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Pregnancy outcomes after fresh-D3 versus frozen-D5 embryo transfer in women with an ectopic pregnancy history: a retrospective cohort study

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Summary

Background: Few studies have investigated the differences between fresh D3 embryo transfer (Fre-D3) and cryopreserved-thawed D5 embryo transfer (Fro-D5) cycles in women with an ectopic pregnancy (EP) history. The aim of this study is to evaluate different pregnancy outcomes between fre-D3 and Fro-D5 embryo transfer cycles in women with an EP history. *Materials and Methods:* The authors performed a retrospective cohort study in a university-based reproductive medicine center including 2,777 Fre-D3 cycles and 1,120 fro-D5 cycles from 2009 to 2014. Ectopic pregnancy and delivery rates were measured. *Results:* The incidence of EP after IVF/ICSI in the Fro-D5 was lower than in Fre-D3 cycles (0.48% *vs.* 2.81%).There were significant differences in the clinical pregnancy rate (61.17% *vs.* 53.61%), delivery rate (50.00% *vs.* 43.62%), implantation rate (48.20% *vs.* 32.03%), and multiple pregnancy rate (26.25% *vs.* 36.68%) between Fro-D5 and Fre-D3. The Fro-D5 group had a significantly lower EP rate (0.45% *vs.* 2.84) and higher delivery (52.23% *vs.* 43.28%) and implantation rates (55.98% *vs.* 35.42%) than the Fre- D3 group. *Conclusion(s):* The results suggest that women who have an EP history have a lower recurrence risk of EP and a better pregnancy outcome, including delivery rate during Fro-D5 cycles than Fre-D3 cycles.

Key words: Ectopic pregnancy; Blastocyst transfer; In vitro fertilization; Pregnancy outcome.

Introduction

Ectopic pregnancy (EP) is a considerable source of morbidity and mortality for women during childbearing age. Previous research results concluded that EP was a risk factor for recurrent EP. Shaw *et al.* showed that women with a previous history of EP had an increased risk of recurrence [1]. Other literature reported that a higher incidence of EP occurred after various methods of assisted reproductive technology (ART, 2.2% to 4.5%) than in spontaneous pregnancy [2, 3]. The present authors' previous studies [4] have shown that women with a prior history of EP have a higher recurrence risk of EP following IVF in comparison with women with no history of EP during fresh embryo transfer (Fre-ET) cycles.

It was reported that the IVF outcomes were significantly better in the group using the freeze-all policy, compared with the group using Fre-ET [5]. The literature has stated that Fro-ET has a significantly reduced EP rate compared with Fre-ET [6-11]. However, Levi Setti *et al.* [12] reported that no significant differences were found between the use of fresh and frozen oocytes in the rates of EP and a significantly higher rate of spontaneous abortions at ≤ 12 weeks was observed in the Fro-ET group. Cheng *et al.* [13] also reported that Fro-ET was not associated with a lower incidence of EP than Fre-ET and embryo stage did not affect the rate of EP.

Few studies have investigated the differences between Fre-ET and Fro-ET cycles in women with an EP history. In this article, the authors performed a retrospective cohort study evaluating the incidence of EP in patients with history of a prior EP from spontaneous pregnancy compared to subsequent in vitro fertilization with a Fresh Day3 stage (Fre-D3) embryo versus a Froze Day 5 stage (Fro-D5) embryo transfer. In addition, the authors also investigated the main IVF/ICSI outcomes between Fre-D3 and Fro-D5 cycles, such as the clinical pregnancy, delivery, and implantation rates.

