Factors affecting endometrial and subendometrial blood flow measured by three-dimensional power Doppler ultrasound during IVF treatment

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BACKGROUND: No information exists in the literature regarding the factors affecting the blood flow towards the endometrial and subendometrial regions during IVF treatment. METHODS: We examined the effect of women’s age, their smoking habits, their type of infertility (i.e. primary or secondary) and parity, causes of infertility and serum estradiol (E2) concentration on endometrial and subendometrial blood flows as measured by a three-dimensional (3D) power Doppler ultrasound during IVF treatment. All patients received a standard long protocol of ovarian stimulation and serum E2 concentration was determined on the day of hCG. 3D ultrasound examination with power Doppler was performed on the day of oocyte collection to determine vascularization index (VI), flow index (FI) and vascularization flow index (VFI) of endometrial and subendometrial regions. RESULTS: The age of women, their smoking habits, their types of infertility and parity and causes of infertility had no effect on the endometrial and subendometrial 3D power Doppler flow indices. There was a negative correlation between serum E2 concentration and endometrial FI (r = –0.109; P = 0.006). CONCLUSIONS: Endometrial blood flow in IVF treatment was negatively affected by serum E2 concentration only.

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

Introduction

IVF/embryo transfer (IVF/ET) treatment involves development of multiple follicles, oocyte retrieval and ET after fertilization. Successful implantation depends on a close interaction between the blastocyst and the receptive endometrium. Endometrial receptivity can be evaluated by histological examination of an endometrial biopsy (Noyes et al., 1950), endometrial proteins in uterine flushing (Li et al., 1998) or more commonly noninvasive ultrasound examination of the endometrium (Turnbull et al., 1995; Friedler et al., 1996). Different ultrasound parameters have been used to assess endometrial receptivity during IVF treatment, including endometrial thickness, endometrial pattern, endometrial volume, Doppler study of uterine arteries and endometrial blood flow.

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). Endometrial and subendometrial blood flow varies in different phases of the menstrual cycle (Gannon et al., 1997; Fraser et al., 1987; Raine-Fenning et al., 2004a). Fraser et al. (1987) determined endometrial blood flow with the use of the clearance of radiolabelled xenon-133 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. Gannon et al. (1997) used an intrauterine laser Doppler technique to measure endometrial microvascular blood flow, which significantly increased during early follicular and early luteal phases.

Uterine blood flow is assumed in many studies to reflect the blood flow towards the endometrium. It is assessed by colour Doppler ultrasound and is usually expressed as downstream impedance to flow because measurement of blood flow volume is difficult and inaccurate, depending on the angle of insonation, accurate measurement of vessel’s diameter and tortuosity of the vessels (Dickey, 1997). The 3D ultrasound with 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; Ng et al., 2004a,b, 2005; Raine-Fenning et al., 2004a,b). Raine-Fenning et al. (2004a) performed 3D ultrasound throughout a spontaneous cycle of 27 normal women.
Endometrial and subendometrial blood flow increased during the proliferative phase, peaking around 3 days prior to ovulation before decreasing to a nadir 5 days post-ovulation. Moreover, the subendometrial flow index (FI) was significantly lower in women ≥31 years and significantly higher in parous women. Smoking was associated with a significantly lower subendometrial vascularization index (VI) and vascularization flow index (VFI).

No information exists in the literature regarding the factors affecting the blood flow towards the endometrial and subendometrial regions during IVF treatment. This prospective study aimed to examine the effect of age of women, their smoking habits, their types of infertility and parity, causes of infertility and serum estradiol (E₂) concentration on endometrial and subendometrial blood flows as measured by a 3D power Doppler ultrasound during IVF treatment.

Materials and methods
Infertile patients attending the Assisted Reproduction Unit of the Department of Obstetrics and Gynecology, The University of Hong Kong between November 2002 and December 2004 for IVF treatment were recruited if they had normal uterine cavity detected on the day of transvaginal ultrasound-guided oocyte retrieval (TUGOR). Every patient gave a written informed consent prior to participating in the study, which was approved by the Ethics Committee, Faculty of Medicine, The University of Hong Kong. They were evaluated only once during the study period and did not receive any monetary compensation for participation in this study.

During the study period, a total of 662 patients received ovarian stimulation for IVF treatment, but merely 645 patients proceeded to TUGOR because of cycle cancellation among 17 patients who had poor ovarian response. Three patients declined to participate because of personal reasons, and another four were excluded after scanning because of the distortion of uterine cavity by uterine fibroids (n = 3) and congenital uterine abnormality (n = 1). Therefore, 638 patients out of the original 662 were included in the analysis, and the breakdown of patients according to indications for IVF was as follows: 134 for tubal factors, 82 for endometriosis, 322 for male infertility, 61 for unexplained causes and 39 for mixed causes. Five hundred and twenty-five patients had been presented in our previous studies (Ng et al., 2004a,b, 2005, in press).

