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Evaluation of endometrial receptivity during *in-vitro* fertilization using three-dimensional power Doppler ultrasound

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KEYWORDS: subendometrial flow; triple line; vascularization index

ABSTRACT

Objective *To compare sonographic endometrial characteristics in* in-vitro *fertilization (IVF) cycles between women who conceive and those who do not.*

Methods *Thirty-five women undergoing IVF treatment participated in the study. Using three-dimensional (3D) power Doppler ultrasound, we assessed endometrial patterns, volume and vascularization, after follicle stimulating hormone (FSH) stimulation but before human chorionic gonadotropin (hCG) administration (referred to hereafter as 'after FSH stimulation') and again on the day of oocyte retrieval.*

Results *The pregnancy rate was 37% (13/35). After FSH stimulation, 29 of the 35 women had a triple-line endometrial pattern, compared with five out of 35 on the day of oocyte retrieval. In those who had a triple-line pattern after FSH stimulation the pregnancy rate was 44.8% (13/29) and it was 0% (0/6) in those with a homogeneous pattern (chi-square test,* $P = 0.039$*). If a triple-line pattern was present on the day of oocyte retrieval the pregnancy rate was 80.0% (4/5), whereas if the pattern was homogeneous the pregnancy rate was 30.0% (9/30) (*$P = 0.032$*). There were no differences between those who conceived and those who did not in endometrial thickness, volume or vascularization on either day examined. Endometrial volume decreased significantly after hCG injection in women who conceived, but not in those who did not conceive. In both groups endometrial and subendometrial vascularization decreased after hCG injection, while the endometrial thickness remained unchanged.*

Conclusions *The existence of a homogeneous endometrial pattern after FSH stimulation seems to be a prognostic sign of an adverse outcome in IVF, while a triple-line pattern after FSH stimulation and a decrease in endometrial volume appear to be associated with conception.*

INTRODUCTION

Despite the fact that top-quality embryos may be available for transfer during *in-vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles, only a maximum of one third of the embryos transferred finally implant[1]. In addition to embryo quality the receptivity of the endometrium plays a role in the implantation process. In order not to damage the endometrium, endometrial receptivity should be evaluated before embryo transfer using a non-invasive method, such as sonography. Several sonographic parameters have been used to assess receptivity, including endometrial thickness, endometrial pattern and endometrial and subendometrial blood flow[2]. Three-dimensional (3D) ultrasound offers the possibility of evaluating the endometrial volume and relating it to the outcome of IVF cycles[3–5]. The presence of a multilayer or triple-line pattern after follicle stimulating hormone (FSH) stimulation and a minimal endometrial thickness at the time of embryo transfer appear to favor implantation[2].

Gonadotropin stimulation in IVF is known to affect luteal-phase function and there is evidence that premature secretory changes taking place in the postovulatory- and

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early luteal-phase endometrium may affect endometrial receptivity[6]. The aim of our study was to evaluate the endometrium after FSH stimulation and on the day of oocyte retrieval, using 3D power Doppler ultrasound. In particular, we wanted to relate the endometrial changes to the outcome of IVF treatment.

METHODS

We recruited 35 consecutive women undergoing IVF treatment between August 2000 and June 2001. Ethics committee approval and specific consent to participate in the study were obtained. Women with uterine fibroids, known endometriosis or a single ovary and those who had undergone a previous operation on the uterus or salpingectomy were excluded from the study.

A standard long gonadotropin releasing hormone (GnRH) agonist protocol for IVF therapy was used. Each woman had had her circulating FSH and luteinizing hormone (LH) levels assayed once during the previous 6 months. Pituitary suppression was achieved using GnRH agonist treatment (daily subcutaneous injections of 0.5 mg Buserelin acetate), which was started on cycle day 21 in cases in which no pathological findings in the uterus or ovaries were observed. If any abnormality was detected, the IVF treatment was cancelled and the patient was excluded from the study. Two weeks later the women underwent sonography and if the endometrium was thinner than 4 mm and if no follicular activity was detected, stimulation of the ovaries using FSH was initiated. Otherwise, the women continued using the GnRH agonist and the ultrasound examination was repeated 1 week later. The standard starting dose of FSH was 150–375 IU depending on the subject's age, previous response and early follicular-phase serum concentration of FSH. The dose of FSH was adjusted during stimulation according to the follicular response as assessed by ultrasound. When two or three leading follicles were at least 18 mm in diameter, a human chorionic gonadotropin (hCG) injection of 10 000 IU was given. If the total number of follicles after FSH stimulation exceeded 15, an hCG injection of 5000 IU was given. Oocyte retrieval was performed 36 h after hCG injection. A maximum of three embryos were transferred 2 days after oocyte retrieval. The cumulative embryo score was calculated according to Steer *et al.*[7]. Clinical pregnancy was diagnosed after a positive pregnancy test and an intrauterine gestational sac seen at ultrasound examination 3 weeks after embryo transfer.

