General Section

Single curettage endometrial biopsy injury in the proliferative phase improves reproductive outcome of subsequent in vitro fertilization-embryo transfer cycle in infertile patients with repeated embryo implantation failure

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Summary

Purpose of Investigation: To evaluate the effectiveness of single curettage endometrial biopsy injury (EBI) in the proliferative phase for *in vitro* fertilization-embryo transfer (IVF-ET) outcome of the subsequent cycle in infertile patients with repeated embryo implantation failure (EIF). *Materials and Methods:* Of 89 patients who repeated EIF three times following transfer of morphologically good embryos and/or blastocysts, 40 patients chose curettage EBI prior to the subsequent IVF-ET cycle. Using a three-mm wide curette, EBI was performed once between days 6 and 12 of the spontaneous cycle. Their IVF-ET outcomes in the subsequent cycle were compared with those in 49 patients who did not opt for EBI. *Results:* The clinical pregnancy rate (37.5% vs 12.2%), embryo implantation rate (23.6% vs 6.3%), and ongoing pregnancy rate (25.0% vs 8.2%) were significantly higher in the EBI group than in the non-EBI group. No serious complaints and complications were noted. *Conclusion:* Single curettage EBI in the proliferative phase of the preceding cycle significantly improved IVF-ET outcome in infertile patients with repeated EIF.

Key words: Endometrial injury; Proliferative phase; Repeated embryo implantation failure; Single curettage biopsy.

Introduction

Despite marked progress in assisted reproductive technology (ART), the pregnancy rate per *in vitro* fertilization-embryo transfer (IVF-ET) cycle stagnates at about one-third over the last decade [1]. One of the major unsolved problems in human reproduction is embryo implantation failure (EIF), which is recognized as negative conception following transfer of morphologically-good embryos/blastocysts. Given that the blastocyst euploidy rate obtained in ART are 50%-60% [2, 3], development of efficient therapeutic approaches to overcome EIF has a potential to improve IVF-ET outcome by 15%-25%, and is thereby eagerly awaited.

Endometrial biopsy has been widely used in gynecological practice to sample the uterine lining for histopathologic diagnosis of malignant conditions, as well as morphological dating of luteal phase in infertile women. While the diagnostic accuracy of luteal phase defect has been questioned in endometrial morphological dating [4], local injury by endometrial biopsy is emerging as a promising medical intervention to increase the successful pregnancy rate in infertile patients with repeated EIF [5].

The mechanisms underlying improvement of receptivity by endometrial biopsy injury (EBI) are not fully understood. EBI was shown to modulate the local expression of certain immunomodulators including chemokines (CCL4 and interleukin-15), cytokines (tumor necrosis factor-α), adhesion molecules (transmembrane mucin-1, laminin 4, and integrin 6), and membrane-bound proteins (uroplakin Ib, adipose differentiation-related protein, and lysosomal associated membrane protein-2), which are postulated to play a role in preparation of favorable endometrial conditions for embryo implantation [6-11].

The optimal conditions for EBI including timing, number, and techniques remain to be determined. Early reports adopted multiple EBI in the secretory phase of the preceding cycle [12, 13], but it is unknown if this phase is more beneficial than other phases. Given the burdens and costs on the patients, fewer biopsies are more acceptable in clinical practice. In the current study, the authors aimed to investigate the effectiveness and safety of single curettage EBI in the proliferative phase of the preceding cycle for IVF-ET outcome in infertile patients who repeated EIF three times following transfer of morphologically good early-cleavage embryos and/or blastocysts.

Materials and Methods

IVF protocols

Gonadotropin-releasing hormone agonist (GnRHa) short protocol or antagonist protocol was used for controlled ovarian stimulation. In the former protocol, intranasal spray of buserelin acetate (Buserecure, 600 μg/day) was initiated on day 1 of the menstrual cycle, whereas intramuscular injection of 300 IU human menopausal gonadotropin (HMG) was started on day 3. In the latter protocol, intramuscular injection of 300 IU HMG was initiated on day 3, while cetrorelix acetate was injected subcutaneously when one or more leading follicles reached a

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Table 1. — *Demographics and reproductive outcomes of infertile patients with repeated EIF.*

