flushing (Li et al., 1998) or more commonly a non-invasive ultrasound examination of the endometrium. Different ultrasound parameters have been used to assess endometrial receptivity during IVF treatment and these include endometrial thickness, endometrial pattern, endometrial volume, Doppler study of uterine arteries and endometrial blood flow. Endometrial thickness and pattern have low positive predictive value and specificity in the prediction of IVF outcome (Turnbull et al., 1995; Friedler et al., 1996).

Angiogenesis plays a critical role in various female reproductive processes such as development of a dominant follicle, formation of a corpus luteum, growth of endometrium and implantation (Abulafia and Sherer, 2000; Smith, 2001). A good blood supply towards the endometrium is usually considered as an essential requirement for implantation. Gannon et al. (1997) used an intrauterine laser Doppler technique to measure endometrial microvascular blood flow, which differed between phases of the menstrual cycle with significant increase in blood flow during early follicular and early luteal phases. Endometrial microvascular blood flow which is determined by an intrauterine laser Doppler technique in the early luteal phase of the cycle preceding an IVF cycle has been shown to be predictive of pregnancy and superior to other conventional parameters predicting endometrial receptivity (Jinno et al., 2001). Endometrial blood flow can be non-invasively evaluated by a colour and power Doppler ultrasound. Power Doppler imaging is more sensitive than colour Doppler imaging at detecting low velocity flow and hence improves the visualization of small vessels (Guerrero et al., 1999). In combination with a three-dimensional (3D) ultrasound, power Doppler provides a unique tool with which to examine the blood supply towards the whole endometrium and the subendometrial region (Schild et al., 2000; Kupesic et al., 2001; Wu et al., 2003; Raine-Fenning et al., 2004a; Ng et al., 2004, 2005).
The aim of this study was to evaluate the role of endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound in the prediction of pregnancy during IVF treatment. We compared the endometrial and subendometrial blood flows between pregnant and non-pregnant patients in their first IVF cycle. Implantation and pregnancy rates were also compared between those with and without endometrial and subendometrial blood flows. The hypothesis was that endometrial and subendometrial blood flows were significantly higher in pregnant patients than non-pregnant patients.

Materials and methods

Infertile patients undergoing the first IVF cycle in the Assisted Reproduction Unit of the Department of Obstetrics and Gynaecology, The University of Hong Kong between November 2002 and December 2004 were recruited if they had normal uterine cavity detected on the day of transvaginal ultrasound-guided oocyte retrieval (TUGOR). Indications for IVF included tubal, male, endometriosis, unexplained and mixed factors. ICSI was performed for couples with severe semen abnormalities where <100,000 motile sperm were recovered after sperm preparation. In cases of obstructive or non-obstructive azoospermia, surgically retrieved sperm from epididymis or testis respectively were used for ICSI. Serum basal FSH concentration was measured on the day of hCG administration. Serum E2 concentration was measured on the day of hCG administration. There were at least three follicles of ≥16 mm in diameter and there were at least three follicles of ≥16 mm in diameter and there were at least three follicles of ≥16 mm in diameter and there were at least three follicles of ≥16 mm in diameter.

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All ultrasound measurements were performed by E.H.Y.N. on the day of TUGOR prior to the procedure using Voluson 730® (Kretz, Zipf, Austria) at around 08:00–10:00 after they had emptied their bladder. The details of 3D ultrasound and data analysis were as previously described (Ng et al., 2000). In short, they were pre-treated with busulberein (Suprecur; Hoechst, Frankfurt, Germany) nasal spray 150 mg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle and they also received HMG (Pergonal; Serono, Geneva, Switzerland) for ovarian stimulation. HCG (Profasi; Serono, Geneva, Switzerland) was given i.m. when the leading follicle reached 18 mm in diameter and there were at least three follicles of ≥16 mm in diameter. Serum E2 concentration was measured on the day of hCG administration. TUGOR was scheduled 36 h after the hCG injection.

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Using colour Doppler in the 2D mode, flow velocity waveforms were obtained from the ascending main branch of the uterine artery on the right and left side of the cervix in a longitudinal plane before they entered the uterus. The gate of the Doppler was positioned when the vessel with good colour signals was identified on the screen. PI and RI of the uterine arteries were calculated electronically when similar consecutive waveforms of good quality were obtained (Ng and Ho, 2002). As there were no differences in uterine PI and RI between the left and the right sides, the averaged uterine PI and RI were given.