Materials and Methods

The authors performed this retrospective cohort study at the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University and collected electronic records of women who underwent IVF/ICSI treatments from January 2009 to December 2014. The method of searching and group di-

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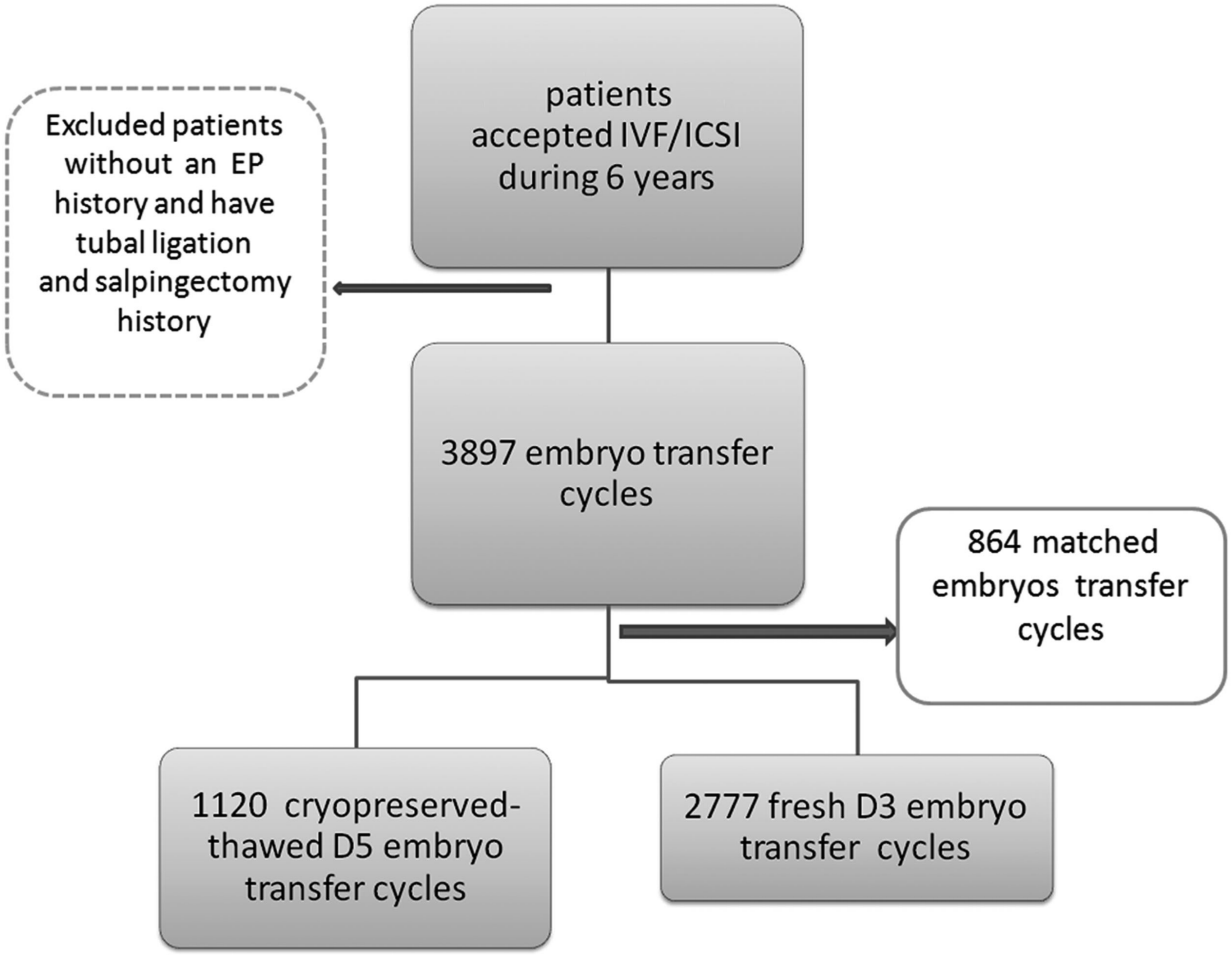

Figure 1. — Flow chart of the database searching pathway and group divisions.

visions are shown in Figure 1.