The details of the long protocol of ovarian stimulation regimen were as previously described (Ng et al., 2000). In short, they were pre-treated with Buserelin (Suprerc, Hoechst, Frankfurt, Germany) nasal spray 150 μg four times a day from the midluteal phase of the cycle preceding the treatment cycle and received human menopausal gonadotrophin (HMG; Pergonal, Serono, Geneva, Switzerland) for ovarian stimulation. Human chorionic gonadotrophin (hCG; Profasi, Serono, Geneva, Switzerland) was given intramuscularly when the leading follicle reached 18 mm in diameter, and there were at least three follicles ≥16 mm in diameter. Serum E₂ concentration was measured on the day of hCG administration using commercially available kits (Automated Chemiluminescence System; Bay Corporation, New York, NY, USA). The intra- and inter-assay coefficients of variation were 8.1% and 8.7%, respectively.

Transvaginal ultrasound-guided oocyte retrieval (TUGOR) was scheduled 36 h after the hCG injection. 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 8–10 a.m. after they had emptied the bladder. The details of 3D ultrasound and data analysis with the intraobserver reliability were as previously described (Ng et al., 2004a). The setting conditions for this study were as follows: frequency, mid; dynamic set, 2; balance, G > 140; smooth, 5/5; ensemble, 12; line density, 7; power Doppler map, 5. Meanwhile, the setting conditions for the subpower Doppler mode were as follows: gain, 6.0; balance, 140; quality, normal; wall motion filter, low 1; velocity range, 0.9 kHz. The resultant truncated sector covering the endometrial cavity in a longitudinal plane of the uterus was adjusted and moved and the sweep angle 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 resultant multiplanar display was examined to ensure that the area of interest had been captured in its entirety. If the volume measurement was complete without 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 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 this is expressed as a percentage (%) of the endometrial volume. Flow index, 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 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 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., 2004a). VI, FI and VFI of the subendometrial region were obtained accordingly (Figure 1).

Statistical analysis
The primary outcome measures were the VI, FI and VFI of endometrial and subendometrial regions. Continuous variables were not normally distributed and were given as median (interquartile range), unless indicated. Statistical tests were carried out using Mann–Whitney U-test and Kruskal–Wallis test, whenever these were appropriate. The correlation was assessed by using the Spearman rank method. Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS Inc., Version 12.0, Chicago, IL, USA). The two-tailed value of P < 0.05 was considered statistically significant.

Results
Table I summarizes the demographic data, ovarian response, treatment outcomes, endometrial and subendometrial 3D power Doppler flow indices.

Age of women
Endometrial and subendometrial 3D power Doppler flow indices were not correlated with age of women. Patients were classified into four age groups: ≤30 years (n = 32); 31–35 years (n = 274); 36–40 years (n = 267) and ≥41 years (n = 15). VI, FI and VFI of endometrial and subendometrial regions were similar among these four age groups (Figure 2).
Only 23 (3.6%) patients were smokers and were significantly younger than nonsmokers (32.0 versus 35.0 years, respectively; \(P = 0.002\), Mann–Whitney U-test). No significant differences in VI, FI and VFI of endometrial and subendometrial regions were shown between smokers and nonsmokers (data not shown).

**Type of infertility and parity**

Primary and secondary infertility were present in 438 (68.7%) and 200 (31.3%) patients, respectively. No significant differences in VI, FI and VFI of endometrial and subendometrial regions were shown between primary and secondary infertility (data not shown). Only 23 (3.6%) patients were parous women, and their endometrial and subendometrial VI, FI and VFI were comparable to those of nulliparous women (data not shown).

**Causes of infertility**

There were no significant differences in VI, FI and VFI of endometrial and subendometrial regions among various causes of infertility (Figure 3).
Serum $E_2$ concentration
Only endometrial FI was negatively correlated with serum $E_2$ concentration ($r = -0.109; P = 0.006$) (Figure 4).

Uterine fibroids were encountered in 163 cycles (25.5%; 163/638). A subgroup analysis of patients without uterine fibroids revealed the same findings as described above.
Figure 3. Boxplot of endometrial and subendometrial 3D power Doppler flow indices in different causes of infertility.
Endometrial and subendometrial blood flow during IVF

Discussion

This is the first study examining the effect of age of women, their smoking habits, their types of infertility and parity, causes of infertility and serum E₂ concentration on endometrial and subendometrial blood flows as measured by 3D power Doppler ultrasound during IVF treatment. All our patients received a standard long protocol of ovarian stimulation and were scanned early in the morning after an overnight fast prior to TUGOR. Those with uterine fibroids were not excluded because we have recently demonstrated that endometrial and subendometrial 3D power Doppler flow indices in patients with intramural fibroids were similar to controls matched with respect to age, type of infertility and serum E₂ concentrations (Ng et al., 2005). We chose 1 mm as the subendometrial region because the subendometrial region may extend beyond the uterine contour especially in the cornual region if 5 mm was taken. The fibroids, if present, would not be included as well when 1 mm was used. Moreover, only the myometrium immediately underlying the endometrium exhibits a cyclic pattern of steroid receptors expression as that of the endometrium (Noe et al., 1999). Our results showed that the age of women, their smoking habits, their types of infertility and parity and causes of infertility had no effect on endometrial and subendometrial 3D power Doppler flow indices. Only endometrial FI was negatively correlated with serum E₂ concentration.