The ultrasound machine was switched to the 3D mode with power Doppler. The setting condition for this study was as follows: frequency mid; dynamic set 2; balance G >140; smooth 5/5; ensemble 12; line density 7; power Doppler map 5. The setting condition for the sub-power Doppler mode was as follows: gain 6.0; balance 140; quality normal; wall motion filter low 1; velocity range 0.9 kHz. The resulting truncated sector covering the endometrial cavity in a longitudinal plane of the uterus was adjusted and moved and the sweep angle was set to 90° to ensure that a complete uterine volume encompassing the entire subendometrium was obtained. The patient and the 3D transvaginal probe remained as still as possible during the volume acquisition. A 3D dataset was then acquired using the medium speed sweep mode. The resulting multi-planar display was examined to ensure that the area of interest was captured in its entirety. If the volume measurement was completed without a power Doppler artefact, the dataset was stored for later analysis by E.H.Y.N.

The built-in VOCAL® (Virtual Organ Computer-Aided Analysis) Imaging Program for the 3D power Doppler histogram analysis was used in the analysis, along with computer algorithms, to measure the endometrial volume and indices of blood flow within the endometrium. Vascularization index (VI), which measures the ratio of the number of colour voxels to the total number of voxels, is thought to represent the presence of blood vessels (vascularity) in the endometrium and is expressed as 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 (Pairleitner et al., 1999). During the analysis and calculation, the manual mode of the VOCAL Contour Editor was used to cover the whole 3D volume of the endometrium with a 15° rotation step. Hence, 12 contour planes were analysed for the endometrium of each patient to cover 180°. Following the assessment of the endometrium itself, the subendometrium was examined through the application of ‘shell-imaging’, which allows the user to generate a variable contour that parallels the originally defined surface contour. In the present study, the subendometrial region was considered to be within 1 mm of the originally defined myometrial–endometrial contour (Ng et al., 2004). VI, FI and VFI of the subendometrial region were obtained accordingly.

Patients were advised to have two embryos replaced into the uterine cavity 48 h after the retrieval but replacing three embryos was allowed. Excess good quality embryos were frozen. All fresh embryos were cryopreserved if serum E2 on the day of hCG injection was >20000 pmol/l in order to reduce the risks of ovarian hyperstimulation syndrome (OHSS). Luteal phase was supported by two doses of HCG or vaginal progesterone (Cyclogest; Cox Pharmaceuticals, Barnstaple, UK). A urine pregnancy test was done 16 days after embryo transfer. If it was positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present. Only clinical pregnancies defined by the presence of one or more gestational sacs or the histological confirmation of gestational product in miscarriages were considered. Ongoing pregnancies were those pregnancies beyond 10–12 weeks of gestation, at which stage the patients were referred for antenatal care. Implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac.

The intra-observer reliability was expressed as the mean intra-class correlation coefficient (ICC) with 95% confidence interval (CI). The mean ICC (95% CI) was 0.970 (0.920, 0.989) for endometrial thickness, 0.973 (0.912, 0.992) for PI and 0.951 (0.838, 0.985) for RI. The
mean ICC for 3D scanning of endometrial volume, VI, FI and VFI were 0.9509 (0.8859, 0.9838), 0.9896 (0.9689, 0.9966), 0.8957 (0.7157, 0.9649) and 0.9916 (0.9750, 0.9973) respectively. The mean ICC for data acquisition of endometrial volume, VI, FI and VFI were 0.9923 (0.9746, 0.9917), 0.9827 (0.9437, 0.9949), 0.9884 (0.9619, 0.9966) and 0.9852 (0.9517, 0.9957) respectively. Serum FSH and E2 concentrations were measured using commercially available kits (Automated Chemiluminescence System; Bay Corporation, NY, USA). The inter-assay and intra-assay coefficients of variation (CV) for serum FSH concentration were 2.8 and 1.7% respectively. The intra- and inter-assay CV for serum E2 concentration were 8.1 and 8.7% respectively.