The Fre-D3 and Fro-D5 groups met the following inclusion criteria: (1) a history of a previous EP by surgical or ultrasound diagnosis from a spontaneous pregnancy; (2) intact bilateral fallopian tubes (salpingostomy or medical treatment) without mutilation of the tubes (such as tubal ligation and salpingectomy); (3) the embryo stage of Fre-ET was D3 blastomere and the embryo stage of Fro-ET was D5 blastocyst, and (4) age between 22 and 41 years at the time of the commencement of IVF/ICSI treatment. All embryos were transferred in autologous cycles. The authors excluded patients with recurrent implantation failure (RIF) from both groups. Patients with implantation failure over three cycles in which reasonably good embryos were transferred are defined as RIF in the present hospital.

The outcome rates, including ectopic pregnancy rate (EPR), clinical pregnancy rate (CPR), delivery rate (DR), miscarriage rate (MR), implantation rate (IR), and multiple pregnancy rate (MPR), were calculated according to the authors' former study [14]. Furthermore, they extracted 864 patients who had both Fre-ET and Fro-ET after a period of time in the same egg retrieval cycle. They also analyzed the pregnancy outcomes in this self-matched cohort.

Follow up regarding pregnancy outcomes was performed by routine clinic visit and phone and recorded in the electronic medical software. The end of the follow-up was August 31, 2015.

Statistical analysis was conducted by SPSS version 19.0. The *t*-test and Chi-test or Fisher's exact test were applied to obtain group comparisons. $P < 0.05$ was considered statistically.

This study was a retrospective analysis of the clinical practice outcomes, and the analysis of the data was approved by the Institutional Review Board of Shandong University.

Results

A total of 3,897 cycles were recruited with 1,120 Fro-ET cycles and 2,777 Fre-ET cycles. The baseline characteristics of all transfer types are shown in Table 1.The mean age of the Fro-ET group was lower than that of the Fre-ET group (30.8 ± 4.0 *vs.* 31.4 ± 4.2, $p = 0.000$). The proportion of PCOS patients was smaller in the Fre-ET group than in the Fro-ET group ($p = 0.000$). The pre-implantation endometrial thickness was 0.98 ± 0.35 cm in the Fro-ET group and 1.07 ± 0.19 cm in the Fre-ET group ($p = 0.000$). The main outcomes of the Fre-ET and Fro-ET cycles are shown in Table 2. The incidence of EP after IVF/ICSI in the Fro-ET group was significantly lower than in the Fre-ET group (0.48% *vs.* 2.81%, $p = 0.000$). There were also

Table 1. — *The main characteristic between frozen-thawed embryos transfer cycles and fresh embryos transfer cycles.*

ITEMS	Fro-D5 group	Fre-D3 group	*p*
Age	30.7±4.0	31.4±4.3	0.000*
BMI (kg/m^2)	23.05±3.35	23.24±3.36	0.109
Endometrial thickness	0.98±0.36	1.07±0.20[a]	0.000*
Endometriosis	13/1120(1.16%)	42/2777(1.51%)	0.400
PCOS	125/1120(11.16%)	209/2777(7.53%)	0.000*
Untreated hydrosalpingx	20/1120(1.79%)	39/2777(1.40%)	0.378

** Statistical significance.*

[a] Endometrium thickness in fresh embryos transfer cycles refer to the endometrial thickness on hCG day.

Table 2. — *The outcomes of IVF/ICSI between cryopreserved-thawed d5 embryo transfer cycles and fresh d3 embryo transfer cycles.*

ITEMS	Fro-D5 group	Fre-D3 group	*p*
CPR	712/1120(63.57%)	1498/2777(53.94%)	0.000*
EPR	5/1120(0.45%)	79/2777(2.84%)	0.000*
MR	91/1120(8.13%)	253/2777(9.11%)	0.326
DR	585/1120(52.23%)	1202/2777(43.28%)	0.000*
IR	978/1747(55.98%)	2009/5672(35.42%)	0.000*

** Statistical significance.*

EPR: ectopic pregnancy rate; CPR: clinical pregnancy rate; DR: delivery rate; MR: miscarriage rate, IR: implantation rate.