	EBI group (n = 40)	Non-EBI group (n = 49)	*p* value *b
Age (yrs), mean ± SD	36.0 ± 2.8	35.2 ± 3.0	0.76
BMI (kg/m^2), mean ± SD	20.6 ± 2.0	20.5 ± 1.9	> 0.9
Category			0.78
Primary	32 (80.0%)	41 (83.7%)	
Secondary	8 (20.0%)	8 (16.3%)	
Infertility etiology *a			
Male factor	13 (32.5%)	18 (36.8%)	0.66
Polycystic ovarian syndrome	4 (10.0%)	3 (6.1%)	0.70
Endometriosis	6 (15.0%)	8 (16.3%)	> 0.9
Tubal factor	10 (24.0%)	9 (18.4%)	0.61
Unexplained	12 (30.0%)	14 (28.6%)	> 0.9
No. of past EIF	3	3	1
No. of past history of assisted hatching	1.0 ± 0.7	1.1 ± 0.6	> 0.9
No. of embryos/blastocysts transferred in three failed ET cycles, mean ± SD	3.5 ± 1.0	3.5 ± 0.2	> 0.9
No. of patients with a history of past miscarriage	5 (12.5%)	2 (4.1%)	0.24
Pregnancy outcome in the subsequent IVF-ET cycle			
Positive pregnancy test rate	19/40 (47.5%)	6/49 (12.2%)	0.0002
Clinical pregnancy rate	15/40 (37.5%)	6/49 (12.2%)	0.0064
Embryo implantation rate	13/55 (23.6%)	4/63 (6.3%)	0.0091
Ongoing pregnancy rate (> 12 weeks of gestation)	10/40 (25.0%)	4/49 (8.2%)	0.041

*a: Totals are not 100 percent due to some patients having more than one diagnosis.
*b: Each *p* value represents the univariate analysis.

maximal diameter of 15 mm. On the day that at least two leading follicles reached a maximal diameter of 18 mm, 5,000 IU human chorionic gonadotropin was administrated intramuscularly. Transvaginal ultrasound-guided (TVUS-guided) oocyte pickup was performed 35 to 36 hours following hCG administration.

After being preincubated for three to four hours, the oocytes were subjected to conventional insemination or intracytoplasmic sperm injection (ICSI). On the following day, fertilization was confirmed by the presence of two pronuclei. The embryos were subjected to daily morphological evaluation. According to Veeck's classification [14], good embryo was defined as grade 1 or 2 embryo (equally cleaved blastomeres). On day 3 following insemination, one of the good embryos was transferred transvaginally into the uterine cavity using ET catheter under the guidance of transabdominal ultrasound. The remaining embryos were further cultivated in blastocyst medium. The embryos that developed to the stage of blastocysts [15] were vitrificated and frozen on day 5 following insemination. Assisted hatching using zona drilling technique was introduced to the patients and performed by the patients' preference.

Hormone replacement therapy was used in cryopreserved-thawed ET cycles. Oral conjugated equine estrogen, 1.25 mg twice daily, was introduced on day 2 of the menstrual cycle, and increased to 2.5 mg, twice daily, on day 6. Patients returned regularly for TVUS measurement of endometrial thickness on day 12 onwards. Progestogen, two mg twice daily, was introduced if endometrial thickness measured eight mm or greater. On day 5 following progestogen initiation, the blastocysts were thawed and transferred as described above.

Serum hCG concentration was measured on day 11 following ET or on day 9 following blastocyst transfer using an automated enzyme immunoassay. According to the manufacturer's guidance, the value with 2 IU/l or more was regarded as a positive pregnancy test. Luteal support with progestogen was continued until nine weeks of gestation. Clinical pregnancy was considered as the presence of intrauterine gestational sac at five weeks of gestation. Embryo implantation rate was calculated as the proportion of the ETs with a documented fetal heartbeat at seven to eight weeks of gestation. Ongoing pregnancy was defined as a viable pregnancy at 12 weeks of gestation. EIF was defined as a negative pregnancy test following transfer of high-grade early cleavage embryos and/or blastocysts.