**Statistical analysis**

The primary outcome measure was a clinical pregnancy. Continuous variables were not normally distributed and were given as median (interquartile range), unless indicated. Statistical comparison was carried out by Mann–Whitney, χ² and Fisher’s exact tests, where appropriate. Multiple logistic regression analysis and the receiver operator characteristic (ROC) curve analysis were applied to determine the best predictive variables (Altman and Bland, 1994). Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS Inc., Version 12.0, Chicago, USA). The two-tailed value of P < 0.05 was considered statistically significant.

**Results**

During the study period, a total of 542 patients received ovarian stimulation for their first IVF cycle but only 525 patients proceeded to TUGOR because of cycle cancellation in 17 (3.1%, 17/542) patients who had poor ovarian response. Three patients declined to participate because of personal reasons and another three patients were excluded after scanning because of distortion of uterine cavity by uterine fibroids. No oocyte was obtained in one cycle, failed fertilization was encountered in another 20 cycles and in another 18 cycles embryos failed to cleave. Embryo transfer was postponed in 29 cycles because of the risk of OHSS. Therefore, embryo transfer was performed in 451 cycles and 94 clinical pregnancies resulted. The pregnancy rate was 20.8% per transfer. Uterine fibroids were encountered in 120 transfer cycles (26.6%, 120/451).

**All transfer cycles**

Table I summarizes the demographic data and ovarian responses of non-pregnant and pregnant patients. There were no significant differences in age of women, primary/secondary infertility, duration of infertility, cause of infertility, presence of fibroids, the insemination method, body mass index (BMI), basal serum FSH concentration, HMG dosage and duration, serum E2 concentration, number of oocytes obtained and number of embryos replaced between the non-pregnant group and the pregnant group. Patients in the pregnant group had significantly lower uterine RI, endometrial VI and VFI than those in the non-pregnant group. Endometrial thickness, endometrial volume, endometrial pattern, uterine PI, endometrial FI and subendometrial VI, FI and VFI were similar between the non-pregnant and pregnant groups (Table II).

Endometrial blood flow was absent in 31 patients and subendometrial blood flow was absent in 23 patients. Twenty patients had no endometrial and subendometrial blood flows. Implantation and pregnancy rates were higher in patients without endometrial and subendometrial blood flows than those with endometrial and subendometrial blood flows, although the difference did not reach statistical significance (Table III).

When age of women, type of infertility, duration of infertility, BMI, number of oocytes obtained, serum E2 concentration, number of embryos replaced, uterine PI, uterine RI, endometrial thickness, endometrial pattern, endometrial volume, 3D

| Table I. Comparison of demographic data and ovarian responses between non-pregnant and pregnant patients |
|-----------------|-----------------|
|                  | Non-pregnant (n = 357) | Pregnant (n = 94) |
| Age of women (years) | 35.0 (32.0–37.0) | 35.0 (33.0–37.0) |
| Primary infertility [n (%)] | 244 (68.3) | 68 (72.3) |
| Infertility duration (years) | 4.0 (3.0–6.0) | 4.0 (3.0–6.0) |
| Causes of infertility [n (%)] |                  |                  |
| Tubal            | 78 (21.8)      | 13 (13.8)       |
| Male             | 185 (51.8)     | 49 (52.1)       |
| Endometriosis    | 40 (11.2)      | 15 (6.0)        |
| Unexplained      | 32 (9.0)       | 11 (11.7)       |
| Mixed            | 22 (6.2)       | 6 (6.4)         |
| Insemination method [n (%)] |            |                  |
| Conventional     | 245 (68.6)     | 70 (74.5)       |
| ICSI             | 112 (31.4)     | 24 (25.5)       |
| Body mass index (kg/m²) | 21.3 (19.9–22.8) | 21.6 (20.0–22.9) |
| Basal FSH concentration (IU/l) | 6.5 (5.3–7.6) | 6.3 (5.0–7.2) |
| HMG dosage (IU)  | 1950 (1650–2250) | 1950 (1650–2138) |
| HMG duration (days) | 11.0 (9.0–12.0) | 11.0 (9.8–12.0) |
| Serum estradiol (pmol/l) | 9737 (6099–14365) | 9032 (6995–13297) |
| No. of oocytes obtained | 8.0 (5.0–13.0) | 9.0 (6.0–13.0) |
| No. of embryos transferred [n (%)] |                  |                  |
| 1                | 38 (10.6)      | 4 (4.3)         |
| 2                | 307 (86.0)     | 83 (88.3)       |
| 3                | 12 (3.4)       | 7 (7.4)         |

Data are given as median (interquartile range) unless otherwise specified. No differences between groups were statistically significant.
power Doppler flow indices of endometrial and subendometrial regions were entered in a conditional forward fashion in multiple logistic regression analysis, only the number of embryos replaced and endometrial VI significantly improved the chance of pregnancy with odds ratios of 2.39 [95% confidence interval (CI): 1.17–4.85, \( P = 0.016 \)] and 0.87 [95% CI: 0.76–0.99, \( P = 0.037 \)] respectively. Other parameters were not predictive of pregnancy.