The 3D sonographic measurements were performed after FSH stimulation but before hCG administration (hereafter referred to as 'after FSH stimulation') and on the day of oocyte retrieval. All the examinations were performed using Kretztechnik Voluson 530D equipment (Kretztechnik-Medison, Zipf, Austria). Identical preinstalled instrument settings (color gain, 45.6; pulse repetition frequency, 0.5; color-power (C-PWR), 2; wall motion filter, 72; frame rate, 4–6) were applied for all patients. The machine was equipped with a transvaginal 3–7-MHz volume transducer with a 100° field of view. First the uterus was visualized in two-dimensional (2D) B-mode. The power Doppler mode was switched on and the power Doppler box was positioned to cover the whole uterus. The 3D facility was engaged by switching to 'volume mode'. The volume sector angle was preset to 90° and the fast volume acquisition (low resolution) setting was selected to avoid artifacts. The duration of volume acquisition was between 10 and 20 s, depending on the dimensions of the volume. Movement of the vaginal probe was avoided during volume acquisition. Acquired 3D volumes were stored on the hard disk and later transferred to a personal computer using a DICOM (digital imaging and communications in medicine) connection. The data were not compressed at any time.

Analysis of stored volumes was performed using the VOCAL™ imaging program (virtual organ computer-aided analysis) version 4.0, which is integral to the Kretztechnik Voluson 530D sonography system. First, endometrial thickness including both endometrial layers was measured where the endometrium appeared to be at its thickest on a longitudinal scan through the uterus. The endometrial pattern was assessed on the longitudinal section and described as triple-line or homogeneous. The volume of the endometrium was determined by manually drawing the endometrial outlines. The longitudinal view was used as a reference image and the rotation step was selected at 15°, resulting in the definition of 12 contours for each endometrium. Once a contour was defined in all image planes, the volume of the endometrium was calculated automatically using the VOCAL program. The subendometrial volume was likewise calculated after we had defined the subendometrial region as the region 10 mm beneath the myometrial–endometrial junction. The VOCAL program then calculated the vascularization index (VI), which measures the ratio of color voxels to all the voxels in the region of interest. It represents the density of vessels in the tissue and is expressed as a percentage.

The reproducibility of volume and color index measurements using transvaginal 3D power Doppler ultrasound has been determined[8]. The intraobserver correlation coefficient for volume measurements is 1.0 and for VI it is 0.89[8].

Statistical analysis was performed using the Statistical Package for the Social Sciences (Release 11.5, SPSS Inc., Chicago, IL, USA). Departure from a normal distribution was assessed using the Kolmogorov–Smirnov test. Paired *t*-tests were used for normally distributed data and the Mann–Whitney *U*-test and Wilcoxon's test (the signed-rank test) for skewed data. Correlation was estimated using Pearson's correlation coefficient. Proportions were compared using the chi-square test. A *P*-value (two-tailed) of < 0.05 was considered significant.

RESULTS

The pregnancy rate was 37% (13/35). For further analysis the women were divided into two groups according to whether or not they had conceived. The two groups did not differ in age, number of previous deliveries or

Table 1 Characteristics of the study groups

Characteristic	*Conception (n = 13)*	*No conception (n = 22)*
Age (years, mean (SD))	33.5 (4.5)	35.4 (4.2)
Previous deliveries (*n* (%))	4/13 (30.8)	5/22 (22.7)
Infertility (years, mean (SD))	5.2 (3.6)	3.4 (2.2)
Previous *in-vitro* fertilization cycles (*n*, mean (SD))	0.4 (0.7)	1.0 (1.6)
Total dose of follicle stimulating hormone (IU, mean (SD))	2226 (645)	2570 (902)
Length of stimulation (days, mean (SD))	14 (4)	12 (4)
Eggs retrieved (*n*, mean (SD))	13 (6)	14 (9)
Embryos transferred (*n*, mean (SD))	2.3 (0.7)	2.2 (0.9)
Total embryo score (mean (SD))	37 (16)	35 (18)

No statistically significant differences were observed between conception and non-conception groups.

