Effect of endometrial cavity fluid on clinical pregnancy rate in tubal embryo transfer (TET)

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Purpose: In this study, we assess whether the endometrial cavity fluid (ECF) generated physiologically by the genital tract have negative effect on the pregnancy rate during tubal embryo transfer (TET) in patients who do not have hydrosalpinx or bilateral tubal obstruction.

Methods: We retrospectively collected samples from 176 women with 195 cycles receiving TET due to male factor, unexplained infertility or endometriosis from June 1999 to Dec. 2003, and divided them into two groups (group I: patient with fluid accumulation >1 mm in the anterior–posterior diameter in the uterine cavity; group II: patient without fluid accumulation in the uterine cavity). Endometrium thickness was measured as a maximal distance between anterior and posterior myometrium–endometrium interface under the long-axis view. The A–P diameter of ECF was measured via vaginal sonar on the day of ovum pick-up (OPU).

Results: From a total of 195 ART cycles involving 176 patients, the accumulation of ECF was detected in 10 cycles (5.1%). Seven of ten cycles (70%) with the accumulation of ECF were proved to be pregnant clinically. However, in the rest 185 cycles (group II), 98 of them (53%) were proved to be pregnant. At the mean time, the implantation rate was 29.03% and 25.71% in the groups I and II, respectively. No significant difference of the clinical pregnancy rate and the implantation rate was found between the two groups.

Conclusions: According to our study, if the ECF was generated physiologically by the genital tract during controlled ovarian hyperstimulation (COH), the clinical pregnancy rate is not worse and no influence of embryo implantation was found.

KEY WORDS: Clinical pregnancy rate; COH; endometrial cavity fluid.

INTRODUCTION

Endometrial cavity fluid (ECF), which came from the reflux of hydrosalpinx or was generated by endometrial cavity, was occasionally found during controlled

ovarian hyperstimulation (COH) in vitro fertilization (IVF). The origin of endometrial cavity fluid was controversial but it was associated with hydrosalpinges, polycystic ovarian disease, subclinical uterine infections and was also generated physiologically by the genital tract. The fluid flushed backwards from hydrosalpinx to intrauterine cavity may be embryotoxic due to its microorganisms, endotoxins (1–3), cytokines (4,5), oxidative stress, and lack of nutrients (6,7). Mechanisms which had been suggested were either direct or systemic influenced by inflammatory mediations on the developing embryo in uterus or on the endometrium prior to or during implantation. It was suspected to have toxic effect on embryo

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development and interfere the implantation of embryos, and resulted in decreasing pregnancy rate (8–11). Most relative studies which revealed ECF with hydrosalpinges will have negative effect on pregnancy rate (9–11). The more extensive the hydrosalpinx, the lower the implantation rates (12).

From the above-mentioned, there may have two origins of ECF and these may have different outcomes for pregnancy rate. In our study, we were going to assess that whether the ECF generated physiologically by endometrial cavity have negative effect on pregnancy rate during tubal embryo transfer (TET) in patients who did not have hydrosalpinx or bilateral tubal obstruction.

MATERIALS AND METHODS

We retrospectively collected samples from 176 women with 195 cycles receiving TET due to male factors, unexplained infertility, or endometriosis from June 1999 to Dec. 2003, and divided them into two groups (group I: patient with fluid accumulation >1 mm in A–P diameter within uterine cavity, group II: patient without fluid accumulation within uterine cavity). There were 10 women with 10 cycles (Table I), who have ECF in group I, and the rest 166 women with 185 cycles were in group II. Women who were older than 40 years, or infertile due to tubal factors (i.e., hydrosalpinx or tubal occlusion) were all excluded. Hysterosalpingography (HSG) was performed before TET programs and all revealed bilateral tubal patency.

In long protocol, subcutaneous injection of leuprolide acetate (Lupron; Abbott laboratories, Illinois), 1 mg per day for pituitary desensitization was started on the 21st day of the previous menstrual cycle until the day of HCG intramuscular injection. Gonadotrophins were given from day 3 of menstruation, with combination of human menopausal gonadotropin (HMG, Pergonal; Serono, Rome, Italy) and recombinant follicle-stimulating hormone (rFSH, Gonal-F; Serono, Aubonne, Switzerland). Transvaginal ultrasonography was used to monitor follicular growth, starting on menstrual day 7 and repeated as necessary. When two to three leading follicles reached a mean diameter of 19 mm, 10,000 IU of human chorionic gonadotropin (HCG, Pregnyl; Organon, Oss, The Netherlands) was given. Transvaginal ovum pickup (OPU) was carried out under sonographic guidance for 34–36 h later at operating room. Classification for embryo assessment 2 days later was introduced by Veeck (13).