The ROC curve analysis showed that only endometrial thickness and subendometrial FI had the area under the curve slightly >0.5 (Table IV).

### Transfer cycles with good prognosis
A total of 166 patients were included in the good prognosis group defined as patients aged ≤35 years with endometrial...
thickness $>8$ mm, transfer of two or more good quality embryos and the availability of frozen embryo(s). There were no significant differences between the non-pregnant and pregnant groups in demographic data, ovarian responses and ultrasound parameters for endometrial receptivity including 3D power Doppler flow indices of endometrial and subendometrial regions (data not shown).

In the multiple logistic regression analysis, none of the above parameters was predictive of pregnancy. The ROC curve analysis showed that the area under the curve was also $<0.5$ for the ultrasound parameters of endometrial receptivity (Table IV).

**Discussion**

This study evaluated the role of the endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound in the prediction of pregnancy during IVF treatment. In order to avoid confounding variables, our patients were in their first IVF cycle and received a standard long protocol of pituitary down-regulation. Except those with distortion of uterine cavity, patients with uterine fibroids were recruited because we could not find an adverse effect of intramural fibroids on the pregnancy rate following IVF (Ng and Ho, 2002). Moreover, endometrial and subendometrial 3D power Doppler flow indices were similar in patients with and without small intramural fibroids (Ng et al., 2005). All patients in the present study were scanned early in the morning after an overnight fasting although there was lack of control for exogenous substances such as caffeine or antihistamine, which might have had an impact on blood flow measurements.

The role of the endometrial and subendometrial blood flows was examined in all transfer cycles and those with good prognosis. In all transfer cycles, the pregnant group had significantly lower uterine RI, endometrial VI and VFI than the non-pregnant group whereas the number of embryos replaced and endometrial VI were the only two predictive factors for pregnancy. In the good prognosis group, none of the parameters were predictive of pregnancy. ROC curve analysis revealed that the area under the curve was $<0.5$ for all ultrasound parameters for endometrial receptivity in all transfer cycles and good prognosis cycles. The endometrial volume measured by 3D ultrasound has not been shown to be predictive of pregnancy (Raga et al., 1999; Yaman et al., 2000; Schild et al., 2001). Doppler flow measurements of spiral vessels were not performed in this study because of inconsistent waveforms obtained from colour Doppler signals of these tiny spiral vessels. Doppler flow indices of spiral arteries are not predictive of pregnancy (Zaidi et al., 1995; Yuval et al., 1999; Schild et al., 2001), although Battaglia et al. (1997) and Kupesic et al. (2001) found significantly lower spiral artery PI in pregnant cycles than non-pregnant ones.

Blood flow in the endometrium and the subendometrial region can be objectively measured by the use of 3D ultrasound with power Doppler (Schild et al., 2000; Kupesic et al., 2001; Wu et al., 2003; Ng et al., 2004, 2005; Raine-Fenning et al., 2004a). In 75 IVF cycles, Schild et al. (2000) showed that all 3D subendometrial power Doppler flow indices performed on the first day of ovarian stimulation 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 outcome among the tested sonographic parameters. Kupesic et al. (2001) found in 89 patients that subendometrial FI on the day of embryo transfer was significantly higher in pregnant cycles whereas subendometrial VI and VFI were similar between pregnant and non-pregnant patients. Wu et al. (2003) also demonstrated in 54 patients that subendometrial VFI on the day of hCG was significantly higher in the pregnant group and was superior to endometrial volume, subendometrial VI and VFI in predicting the outcome. Areas under the ROC curve for VFI, VI, FI and endometrial volume were 0.8912, 0.6011, 0.6373 and 0.6674 respectively. The best predictive rate was achieved by a subendometrial VFI threshold value of $>0.24$.

