Endometrial thickness and volume by three-dimensional ultrasound one week after embryo transfer to detect pregnancy

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Abstract *Purpose:* Determine if the evaluation of endometrium one week after embryo transfer can predict pregnancy.

Methods: Endometrial volume and thickness were evaluated by three-dimensional ultrasound in 40 patients one week after embryo transfer. These results were compared to serum pregnancy test performed one week later.

Results: Eighteen patients have achieved pregnancy. A significant difference was found for endometrial volume: 6.49 ± 1.97 mL versus 3.40 ± 1.11 mL (pregnant versus not pregnant); and thickness: 11.15 ± 2.75 mm versus 9.77 ± 1.85 mm. The ROC curve was used to detect the best cutoff values: endometrial volume of 3.48 mL (sensitivity-100%, specificity-68.2%) and endometrial thickness of 10.3 mm (sensitivity-72.2%, specificity-77.3%). The area under curve was significant higher for endometrial volume (0.909 versus 0.745, $p=0.027$). No pregnancy was achieved in women who had an endometrial volume <3.8 mL (15 patients) or thickness <7.9 mm (3 patients).

Conclusions: The endometrial volume and thickness were significant higher in pregnant women and this difference was more prominent for endometrial volume.

Keywords Ultrasound · Assisted reproduction techniques · Endometrium

Introduction

The period between embryo transfer (ET) after *in vitro* fertilization (IVF) and the pregnancy test (serum β -hCG) normally lasts two weeks [1]. Yong et al. [2] have demonstrated that in this period (between ET and the pregnancy test) the women submitted to IVF are most vulnerable to psychological stress, presenting the highest levels of hostility, depression, and state anxiety scores (compared with the period before treatment and before embryo transfer).

The diagnosis of pregnancy by ultrasound (US) is even later and is done by the detection of the gestational sac at least 3 weeks after ET [3]. However, the endometrial engorgement might be detected by ultrasound prior to serum β -hCG test and then minimize the time of psychological distress that they are exposed to. It was clearly demonstrated that three dimensional US (3DUS) is more precise to determine volume than conventional US and that the rotational method (VOCAL[®]) is superior to conventional 3DUS measurements even for *in vitro* [4] as for *in vivo* [5] models. As far as we know, there are no reports on 3DUS in this period. We aimed in this study to determine if, one week after ET, the measurement of endometrial volume (by rotational method) or thickness by 3DUS could predict pregnancy.

Methods

Patients

Forty consecutive patients submitted to IVF and ET were evaluated one week after ET. Inclusion criteria were: age

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≤ 39 years; body mass index ≤ 25 Kg/m²; first IVF cycle, no estrogen supplementation, absence of uterine pathologies and acceptance to join the research. All the patients were down-regulated with GnRH agonist (long protocol) and stimulated with recombinant FSH (Gonal[®], Fleet Laboratories Limited, Watford, UK or Puregon[®], Organon, Roseland, NJ, USA). Recombinant hCG (Ovidrel[®], Fleet Laboratories Limited, Watford, UK) was administered when there was at least one follicle with diameter ≥ 18 mm. The oocyte retrieval was performed 36 hours after the hCG administration and the embryo transfer 2–3 days later. Luteal phase were supplemented with 90 mg progesterone gel (8% Crinone[®], Fleet Laboratories Limited, Watford, UK). The β -hCG test was performed two weeks after ET.

The research protocol has been conducted in accordance with the guidelines in The Declaration of Helsinki and it was approved by the Ethics and Research Committee of the *Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo*. Informed written consent was obtained from all women. The overall pregnancy rate was 45% (18 of 40 patients, 10 singleton and 8 twins). No patients have been excluded in this study and all of them have been evaluated until the 20th week of pregnancy. No miscarriage has occurred in these 40 patients.

Ultrasound

All 3D scans were obtained one week after the ET using an 8–5 MHz vaginal probe Sonoacer[™] 9900 (Medison Co., Ltd.) by a single operator (WPM). All scanned volumes were stored on a hard disk and CDs, but all measurements were made soon after the end of exam by the same observer (WPM). Endometrial thickness was measured from central part of endometrium using the multiplanar display just to avoid oblique or exocentric measurements by placing the calipers on the miometrial-endometrial borders at the junction of the upper and lower two-thirds of the endometrial cavity.