Table 2 Sonographic parameters after follicle stimulating hormone (FSH) stimulation and on the day of oocyte retrieval

	After FSH (mean (SD))	*Day of oocyte retrieval (mean (SD))*	P
Conception cycles ($n = 13$)			
Endometrium			
Thickness (mm)	12.5 (3.2)	12.5 (3.5)	NS
Volume (mL)	7.1 (3.5)	6.1 (2.7)	0.033
VI	1.0 (1.3)	0.3 (0.4)	0.016
Subendometrium VI	5.9 (6.1)	2.0 (1.7)	0.016
Non-conception cycles ($n = 22$)			
Endometrium			
Thickness (mm)	11.5 (2.5)	12.8 (4.2)	NS
Volume (mL)	5.5 (2.9)	5.7 (3.5)	NS
VI	1.1 (1.6)	0.5 (0.8)	0.013
Subendometrium VI	6.7 (6.3)	2.9 (2.5)	0.006

No statistically significant differences were observed between conception and non-conception cycles. NS, not significant; VI, vascularization index.

number of previous IVF treatment cycles, and there was no difference in the total dose of FSH required, number of days of stimulation, number of oocytes retrieved, number of embryos transferred or cumulative embryo score in the transferred embryos (Table 1). The ultrasound examination after FSH stimulation was performed on the same day that hCG was injected in 18 out of 35 (51.4%) patients and one day before the hCG injection in 12 out of 35 (34.3%). The remaining five patients (14.3%) underwent ultrasound examination 2 days before the hCG injection, and therefore they had one more FSH injection on the day following the scan. The mean period in days between the 'after FSH stimulation' scan and hCG injection was 0.63 (SD, 0.73; range, 0–2) days and there was no difference in this period between those who conceived and those who did not. The ultrasound examination after hCG injection was performed in all patients just before ultrasound-guided oocyte retrieval, approximately 36 h after the hCG injection.

In Table 2 we present the results concerning endometrial thickness, volume and VI after FSH stimulation and on the day of oocyte retrieval. There were no differences between the women who conceived and those who did not at either examination. Endometrial volume decreased significantly after hCG injection in those women who conceived. This was not the case in those who did not conceive. In both groups endometrial and subendometrial VI decreased after the hCG injection.

After FSH stimulation 29 of the 35 women had a triple-line endometrial pattern, whereas this frequency was five out of 35 on the day of oocyte retrieval. In women with a triple-line pattern after FSH stimulation the pregnancy rate was 44.8% (13/29) vs. 0% (0/6) in those with a homogeneous pattern (chi-square test, $P = 0.039$). After FSH stimulation the endometrial VI was higher in women with a triple-line pattern (1.2 ± 1.6) than it was in those with a homogeneous pattern (0.2 ± 0.2, $P = 0.004$), with no other differences between those with a triple-layer and those with a homogeneous pattern. Women with a triple-line pattern on the day of oocyte retrieval conceived in 80.0% of cases (4/5), while if the pattern was homogeneous the pregnancy rate was 30.0% (9/30) (chi-square test, $P = 0.032$). After hCG injection there was no difference in VI or endometrial volume between women with an endometrial triple-layer pattern and those with a homogeneous pattern.

Women with secondary infertility had higher ($P < 0.05$) subendometrial VI values after FSH stimulation (8.3 ± 7.5) and on the day of oocyte retrieval (3.3 ± 2.7) than did those with primary infertility (4.1 ± 3.7 and 1.7 ± 1.4, respectively), but there was no difference in the pregnancy rate between women with primary and those with secondary infertility.

No correlation was observed between the number of oocytes retrieved and endometrial volume or VI.

DISCUSSION

The presence of a triple-line pattern in the endometrium after FSH stimulation was strongly associated with the outcome of the IVF cycle. A triple-line pattern at ultrasound examination reflects endometrial proliferation. During the secretory phase the triple-line pattern no longer exists[9]. Most (83%) of our subjects had a triple-line pattern after FSH stimulation. The presence of such a pattern after FSH stimulation has been found to be associated with a higher pregnancy rate than is the absence of this pattern[10–14]; our study confirmed this. The absence of a triple-line pattern may be a sign of premature secretory changes in the endometrium and that the time of maximal endometrial receptivity has passed[6].