This is the largest study in the literature involving 451 transfer cycles, whereas a much smaller number of subjects was studied by Kupesic et al. (2001) and Wu et al. (2003). Our results were clearly contradictory to those of Kupesic et al. (2001) and Wu et al. (2003). These studies were different in patients’ characteristics, the day of ultrasound examination and the selection of the subendometrial region. In the present study, all patients were in the first IVF cycle and had two to three embryos replaced at the early cleavage stage 2 days after TUGOR following a standard protocol of ovarian stimulation. Kupesic et al. (2001) recruited patients undergoing repeated IVF attempts following a long protocol of pituitary down-regulation, who had serum basal FSH concentration $<10$ IU/l, no uterine fibroids, ovarian cysts or ovarian endometriomas. One or two good quality blastocysts were replaced 5 days after TUGOR. Wu et al. (2003) examined patients in their first IVF cycle who were aged $<38$ years with basal FSH concentration $<15$ IU/l and had two or more good quality embryos transferred. The details of ovarian stimulation and day of embryo transfer were not described in the study.

Ultrasound examination was performed on the day of hCG (Wu et al., 2003), TUGOR (the present study) and embryo transfer (Kupesic et al., 2001). In the studies of Kupesic et al. (2001) and Wu et al. (2003), 3D power Doppler indices of the endometrial region were not given and the subendometrial region included 5 mm of the myometrial–endometrial interface. We reported endometrial and subendometrial blood flow separately and the subendometrial region was defined as a shell within 1 mm of the myometrial–endometrial interface. We chose 1 mm as the subendometrial region because the subendometrial region may extend beyond the uterine contour especially in the corneal region if 5 mm was taken. The fibroids, if present, would not also be included when 1 mm was used. Moreover, only the myometrium immediately underlying the endometrium exhibits a cyclic pattern of steroid receptor expression like that of the endometrium (Noe et al., 1999). There is no standard definition of subendometrial region in the literature, ranging from 5 mm (Schild et al., 2001; Wu et al., 2003) to 10 mm (Chien et al., 2002). Zaidi et al. (1995), Battaglia et al. (1997) and Maugé-Laulom et al. (2002) did not specify the subendometrial region. We are still uncertain whether these differences in patients’ characteristics, the day of ultrasound examination and the selection of the subendometrial region could explain the contradictory findings of these studies.
Our findings also appeared to be in great contrast to the general belief that a good blood supply towards the endometrium is essential for successful implantation. Indeed, they are in keeping with studies that specifically examined perfusion within the human endometrium during the menstrual cycle. Fraser et al. (1987) determined endometrial blood flow through the menstrual cycle in non-pregnant women with the use of the clearance of radiolabelled $^{133}$Xe 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 al. (2004a) showed that endometrial and subendometrial blood flows by 3D ultrasound increased during the proliferative phase, peaking around 3 days prior to ovulation before decreasing to a nadir 5 days post-ovulation. Therefore, there is a period of relatively reduced perfusion in the immediate post-ovulatory period, extending to the implantation period in spontaneous cycles.

It is proposed that the degree of change in endometrial perfusion from the late follicular phase through to the early luteal phase is a more important determinant of endometrial receptivity (Raine-Fenning et al., 2004b). It would not be too surprising if there were a non-significant trend of higher implantation and pregnancy rates in our patients with absent endometrial or subendometrial blood flow. On the other hand, absent endometrial or subendometrial flow detected by colour or power Doppler in two-dimensional ultrasound is associated with no pregnancy (Zaidi et al., 1995; Battaglia et al., 1997) or much reduced pregnancy rate (Chien et al., 2002; Maugue-Laulom et al., 2002). Hypoxia in the endometrium may play a beneficial role for implantation, as the expression of vascular endothelial growth factor is up-regulated by hypoxia (Sharkey et al., 2000) and relatively low oxygen tension was present around the blastocyst during the time of implantation (Graham et al., 2000). There is still no consensus when the ultrasound examination for assessing endometrial receptivity in IVF treatment should be done. It may be much better to measure endometrial and subendometrial blood flow during the follicular phase and early luteal phase to determine the changes in order to delineate the role of endometrial and subendometrial blood flows.

In conclusion, endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound were not good predictors of pregnancy if they were measured at one time-point during IVF treatment. Further longitudinal studies in the late follicular phase and early luteal phase should be performed.

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