VOCAL[®] imaging program (Virtual Organ Computer-aided Analysis) which is an extension of 3D-VIEW[®] (Kretz Technik, Zipf, Austria) was used to measure the endometrial volume in the manual mode using 15 degrees of rotation, described as satisfactory [6] for endometrial volume measures. For these rotational measurements, two calipers were placed at the upper and lower aspects of the endometrial cavity and the volume rotated about these to ensure they were appropriately sited and that all of the endometrium had been included. When the calipers were judged to be correctly positioned, the degree of rotation was determined by selecting the rotation angle and the manual mode of measurement entered. The junction between the miometrium and

endometrium was then traced in a clockwise fashion using a track ball.

Statistical analysis

Statistical analysis was undertaken using GraphPad Prism[®] 4 (GraphPad Software Inc) and MedCalc[®] Version 7.2.1 for Windows (Frank Schoojans). The data (endometrial volume and thickness) of each group (pregnant and not pregnant) were submitted to Kolmogorov-Smirnov test to verify the normality of distribution. So, the mean, standard deviation, coefficient of variation and 95% confidence interval were calculated. F test were performed to compare the variances. An unpaired *t* test with Welch correction (since the variances were significantly different) was performed to compare the two groups. The receiver operating characteristics (ROC) curve analysis and comparison were performed to determine the sensibility and specificity of the endometrial volume and thickness to predict pregnancy [11]. The estimation of the sample size was performed using an endometrial volume of 2.82 ± 0.85 mL (mean $\pm$ standard deviation) [10]. Using $\alpha = 0.05$ and power of 80% with 18 patients per group a difference of 30% (0.82 mL) would be detected.

Results

No significant difference in age (33.02 ± 4.52 years vs. 34.37 ± 3.74 years; pregnant vs. not pregnant; $P = 0.31$), weight (56.94 ± 4.04 kg vs. 59.73 ± 6.54 kg; pregnant vs. not pregnant; $P = 0.12$) and body mass index (22.03 ± 2.06 kg/m² vs. 22.9 ± 1.96 kg/m²; pregnant vs. not pregnant; $P = 0.18$) was found. A significant difference between the pregnant and not pregnant patients was found both in endometrial volume and endometrial thickness evaluated by 3DUS (Table 1).

ROC curves (Fig. 1) were used to detect the best cutoff values: endometrial volume of 3.48 mL (sensitivity = 100%, specificity = 68.2%) and endometrial thickness of 10.3 mm (sensitivity = 72.2%, specificity = 77.3%). A comparison between the two curves shows a significant higher area under curve for endometrial volume (0.909 vs. 0.745, $P = 0.027$). No pregnancy was detected in women who had an endometrial volume < 3.8 mL (15 of 22 not pregnant) or thickness < 7.9 mm (3 women). These rates (68% for endometrial volume and 13.6% for thickness) were significant different ($P = 0.0005$ by Fisher exact test).

There was no significant difference between the pregnant women that conceived singletons (13 women) or twins (5 women): endometrial volume = 6.45 ± 0.47 mL vs. 6.59 ± 1.26 mL, $P = 0.89$; endometrial thickness = 11.02 ± 1.10 mm vs. 12.10 ± 0.80 mm, $P = 0.47$ (singletons vs. twins, respectively).

Table 1 Endometrial volume and thickness evaluated by 3DUS one week after ET

	Pregnant (<i>N</i> = 18)	Not pregnant (<i>N</i> = 22)	<i>P</i> value
Volume - mean $\pm$ SD	6.49 $\pm$ 1.97 mL	3.40 $\pm$ 1.11 mL	<0.0001
Thickness - mean $\pm$ SD	11.80 $\pm$ 2.75 mm	9.77 $\pm$ 1.85 mm	0.0083

Note. *P* value was obtained by unpaired *t* test with Welch's correction.