The day of ovum pick-up was defined as D0, and TET was arranged on D2. The blood E2 level (picogram per milliliter) on the day of HCG injection, number of oocytes retrieved, number of mature oocytes, number of fertilized oocytes, number of embryos obtained, and number of embryos transferred were all measured and listed in Table II. Endometrium thickness was measured as a maximal distance between anterior and posterior myometrium–endometrium interface under the long-axis view, and the A–P diameter of ECF (millimeter) was measured via vaginal ultrasonography on the day of OPU (Fig. 1). If intrauterine cavity fluid is present, then endometrium thickness was the distance, mentioned-above, deducted the maximal fluid A–P diameter.

The patient underwent laparoscopy for TET and both tubes appearance were normal. A GIFT catheter (k-GIFT-1010, COOK, Queensland, Australia) was used to transfer embryos in 20 μ L of G1.2 medium (Vitrolife; Goteborg, Germany). All embryos were transferred into either the right tube and/or the left tube, with the transfer bolus deposited 2–4 cm from the fimbria. The entire process was continuously monitored laparoscopically and was atraumatic. Following TET, the patient remained in supine position for 4 h and was then discharged. Luteal phase support was provided with 2500 IU of HCG injection on day 2, 5, and 8, or 50 mg of progesterone (progesterone, Tai Yu Chemical Pharmaceutical Co., Shing Ju, Taiwan) administered intramuscular daily from the day of OPU. Micronized progesterone (Utrogestan, Laboratories Piette International S.A.; Brussels, Belgium) at a dose of 600 mg/day with intravaginal suppository commenced on the next day after OPU.

Clinical pregnancy rate was indicated by an intrauterine gestational sac seen on ultrasound examination 3.5 weeks after embryo transfer. Implantation rate was defined by the proportion of individual embryos transferred resulting in a gestational sac. Transvaginal ultrasound (HITACHI, EUB-415, Tokyo, Japan) was used at operating room. The procedures including ovum pick-up and ECF measuring were all executed by the same operator.

Statistics

Data are presented as mean $\pm$ standard deviation (SD). Comparisons between the two groups were performed using the Mann–Whitney *U*-test. The statistical test to analyze clinical pregnancy rates and

Table I. Patient Characteristics

Patient	Age (years)	Mean A-P diameter of ECF (mm)	E2 on HCG day (pg/mL)	No. of oocyte re-retrieved	No. of transferred embryos	Implantation sacs	Clinical pregnancy	Living birth
1	24	2.6	4512	17	3	1	Yes	Yes
2	36	4.5	541	2	1	1	Yes	No
3	39	3.6	2486	12	4	1	Yes	Yes
4	32	4.9	2042	18	4	0	No	No
5	28	2.1	4885	20	3	3	Yes	Yes
6	23	3.2	5570	22	3	1	Yes	Yes
7	27	3.5	2316	15	3	0	No	No
8	35	3.6	5245	11	3	0	No	No
9	30	2.7	1208	8	4	1	Yes	Yes
10	33	2.8	4667	16	3	1	Yes	Yes

live birth rates was done using chi-square analysis. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

From a total of 195 TET cycles involving 176 patients, ECF was detected in 10 cycles and a prevalence of ECF was 5.1% (10/195). The number of

oocytes retrieved and number of mature oocytes were considered statistically significantly different (14.10 ± 5.99 versus 10.05 ± 4.23 and 12.06 ± 5.54 versus 8.29 ± 3.56 ($p < 0.05$)) between group I and group II. The total number of embryos per cycle and number of embryos transferred per cycle were not considered statistically significantly different between the two groups. In group I with ECF, 7 of 10 were proved to be pregnant (pregnancy rate: 70%).

Fig. 1. Transvaginal sonogram demonstrating the appearance of endometrial cavity fluid (A-P diameter 2.6 mm) on the day of ovum retrieval. This woman was proved to be pregnant and she delivered a viable male baby (BW: 3,018 g) by Cesarean section.

Table II. Comparison of Clinical Parameters and Pregnancy Outcomes Between ECF Group and Control Group

Parameter	Group I	Group II	<i>p</i>
No. of women	10	166	—
Cycle of COH	10	185	—
Age (years)	30.7 ± 5.25	33.43 ± 3.17	NS
E2 on HCG day (pg/mL)	3348.10 ± 1825.73	2525.92 ± 1460.69	NS
Mean A–P diameter of endometrial fluid (mm)	3.36 ± 0.86	—	—
No. of oocytes retrieved	14.10 ± 5.99	10.05 ± 4.23	<0.05
No. of mature oocytes	12.06 ± 5.54	8.29 ± 3.56	<0.05
No. of fertilized oocytes	8.10 ± 4.04	7.01 ± 3.31	NS
No. of embryos/cycle	6.80 ± 3.52	6.76 ± 3.27	NS
No. of transferred embryos/cycle	3.20 ± 0.90	3.41 ± 0.97	NS
Implantation rate/TET (%)	29.03	25.71	NS
Clinical pregnancy rate/TET (%)	70	53	NS
Living birth/TET (%)	60	41	NS

Note. Group I: patient with fluid accumulation within uterine cavity (>1 mm); Group II: patient without fluid accumulation within uterine cavity.

