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Short Communication

Histological component quantification for the evaluation of endometrial receptivity in women with natural cycles undergoing in vitro fertilization/intracytoplasmic sperm injection

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ABSTRACT

Objective: Our aim was to evaluate the value of the volumetric fraction of vascular endothelial cells (EnVF) for determining endometrial receptivity in women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).
Materials and methods: The records of women undergoing IVF/ICSI between 2006 and 2010 were retrospectively reviewed. An endometrial biopsy was performed in the cycle prior to IVF/ICSI. EnVF was calculated from endometrial biopsy staining.
Results: Twenty-seven patients who did not become pregnant, 8 who had a miscarriage, and 21 with a clinical pregnancy were included. The three groups were similar with respect to infertility and IVF characteristics. An EnVF $\leq$3.85 was associated with not becoming pregnant, an EnVF >5.29 with miscarriage, and a level between 3.86 and 5.29 was associated with clinical pregnancy ($p = 0.001$).
Conclusions: EnVF examined in the prior cycle may be a marker of endometrial receptivity and predict the chance of pregnancy in women undergoing IVF/ICSI.

Introduction

Repeated embryo implantation failure after in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) may be due to endometrial inadequacy; however, endometrial inadequacy is difficult to detect through routine clinical tests. Vaginal ultrasound measurement of endometrial shape and thickness is commonly performed, but many patients with repeated implantation failures have a normal endometrial shape and thickness [1,2]. Serological markers such a progesterone and estrogen have limited clinical value [3,4].

The volumetric fraction of vascular endothelial cells (EnVF) is the proportion of endothelial cells, by volume, in a specific tissue, and is a basic indicator used in the biological studies [5–8]. In a prior study of polycystic ovary syndrome (PCOS) patients, found that a lower EnVF during the embryo implantation window in the first menstrual cycle after discontinuation of oral contraceptive pill (OCP) use was associated with significantly lower clinical pregnancy rates after subsequent ovarian stimulation [9].

The purpose of this study was to determine if EnVF can be used as an indicator of endometrial receptivity in women with natural menstrual cycles undergoing IVF/ICSI.

Patients and methods

The records of patients who received a first cycle of IVF/ICSI between October 2006 and October 2010 at the Reproductive Medicine Centre in the People's Hospital in Peking University, Beijing, China were retrospectively reviewed. This study was approved by the Institutional Review Board of the hospital, and because of the retrospective nature the requirement of informed patient consent was waived. All patients provided informed consent for endometrial biopsies and IVF procedures.

The inclusion criteria were: basal follicle stimulating hormone (FSH) level <10 U/L, total number of antral follicles in two ovaries $\geq$10, age < 37 years, no uterine septum, endometrial polyps, and

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submucosal uterine myoma, an endometrial thickness not less than 7 mm measured by transvaginal ultrasonography in the embryo implantation window (defined as 7–10 days after peak urinary luteinizing hormone [LH] level), and the leading follicle diameter reached ≥18 mm. All patients received an endometrial biopsy prior to beginning IVF (standard practice at our institution). Patients with confirmed endometrial pathology including chronic inflammation and endometrial tuberculosis, with a poor ovarian response to hyperstimulation, and with no transferable quality embryos were excluded. To minimize the impact of embryo and other factors which cause infertility, we attempted to choose patients with similar ovarian function and embryo quality.

Clinical pregnancy was defined as the presence of intrauterine embryonic sac(s) by transvaginal ultrasound 4 weeks after embryo transfer (ET). Spontaneous abortion was defined as pregnancy failure during the first trimester of pregnancy. Patients were divided into three groups: those that achieved pregnancy, did not become pregnant, and that had a miscarriage.

All participants received transvaginal ultrasounds to monitor ovulation. Diagnostic curettage of endometrial tissue was carried out when the leading follicle diameter reached ≥18 mm at 7–10 days after peak urinary LH level in the cycle proceeding the IVF cycle. The endometrial tissue was fixed using 10% neutral formalin and embedded in paraffin for conventional hematoxylin & eosin (HE) microscopic examination and was confirmed as secretory phase endometrium.

In brief, for determination of EnVF the endometrial tissue was rehydrated in phosphate buffered saline (PBS) for 5 min after removing wax. Endogenous peroxidase was removed by mixing with 0.3% hydrogen peroxide, and the tissue was washed three times with PBS. The tissue slice was treated by 20% sheep serum for 30 min, then mixed with CD34 murine monoclonal antibody (1:50) (Serotec, Oxford, UK). The mix was placed in a 37 °C incubator for 1 h and then washed three times with PBT (PBS containing 0.1% Tween) before being mixed with biotin-labeled sheep anti-rat antibody (1:500) (Serotec, Oxford, UK) for 1 h. After washing with PBT, 1:500 diluted streptavidin conjugate horseradish peroxidase (HRP) was added and washed with PBT after 15 min. The tissue was stained with 3, 3′-diaminobenzidine (DAB). Mayer's hematoxylin was used for subsequent staining before the tissue was dehydrated using xylene and sealed.

Three pieces of tissue were randomly selected from all the endometrial tissues of each patient for volume fraction measurement. HE and CD34 dyed slices were examined under 60× magnification. Ten random fields were selected from each piece. A test grid of 250 points was overplayed on the pictures. The numbers of test points falling on CD34 staining positive cells, glandular epithelial cells, gland cavity, and interstitial tissue, respectively, were counted. The volume fraction occupied by each component was then calculated by expressing the number of points hitting that component as a percentage of the total number of test points applied. The volumetric percentage from each piece of tissue was averaged for each patient.