EBI for repeated EIF

The study was approved by the local ethical committee of the Institutional Review Board. The infertile patients with a history of three EIFs were enrolled in the study under informed consent. Based upon the patient's treatment preferences, single EBI was or was not performed once between days 6 and 12 in the spontaneous cycle prior to the subsequent IVF-ET cycle. A thin metal curette (three-mm width) was inserted through the cervical os and advanced gradually into the uterine cavity until resistance was felt. After single scratch in the uterine cavity, curette was removed to confirm endometrial sampling. If endometrial tissue was absent on the curette, an additional scratch was performed. All patients were given a prophylactic two-day oral administration of clarithromycin (400 mg/day).

Statistics

Datasets were compared using two-tailed Student's t-test, nonparametric Mann-Whitney U-test, Fisher's exact test, or two-by-two contingency table in combination with Pearson's χ^2 test. A *p* value less than 0.05 was considered significantly different.

Results

From January 2010 to December 2011, 91 infertile outpatients had three consecutive negative serum pregnancy

tests despite the transfer of good early cleavage embryos and/or blastocysts. Of 89 patients enrolled in the study, 40 patients chose single curettage EBI, while others did not. There were no significant differences in the demographics including age, body mass index (BMI), and infertility etiology between the two groups (Table 1). No serious complaints and complications including uterine perforation, pelvic infection, and persistent hemorrhage were noted during and following the EBI procedure. The number of the embryos transferred in the cycle following EBI was similar ($p = 0.41$) between the EBI group (single embryo transfer in 25 patients and two-embryo transfer in 15 patients) and non-EBI group (single embryo transfer in 35 patients and two-embryo transfer in 14 patients). The number of the past assisted hatching was also similar ($p > 0.9$) between the two groups.

The overall positive pregnancy test rate was significantly higher ($p = 0.0002$, odds ratio 6.5) in the EBI group (47.5%) compared with the non-EBI group (12.2%). There were seven first-trimester miscarriages and one tubal pregnancy (treated with methotrexate administration) in the EBI group, and one first-trimester miscarriage and one second-trimester miscarriage in the non-EBI group. Twin pregnancy was noted in three women in the EBI group and none in the non-EBI group. The clinical pregnancy rate (37.5% vs 12.2%, p = 0.0064, odds ratio 4.3), embryo implantation rate (23.6% vs 6.3%, p = 0.0091, odds ratio 4.6), and ongoing pregnancy rate (25.0% vs 8.2%, $p = 0.041$, odds ratio 3.8) were significantly higher in the EBI group than in the non-EBI group.

Discussion

Based on the findings in rodents that EBI is most effective under the influence of progesterone [16], early clinical trials adopted multiple timed biopsies in the secretory phase (on day 21 and 26) of the preceding cycle [12, 13]. Intrauterine intervention during the secretory phase, however, is fraught with the risk of iatrogenic abortion. The present authors here demonstrated that the proliferative phase of the preceding cycle is an effective and safe period to perform EBI.

One recent preliminary report showed that 45% of infertile patients with a history of repeated EIF conceived clinically in controlled ovarian stimulation/fresh IVF-ET cycles following single EBI in the mid secretory phase of the preceding cycle [17], but this study lacked a control group. The present authors confirmed that single EBI significantly increases clinical pregnancy, embryo implantation, and ongoing pregnancy rates. On the contrary to the EBI in the preceding cycles, single EBI performed on the day of oocyte pickup had a negative impact on endometrial receptivity and reproductive outcome in fresh ET cycles [18, 19]. The current findings suggest that the preceding cycle of the IVF-ET cycle is a good option for single EBI.

As for the devices, many of the previous studies employed disposable flexible suction catheters to injure the uterine lining [5, 17]. These types of EBI devices are currently unauthorized and officially inaccessible in Japan. Although there are apparently no published trials that directly compared the effects between the different types of EBI devices, the current authors obtained satisfactory reproductive outcomes using a conventional curettage. No serious complaints and complications were seen following curettage biopsy. One obvious benefit of curette EBI is cost-effectiveness. The current results implicate that curettage EBI is available to infertile patients suffering from repeated EIF.

In conclusion, single curettage EBI in the proliferative phase of the preceding cycle is a safe and effective method to improve IVF-ET outcome in infertile patients with repeated EIF. The authors suggest the availability of this method in infertility treatment, although further studies are required to optimize the conditions for EBI.

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