The Outcome of Repeated In Vitro Fertilization-Embryo Transfer Based on the Endometrial Thickness

〈*Original Article*〉

The Outcome of Repeated In Vitro Fertilization-Embryo Transfer Based on the Endometrial Thickness

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ABSTRACT

Objective: To determine whether in vitro fertilization embryo transfer (IVF-ET) outcome is affected by endometrial thickness.
Design: Retrospective study.
Setting: IVF-ET unit in Osaka Medical College.
Patients: Twenty-eight patients with tubal infertility who became pregnant following IVF-ET between 1998-2000. All patients underwent 2 cycles of IVF-ET with long down-regulation protocol of Gonadotorophin releasing hormone analogue (GnRHa) and became pregnant following one of them. These fifty-six courses were divided into pregnant and non-pregnant courses.
Main Outcome Measure(s): Endometrial thickness, serum E_2 and LH levels, number of oocytes, fertilization rate, cleavage rate, high quality embryo rate and pregnancy rate were investigated.
Results: The endometrium in the pregnant courses were thicker than those in the non-pregnant courses ($P<0.05$). Serum E_2 level showed direct correlation with endometrial thickness ($R=0.882$, $P < 0.001$). The courses with endometrial thickness greater than or equal to 10 mm showed a significantly higher pregnancy rate than courses with endometrium of less than 10 mm. Although there was no statistical difference in high-quality embryo rate or number of transferred embryo rate between the two groups, the fertilization rate of the courses with endometrium greater than or equal to 10 mm was higher than that in the courses with endometrium less than 10 mm.
Conclusion: Although the number of transferred embryos and embryo quality does not affect the pregnancy rate, endometrial thickness might play an important role in the success of IVF-ET.

Introduction

During the menstrual cycle the endometrium shows cyclic change for implantation based on change of estaradiol in the proliferative phase and progesterone in the secretory phase. Therefore, certain endometrial abnormalities such as Asherman's syndrome prevent the endometrium from changing normally. Several authors have evaluated the effect of endometrial thickness during the pre-ovulatory phase on pregnancy outcome during in vitro fertilization embryo transfer (IVF-ET), but the results have been controversial (CHECK et al, 1991; DICKEY et al., 1997; NOYES et al., 1995; RINALDI et al., 1996; SHIELD et al., 2001). For example, some authors have supposed the existence of a correlation between the degree of endometrial development as determined by ultra-

sound and the probability of embryo implantation (CHECK et al, 1991; DICKEY et al., 1992; NOYES et al., 1995; RINALDI et al., 1996), while others have not supported these findings (De GEYTER et al., 2000; SHIELD et al., 2001). The measurement of endometrial thickness is a simple technique, however, it is still controversial whether endometrial thickness determined by ultrasound-examination is a useful parameter to predict IVF-ET outcome (KHALIFA et al., 1992). The differences of regime and profile of each patient might be a cause of these controversies, therefore, we investigated the difference of endometrial thickness in pregnant and non-pregnant cycle of IVF-ET in the patients who underwent 2 courses of IVF-ET and became pregnant following one of them.

Material and Method

Patients

The medical records of 28 patients with tubal infertility undergoing IVF-ET in the program at Osaka Medical College during 1998 and 2000 were retrospectively reviewed. All patients underwent 2 cycles of IVF-ET with long down-regulation protocol of Gonadotorophin releasing hormone analogue (GnRHa) and became pregnant following one of them. The cycles, 56 in total were divided into pregnant and non-pregnant cycles.

Protocol of controlled ovarian hyperstimulation (COH)

Treatment with the GnRHa was started in the mid-luteal phase of the preceding cycle and was continued through the follicular phase of the next cycle until the day of hCG administration. Protocol of controlled ovarian hyperstimulation (COH) with hMG (150 IU FSH and 150 IU LH per ampoule; Pergonal; Teizou Pharma. Co. Tokyo, Japan) and/or FSH (75 IU FSH per ampoule; Phertinom; Serono, Welwyn Garden City, Herts, UK) was started on day 3 of the current cycle in an individual step-down protocol until a dominant follicle reached a diameter of 18 mm. Ovulation was triggered with 10,000 IU of hCG (Pregnyl; Organon, Cambridge, UK), which was administered 35 hours before oocyte retrieval. Follicular maturation and endometrial thickness were monitored by tarnsvaginal ultrasound examination (Sonovista-EX; Mochida, Tokyo, Japan) from day 6 to the day of hCG-administration. Endometrial thickness was measured in the saggital plane with a 7.5-MHz scan at a distance between the hyperechogenic The assessments of endometrial thickness was measured in the saggital plane with a 7.5-MHz scan, and the distance of approximately 1 cm from uterine fundus at the time of hCG injection. Oocyte retrieval was performed via an ultrasound-guided transvaginal approach 35 hours after hCG administration. Insemination was performed with insemination medium (human tubal factor; Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10% synthetic serum substitute (SSS; Irvine Scientific, Santa Ana, CA, USA) 6 hours after oocyte retrieval, and fertilization was confirmed by identification of pronuclei 16 hours after insemination. All embryos were transferred into growth medium (20% SSS + human tubal factor) and were evaluated using the Veeck criteria (VEEK. 1988). Forty-eight hours after oocyte retrieval, embryos in transfer medium (50% SSS + human tubal factor) were implanted into the uterine cavity, and the recipient then rested in bed for 4 hours. Vaginal progesterone suppositories (200 mg/day) was started on the day of oocyte retrieval to provide luteal support and was continued daily until a pregnancy test was performed. Pregnancy was confirmed by identification of an intrauterine gestational sac by transvaginal ultrasound examination.