However, in the rest 185 cycles without ECF, 98 of them were proved to be pregnant (pregnancy rate: 53%). The implantation rate was 29.03% in group I and 25.71% in group II (Table II). No significant difference of the clinical pregnancy rate and the implantation rate was noted between the two groups.

DISCUSSION

Several studies had shown a strong association between the presence of hydrosalpinges and poor outcome in assisted reproductive technology (ART). ECF was occasionally found during IVF among patients diagnosed as hydrosalpinx (8,14–18). It was thought to have negative effect on embryo implantation, the lower clinical pregnancy rate, and even lead to abortion (1,9,10). These include embryotoxic effects of hydrosalpinx fluid, reduced endometrial receptivity (8,16) and mechanical factors or flushing effects from the reflux of hydrosalpinx fluid into the endometrial cavity to interfere the embryo implantation (14,16,17,19). However, Strandell *et al.* (20) found that hydrosalpinx fluid did not adversely affect the normal development of human embryos and implantation, but they ignored the long-term effect of hydrosalpingeal fluid to embryos.

Most studies showed that the ECF usually developed after receiving HCG injection (8,14,16,17). If fluid accumulation reached a diameter of over 3 mm either before or after HCG was given, it usually persisted until the time of the peri-implantation period and affected embryo implantation (8). Aspira-

tion of the hydrosalpinx fluid prior to an IVF procedure slightly increased implantation rates (21). Some studies showed that salpingectomy prior to IVF may improve the pregnancy rate (22,23). Proximal tubal cauterization is effective in reversing the adverse effects of hydrosalpinges and improves pregnancy rates (24).

The fluid flushed backwards from hydrosalpinx to intrauterine cavity may be embryotoxic (1–5). However, even in patients without hydrosalpinx, fluid accumulation may be seen in uterine cavity. In our study, patients with hydrosalpinx or bilateral tubal obstructions were all excluded. Bilateral fallopian tubes were proved to be healthy morphologically by laparoscopy during TET procedure. Nevertheless, the incidence of ECF was 5.1% and we thought that fluid was generated physiologically by the genital tract during COH. By the way, the clinical pregnancy rate was not worse than the control group without ECF (70% versus 53%). No difference of the implantation rate was found also in the two groups. Therefore, we presumed that physiological endometrial cavity fluid was not embryotoxic and it may contain some kind of growth factors to have benefits to the embryos. We know that the level of certain growth factors such as leukemia inhibitory factor (LIF), colony-stimulating factor-1 (CSF-1), glyodelin, and integrin $\alpha_v\beta_3$ increase greatly in the endometrium during the implantation window, which would facilitate implantation (25). Once the embryo has reached the uterine cavity, a preprogrammed sequence of events occurs. These steps involve the production and secretion of many biochemical factors such as cytokines, growth factors,

and adhesion molecules by the endometrium as well as the embryo, thus leading to the formation of a receptive endometrium.

Chien *et al.* (26) found that fluid accumulation within the uterine cavity reduced pregnancy rates in women undergoing IVF, but they did not have clear informations regarding with origin of ECF. Adversely, our study proposed to the origin of fluid generated physiologically by endometrial cavity and cases of hydrosalpinx or bilateral tubal obstruction were all excluded. Levi *et al.* (27) found that ECF increased cancellation rate in ART cycles. However, in above study, we found that cases with tubal factors and hydrosalpinges were also included. These fluid may contain embryotoxicity and cytokines which were thought to be detrimental for embryos.

According to our recent unpublished preliminary data, we found the ECF disappeared in nine patients who had no tubal disease when embryos were transferred through the cervical canal on the third day after ovum retrieval. It meant the mechanical effect which may interfere in the embryo implantation was eliminated. The incidence of the ECF in patients with healthy fallopian tubes was low. The sample size in this investigation still too small to verify the real effect of ECF on the outcomes of assisted reproductive technology, but we found the ECF occurred during ovum retrieval had no negative effect on the embryo implantation. We need to collect more cases to prove this finding in the future.

In summary, according to our study, if the ECF was not flushed backwards from hydrosalpinx, but was generated physiologically by the genital tract during COH, clinical pregnancy rate was not worse (70% versus 53%, Table II). It was assumed that ECF contained some kind of growth factors which can facilitate the implantation of embryo, but further endeavor had to be made to prove this hypothesis.

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