Statistical methods

Data were expressed as mean (standard deviation) for age, EnVF, gland cavity VF, and interstitial stroma VF; median (interquartile range) for infertility and volumetric percentage of glandular epithelial cells (EpVF); count (percentage) for categorical data. To examine group difference, analysis of variance (ANOVA), Kruskal–Wallis test and chi-square test were implemented for continuous variables with and without normal distributions, and categorical variables, respectively. When there were 20% of cells with expected value less than five, the chi-square test was replaced with Fisher's exact test. A $p < 0.05$ was considered statistical significance. Post-hoc testing was carried out if ANOVA revealed a significant result, and significant level was adjusted to 0.017 (0.05/3) or 0.008 (0.05/6). All statistics were two-sided and estimated using SPSS statistical software (version 22, IBM Corp., Armonk, NY).

Results

Fifty-six patients were included in the study: 27 who did not become pregnant after IVF/ICSI, 8 who had a miscarriage, and 21 who had a clinical pregnancy. The three groups had similar length of infertility, proportion of primary infertility, causes of infertility, EpVF level, gland activity VF level, and interstitial stroma VF level. However, the age of pregnancy group was significantly younger than that of non-pregnant group (29.4 vs. 32.9 years, $p = 0.014$). Compared with the other two groups, the miscarriage group had a higher EnVF level than the non-pregnant group and the pregnant group (6.3 vs. 4.6 and 4.8, respectively, $p = 0.003$ and 0.009). The three groups of patients also were not different in the number of follicles with diameters >1.4 cm, cumulus oocyte complex, fertilized oocytes, embryos obtained, embryos quality and embryos transferred.

EnVF was divided into quartiles, and the pregnancy and miscarriage rates according to quartile were calculated. Women with an EnVF ≤3.85 were more likely to not become pregnant, and those with an EnVF >5.29 were more likely to have a miscarriage ($p = 0.001$) (Table 1).

Discussion

The results of this study suggest that EnVF determined in the prior cycle is associated with pregnancy outcome after IVF, and may be a predictor of endometrial receptivity. All other endometrial parameters measured in this study had no associations with clinical pregnancy rates. Also of note is that the groups were similar with respect to embryo quality, thus excluding this factor as a cause of poor outcomes.

EnVF may be an indirect reflection of the endometrial vascular growth in the implantation window, and therefore an indicator of the development of other endometrial tissue. Studies have shown that human endometrial angiogenesis abnormalities, such as endometriosis and dysfunctional uterine bleeding, can lead to infertility [10,11], and that endometrial angiogenesis is associated with receptivity [12,13]. Interestingly, biopsy-induced inflammatory conditions have been shown to improve endometrial receptivity [14,15]. A low EnVF indicates (1) there is a low vascular density in the uterus or (2) the interstitium is proportionally too great or the glandular lumen is highly enlarged. While EnVF accounts for a small proportion of endometrial cells, it has an important influence on pregnancy outcomes. We also assessed the

Table 1
Pregnancy and miscarriage rates by volumetric fractions of vascular endothelial cells (EnVF) quartile.

	Non-pregnant (n = 27)	Miscarriage (n = 8)	Pregnancy (n = 21)	p
EnVF quartile				**0.001**
≤3.85	11 (78.6)	0 (0)[a]	3 (21.4)[b]	
3.86–4.56	5 (35.7)	0 (0)[a]	9 (64.3)[b]	
4.57–5.29	5 (35.7)	2 (14.3)	7 (50.0)	
>5.29	6 (42.9)	6 (42.9)[a]	2 (14.3)[b]	

Data are presented as number (%).
Bold value indicates significantly different among three groups, $p < 0.05$.
[a] Indicates significant difference from non-pregnant group, $p < 0.008$.
[b] Indicates significant difference from miscarriage group, $p < 0.008$.

EpVF, gland cavity VF, and interstitial stroma VF and found no significant differences between the three groups. This also suggests that even though endometrial vascular endothelial cells are a small component of the endometrial tissue, small variations may significantly affect the implantation process.

There are limitations to the current study that should be considered. The study was retrospective in nature and the sample size was small. To minimize the impact of embryonic and other factors which cause infertility, we attempted to choose patients with similar ovarian function and embryo quality; however, this may have introduced selection bias. Due to the use of ovulation drugs, endometrial receptivity in a natural menstrual cycle may not equal that in an IVF/ICSI cycle. However, it is not appropriate to perform endometrial sampling during a stimulation cycle. We did not examine correlations of hormone levels with EnVF. We also did not examine if EnVF varied from cycle to cycle, or variations during different times in a single cycle. Future study can examine the dynamic changes in different phase of the natural cycle. Lastly, biopsy-induced inflammatory conditions may improve endometrial receptivity. However, given that IVF was not performed in the same cycle as endometrial biopsy it is unlikely, though possible, that this affected the results.

In summary, EnVF determined in the prior cycle may be a useful indicator of endometrial receptivity and predictor of clinical pregnancy of patients undergoing IVF/ICSI with natural cycles. A level between 3.86 and 5.29 was associated with clinical pregnancy, while lower and higher levels were associated with not achieving pregnancy and miscarriage, respectively.

Conflicts of interest

All authors have no commercial or financial conflicts of interest to disclose.

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