Informed consent was obtained for all tests from controls and patients after the study protocol had been approved by a local ethics committee of Osaka Medical College.

Hormone assay

Hormonel assay was carried out using commercially available RIA kits. At the time of hCG-injection, E_2, P, and LH levels in the serum were assayed to determine whether IVF-ET should be progressed. When LH exceeded 10 IU/l before oocyte retrieval, IVF-ET was canceled. Whether there is any correlation between E_2 level and endometrial thickness was investigated.

Statistical analysis

The significance of difference was determined with unpaired t test and χ^2 test. A level of $P < 0.05$ was accepted as statistically significant.

Results

Table 1 represents hormone environment and endometrial thickness at the time of hCG injection in the pregnant and non-pregnant courses. There was no statistical difference in total dose of hMG/FSH and P, however serum E_2 levels in the non-pregnant courses were statistically higher

Table 1 Hormone environment and endometrial thickness at hCG injection of both groups

	Pregnant N=28	Non-pregnant N=28	P value
Total dose of hMG/FSH (IU)	1761.2±419.1	1865.3±446.8	NS
Estradiol (pg/ml)	1654.7±1064.1	2238.0±1057.4	0.0011
Progesterone (ng/ml)	0.6±0.3	0.5±0.2	NS
Endometrial thickness (mm)	11.5±2.2	8.7±2.2	0.00005

Note: Values are mean ±SD. NS = not significant

Table 2 IVF-ET outcome based on thickness less than or greater than / equal to 10 mm

	Less than 10 mm N=30	Greater or equal to 10mm N=26	P value
Estradiol (pg/ml)	2010.3±1024.7	1794.2±115.7	NS
No. of picked up oocyte	6.5±3.5	8.0±4.8	NS
Fertilization rate (%)	69.4 (136/196)	81.4 (184/226)	0.004
No. of high quality embryo(G1+G2) (%)	46.3 (63/136)	45.6 (84/184)	NS
No. of transferred embryo	3.4±1.7	4.0±2.2	NS
Pregnancy rate (%)	20 (6/30)	84.6 (22/26)	0.0000014

Note: Values are mean ±SD. NS = not significant

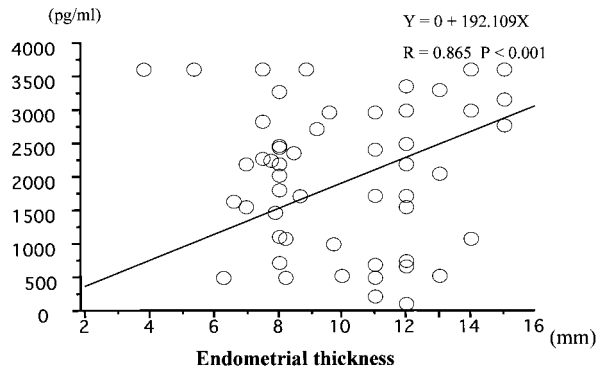

Fig. 1 Correlation between serum E_2 level and endometrial thickness is shown. (Correlation coefficient, 0.865, P<0.001).

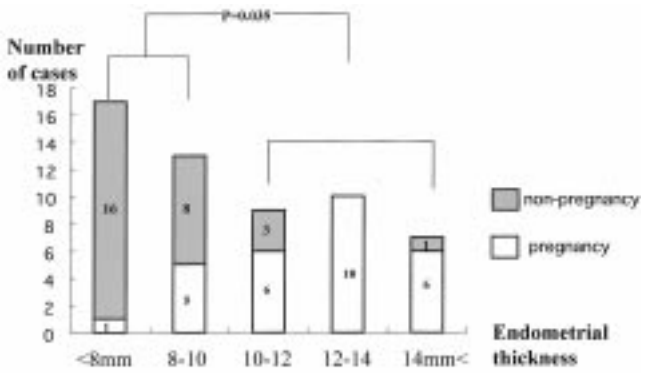

Fig. 2 Endometrial thickness was divided into 5 groups as shown in X-axis. The Y-axis on the left indicates the number of courses. The open bar represents the number of pregnant couses and the shaded bar represents that of non-pregnant courses. Statistical difference in pregnancy rate was found between endometrial thickness <10 mm and >10 mm (P = 0.035).