Kurjak and Kupesic (1995) demonstrated changes in the flow velocity patterns of the ovarian, uterine, radial and spiral arteries with age of women. They suggested that the ageing process initially affected the uterus less than the ovary because the uterine resistance index (RI) did not change significantly in the first post-menopausal years. Uterine Doppler flow indices were significantly reduced in patients undergoing ovarian stimulation than in those evaluated in natural cycles, and the percentage reduction was correlated with the age of women, serum E₂ concentration and number of oocytes (Cacciatore and Tiitinen, 1996).

Although smoking is associated with endothelial dysfunction (Neunteufl et al., 2000) and an increase in arterial wall stiffness (Caro et al., 1987), there is conflicting evidence regarding its effect on uterine blood flow measured by colour Doppler ultrasound. Nordenvall et al. (1991) and Albuquerque et al. (2004) reported an increase in resistance, while Castro et al. (1993) noted a decrease. Others (Newnham et al., 1990; Bruner and Forouzan, 1991; Kimya et al., 1998) found no effect at all. We could not find any effect of smoking on endometrial and subendometrial blood flows in IVF treatment, but there may be too few smokers in the present study to draw a firm conclusion. Other factors such as the duration of smoking and the number of cigarettes per day may also have impact on the endometrial and subendometrial blood flows.

In view of the small number of parous patients, we also examined the type of infertility, which had no effect on endometrial and subendometrial blood flow during IVF treatment. Unexplained infertility appears to be associated with impairment of endometrial perfusion. Women with unexplained infertility demonstrated significantly reduced uterine artery flow velocities in all phases of a spontaneous menstrual cycle and significantly elevated uterine and subendometrial artery impedance in the periovulatory and midluteal phases.
when compared with those with tubal infertility (Edi-Osagie et al., 2004). Similarly, Raine-Fenning et al. (2004b) found that endometrial and subendometrial vascularity of women with unexplained infertility were significantly lower during the mid-late follicular phase of a natural cycle than that of normal fertile women. On the other hand, Isaksson et al. (2003) could not observe any differences in the uterine impedance during the spontaneous or the IVF cycle between unexplained and tubal infertility. We could not observe any significant differences in endometrial and subendometrial blood flow among different causes of infertility.

The absence of the effect on endometrial and subendometrial blood flow by the demographic factors may be explained by ovarian stimulation in IVF treatment. Check et al. (2000) demonstrated that women had similar uterine pulsatility index (PI) and resistance index (RI) measured at baseline, on the day of TUGOR and at the midluteal phase, irrespective of their age. We previously demonstrated that uterine PI and RI in tubal infertility were significantly lower than that in male infertility during natural cycles, but such difference was not found during IVF cycles (Basir et al., 2001). We did not measure serum E2 concentration on the day of TUGOR and therefore could not correlate serum E2 concentration with endometrial and subendometrial blood flows on that day, although there was a strong correlation between serum E2 concentrations on the day of hCG and the day of TUGOR (Chan et al., 2005). The weak negative correlation between serum E2 concentration on the day of hCG and endometrial FI on the day of TUGOR shown in this study appeared to be contradictory to the potent angiogenic and vasodilatation effects of E2 (Losordo and Isner, 2001).

Little information is available regarding the exact mechanisms by which this steroid exerts its function on the process of both physiological and pathological angiogenesis (Kapiteijn et al., 2001). The mechanisms and control of angiogenesis in the endometrium are far from being fully understood (Smith, 2001), and supraphysiological E2 concentration may have opposite effects on endometrial angiogenesis. Endometrial and subendometrial E2 concentration were indeed significantly lower in the stimulated cycle than that in the natural cycle (Ng et al., 2004b). From our laboratory demonstrated that E2 had a biphasic effect on the expression of vascular endothelial growth factor mRNA in endometrial cultures, which was up-regulated by E2 at the concentration of 1–10 nmol/l but down-regulated by E2 at the concentration of 100 nmol/l (Ng, unpublished data).

A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation. Endometrial microvascular blood flow determined by an intravascular 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). However, we have recently shown that endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound were not good predictors of pregnancy (Ng et al., in press), although significantly higher subendometrial blood flows were shown in pregnant IVF cycles (Kupesic et al., 2001; Wu et al., 2003).

In conclusion, we found that during IVF treatment, the age of women, their smoking habits, their types of infertility and parity and causes of infertility had no effect on endometrial and subendometrial 3D power Doppler flow indices measured on the day of oocyte collection. Only endometrial FI was negatively correlated with serum E2 concentration on the day of hCG.

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