Injection of hCG induced disappearance of the triple-line pattern, probably representing normal physiological transformation from the proliferative to the secretory phase. Thirty-six hours after hCG injection only 14% of the women had a triple-line pattern. The presence of a triple-line pattern 36 h after hCG injection, however,

was still associated with a higher pregnancy rate. If absence of a triple-line pattern is associated with secretory transformation in the endometrium, our results suggest that secretory changes may have occurred prematurely in some women, resulting in a non-conception IVF cycle. The presence of a triple-line pattern 36 h after hCG injection may therefore indicate that secretory changes will take place later, which seems to favor implantation and success in IVF.

We observed no difference in endometrial thickness between the conception and non-conception groups. Coulam *et al.*[10] and Serafini *et al.*[11] measured endometrial thickness on the day of hCG injection during IVF cycles. As in our study, they used a GnRH analog for pituitary down-regulation and observed no difference in mean endometrial thickness between conception and non-conception cycles. We also observed that endometrial thickness on the day of oocyte retrieval did not differ between the conception and non-conception groups, confirming the common opinion that measurement of endometrial thickness cannot be used to predict the likelihood of subsequent conception[2].

In our study endometrial volumes did not differ between conception and non-conception cycles, either after FSH stimulation before hCG injection or on the day of oocyte retrieval. Schild *et al.*[4] assessed endometrial volume on the day of oocyte retrieval and Kupesic *et al.*[12] did so on the day of embryo transfer. Both concluded that endometrial volume does not predict conception.

After hCG injection we observed a decrease in mean endometrial volume in the conception cycles, with no change in mean endometrial thickness. In the non-conception cycles no such volume change was observed. It may be that the postovulatory decrease in endometrial volume is part of the secretory changes taking place during the early luteal phase and is required to allow implantation of the embryo. Measurement of endometrial thickness on a 2D image was not sufficiently sensitive to detect this change.

Comparison of endometrial and subendometrial vascularity between the conception and non-conception groups revealed no differences. Kupesic *et al.*[12] investigated subendometrial perfusion on the day of embryo transfer using 3D power Doppler ultrasound. The subendometrial volume they used was a fixed box which could not be adjusted manually to follow the outlines of the myometrial–endometrial border, as in our study. They observed that women who conceived were characterized by a significantly higher flow index in the subendometrial area. The differences between their results and ours may be explained by the difference in the method of assessing blood flow.

In addition to the change in endometrial pattern, we found that hCG induced a decrease in both endometrial and subendometrial vascularization. Gannon *et al.*[13] measured endometrial flow using a laser Doppler technique and observed uterine perfusion to be at its lowest around the time of ovulation. Raine-Fenning *et al.*[14] used 3D power Doppler ultrasound equipment similar to that used here to evaluate endometrial and subendometrial vascularity in natural cycles, noting a decrease in the endometrial vascularity during the periovulatory period similar to our observation. The results they obtained in the subendometrial region also resembled ours, despite the fact that the thickness of the subendometrial layer was only 5 mm, whereas we used a subendometrial thickness of 10 mm. Ng *et al.*[15] used a 1-mm shell to define the subendometrial region and measured endometrial and subendometrial blood flow in IVF cycles to compare the results between moderate and excessive ovarian responders. They took 3D power Doppler measurements on the day of oocyte retrieval and 2 and 5 days thereafter. Using a conventional 2D color Doppler technique they observed a progressive decrease in subendometrial vascularization in both groups without any simultaneous change in uterine artery impedance being measured.

In conclusion, it seems that in the early luteal phase just after ovulation, endometrial and subendometrial blood flow decreases – if we assume that the flow indices reflect blood flow. In natural cycles without implantation flow indices increase again in the mid-luteal phase[14]. This probably also occurs in IVF conception cycles.

According to our results, conception in IVF cycles cannot be predicted by measuring only endometrial volume or vascularization using 3D power Doppler ultrasound. There are, however, certain endometrial characteristics associated with conception in IVF cycles: a triple-line pattern after FSH stimulation and a decrease in endometrial volume and in vascularization after hCG injection. The existence of a homogeneous endometrial pattern after FSH stimulation seems to be a prognostic sign of an adverse outcome.

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