than those in the pregnant courses. Moreover, endometrial thickness in the pregnant courses was statistically thicker compared with that in non-pregnant courses (P = 0.000014) (**Table. 1**). Correlation between serum E_2 level and endometrial thickness in the 56 courses is shown in **Fig. 1** (correlation coefficient, R=0.865, P<0.001). Endometrial thickness was divided into 5 groups as shown in **Fig. 2** and it shows a statistical difference in pregnancy rates between endometrial thickness less than 10 mm and greater than or equal to 10 mm (P = 0.035). **Table 2** shows IVF-ET outcome based on endometrial thickness less than 10mm and greater than or equal to 10 mm. Although there was no statistical difference in serum E_2 levels, number of picked up oocyte, high-quality embryo rate and number of transferred embryos, fertilization rates and pregnancy rates with endometrial thickness greater than or equal to 10 mm were statistically higher than those with endometrial thickness less than 10mm. No patient experienced ovarian hyperstimulation syndrome (OHSS).

Discussion

Ultrasonography is a non-invasive method that can provide serial information about the characteristics of the endometrium. The ultrasonographic evaluation of the endometrium varies in terms of thickness and echogenicity throughout the natural or stimulated cycle. The present study was a retrospective study that examined the difference of endometrial thickness of the same patients who underwent IVF-ET twice and became pregnant following the treatments to determine what impact endometrial thickness has on IVF-ET outcome. Controversy exists regarding the prognostic significance of endometrial thickness and pattern regarding the initiation of pregnancy in infertile women whose cycles are monitored by transvaginal sonography. Some authors have reported that not only endometrial thickness but also pattern contribute to outcome of treatment cycle (CHECK et al, 1991; KHALIFA et al., 1992; DICKEY et al., 1997; SHIELD et al., 2001), however an increased luteal phase progesterone secretion might influence endometrial thickness, and various measurement methods (including outer edge to outer edge, and outer edge to inner edge) could further affect the outcome. The use of various measuring methods of endometrial thickness might provide a further explanation for conflicting results. Some authors reported results by cut-off values (DICKEY et al., 1992; NOYES et al., 1995; RINALDI et al., 1996), while others evaluated by mean endometrial thickness or compared endometrial thickness within percentile group (DICKEY et al., 1992; YUVAL et al., 1999). Therefore, in the present study we focused on only endometrial thickness and evaluated it by mean endometrial thickness.

Reuter et al. reported that no patient conceived with endomterial thickness <8mm (REUTER et al., 1996) and Bergh et al. found that pregnancies did not occur in patients with an endometrial thickness of <9mm (BERGH et al., 1992). Moreover, Khalifa et al. scrutinized the link between conception and endometrial thickness in IVF-ET and reported a minimal endometrial thickness associated with conception as being 7 mm on the day of hCG and the day of embryo transfer (KHALIFA et al., 1992). In the present study, pregnancy rate of treatment cycle with endometrial thickness <8mm was 1.7% (1/56), therefore, thicker endometrium >10mm might be a useful parameter to predict successful implantation. Previous studies found that there was a positive correlation between endometrial thickness and successful implantation, as well as between endometrial thickness and serum E_2 (REMOHI et al., 1997). Kovacs reported pregnancy rates were shown to be higher when endometrial development was one of the factors that played a significant role and other variables such as age embryo quality, number of embryos transferred and stimulation protocol were also shown to have a significant impact on the outcome (KOVACS et al., 2003).

On the other hand, four successful pregnancies in patients with endometrium <7mm and one successful pregnancy in spite of 4mm endometrium have been reported (SUNDSTORM et al., 1998; GEYTER et al., 2000). Therefore, reduced endometrial thickness has a marginal effect on the probability of achieving a pregnancy with assisted reproduction. Furthermore, thin endometrium may be associated with pathologic abnormalities such as squamous cell metaplasia (RUIZ-VALASCO et al., 1997), and the endometrium of patients with this abnormality does not show a normal proliferative response to the rising E_2 levels (GEYTER et al., 2000). Friedler et al. stated that the low predictive value of endometrial thickness might be explained by its weak relationship with uterine receptivity (FRIEDLER et al., 1996). These data are partially consistent with our results that E_2 levels were significantly higher in non-pregnant courses than pregnant ones.

In conclusion, the results of the present study identified a statistically significant difference in mean endometrial thickness between cycles that became preganant and those did not. Endometrial thickness >10mm at the time of hCG administration might be a predictable value for implantation in IVF-ET, however, what affect the endometrial thickness has was undetermined. Further studies including histological examination are required to evaluate what factors affect endometrial development.

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