Discussion

Some studies have shown that endometrial thickness on the day of oocyte retrieval or embryo transfer could be an important factor in predicting the outcome of *in vitro* fertilization (IVF) cycles [9, 10]; other studies, however, have failed to show such relation [11, 12]. Also, there are studies that have reported no direct correlation between endometrial ultrasound parameters and pregnancy rate, even though a minimal endometrial mass was favorable for pregnancy to occur [7, 13]. Schild et al. [12] have demonstrated that the evaluation of endometrial thickness and volume in the day of oocyte retrieval by 3DUS was not able to predict pregnancy and, moreover, the endometrial volume and thickness was even higher (but not significant) in not pregnant women. In a prospective study, Raga et al. [7] have shown that pregnancy and implantation rates were significantly lower ($P < 0.05$) in the group of patients with an endometrial volume < 2 mL. Furthermore, no pregnancy was achieved with an endometrial volume < 1 mL. Similar results were found by Yaman et al. [13]: they have shown that pregnancy rate in patients with an endometrial volume ≥ 2.5 mL was significantly higher than that in patients with an endometrial volume < 2.5 mL ($P < 0.012$), however their results have shown that neither

endometrial volume nor endometrial thickness was directly correlated with pregnancy rate. In a recent publication [14] 3DUS was performed in 451 embryo transfers and no significant difference was found in endometrial thickness (11.6 mm vs. 11.8 mm, pregnant vs. not pregnant) or volume (4.73 mL vs. 4.93 mL, pregnant vs. not pregnant) and the receiver operator characteristics analysis of their data have shown that these values could not predict pregnancy. Based on this controversial literature, it is possible that a minimum endometrial mass could favor the pregnancy rate, but it is unlikely that a significant difference in endometrial thickness or volume really exists before embryo transfer.

In our study, we hypothesize that the evaluation of endometrial volume and thickness by 3DUS could detect pregnancy earlier than β -hCG test. We have found that the endometrial volume and thickness one week after embryo transfer were significant higher in pregnant women. This difference is probably due to endometrial engorgement that occurs in the implantation process what was already demonstrated in primates. Jones and Fazleabas [15] have demonstrated that infusion of physiological doses of hCG in the uterine lumen of baboon results in an increase in number of small blood capillaries in the stroma of the endometrial functionalis what is an evidence that embryonic molecules influence precociously the endometrium in a paracrine manner to commence an angiogenic reaction. It was demonstrated in fecund monkeys [16, 17] an increased vascularity and angiogenesis at the implantation site in the endometrium. A large number of small blood vessels could be seen just after implantation in the stroma underlying the luminal epithelium. Also, the functionalis layer was filled with a number of microcapillaries and the blood vessels in the basalis layer were enlarged and engorged with blood.

In our study the use of endometrial volume was more accurate than endometrial thickness in predicting pregnancy. The implantation and the process of endometrial engorgement can occur in any site of endometrium and the measurement of endometrial thickness (that represents only a thin fragment of endometrium) may fail to demonstrate the endometrial increase in all cases. Such problem is unlikely to occur in endometrial volume measurement. This study was the first attempt to make a more precocious diagnosis of pregnancy after IVF aiming to minimize the psychological distress that is already well established in this period [2]. Besides the importance for the patient mental health, it is also important because pre-existing psychological factors

Fig. 1 ROC (Receiver operating characteristic) curves for endometrial volume and thickness evaluated by 3DUS one week after ET to predict pregnancy

are independently related to treatment outcome in IVF/ICSI [18, 19] and it is known that a considerable proportion of patients will show substantial emotional problems even after 6 months of a non successful cycle [20].

However, even for endometrial volume, no cut off value has permitted to surely predict pregnancy. This was probably due to the wide range of endometrial volume and thickness that is found in patients before ET that will become pregnant or not [21]. The results of our study confirms that endometrial tissue increases after implantation, but other studies are necessary to demonstrate if it is possible to detect the small increase in endometrial volume and thickness that occurs in this short period of time (one week after ET). Perhaps the difference between endometrial volume before ET and one week after ET will be a more precise tool to predict pregnancy.

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