Table 3. — *The outcomes of IVF/ICSI in matched embryos transfer.*

ITEMS	Fro-D5 group	Fre-D3 group	*p*
CPR	507/864(58.68%)	221/864(25.58%)	0.000*
EPR	5/864(0.58%)	44/864(5.09%)	0.000*
MR	69/864(7.99%)	138/864(15.97%)	0.000*
DR	411/864(47.57%)	69/864(7.99%)	0.000*
IR	638/1414(45.12%)	241/1744(13.82%)	0.000*

** Statistical significance.*

significant differences in CPR (61.17% *vs.* 53.61%, p = 0.000), DR (50.00% *vs.* 43.62%, p = 0.000), IR (48.20% *vs.* 32.03%, p = 0.000), and MPR (36.68 *vs.* 25.15%, p = 0.000). However, there were no significant differences in MR (7.56% *vs.* 9.12%, p = 0.102). The EPR was significantly lower and DR, CPR, and IR were all significantly higher in the Fro-D5 group than in the Fre-D3 group.

The results of matched blastocyst transfers are presented in Table 3.The EPR was 0.58% (44/857 women) in Fro-ET and 5.13% (5/857 women) in Fre-ET cycles (p = 0.000, OR=0.1, 0.043-0.275). Significantly lower MR (p = 0.000) and higher DR (p = 0.000) and CPR (p = 0.000) were found in the Fro-ET group compared to the matched Fre-ET group.

Discussion

There has been almost no literature published targeting infertile women with a previous EP history. Therefore, this analysis aimed at comparing the risk of EP after Fre-ET with the risk of EP after Fro-ET. This study suggests that women who have an EP history have a lower recurrence risk of EP and better pregnancy outcomes, including delivery rate, during Fro- Fro-D5 than Fre-D3 cycles.

EP has been a topic of debate in the obstetric field, especially since the inception of ART. It was reported that the proportion of EP and heterotopic pregnancies combined was lower in Fro-ET cycles than in Fre-ET cycles [9]. It was also proposed that the major cause of tubal implantation is malfunction of the tube itself, although embryonic and uterine factors may also be implicated [15]. Tubal factor infertility and previous surgery for endometriosis appear to be risk factors for EP after IVF treatment [16]. Because salpingectomy or tubal ligation could significantly minimize the risk of repeated EP in the same tube and reduce fertility potential, the present authors excluded those patients. A majority of the patients included had salpingostomy or methotrexate treatment and were considered as a whole cohort.

In the present study, the number of patients combined with endometriosis was balanced. The Fro-ET group had a higher ratio of PCOS, possibly because there were more cancelled fresh cycles in PCOS patients. There were no significant differences in the number of untreated hydrosalpinx which would affect the IVF/ICSI outcomes.

An explanation for EP during IVF-ET could be impairment of tubal function and endometrial receptivity due to COH and the subsequent alteration in the hormonal milieu [17]. During Fre-ET cycles, hyperphysiologic hormonal levels caused by COH have been linked to an increased risk of EP [18]. Fro-ET occurs within a hormonal environment that is closer to a natural menstrual cycle. The present authors infer that the underlying mechanisms leading to the above results may mainly be due to the change in hormones which could create the permissive environment that promotes embryo implantation into the uterus.

To keep the interference factors to a minimum, the present study of the matched Fre-Fro comparison showed significant improvement in Fro-ET. The main differences between the two groups were the hormonal level and embryo stage. Most of the potentially confounding variables were not significantly different between the two groups and had no meaningful clinical changes. This cohort met well and the timeframe of analysis was short. Therefore, the authors conclude that the difference in outcomes was probably not due to confounding variables, but more likely due to superior endometrial receptivity in the Fro-ET group.

Both Huang *et al.* [8] and Fang *et al.* [11] reported that Fro-ET cycles were associated with a significantly lower risk of EP when compared with fresh cycles. The present

results also showed that the Fro-D5 group had better clinical outcomes than the Fre-D3 group. The embryo stage may play a more important role than the frozen policy in improving the outcomes of IVF for patients with previous EP. This was different in the Cheng *et al.* study [13].

The present research is valuable because the 1,120 cycles of the experimental group and the 2,777 cycles of the controlled group render this study one of the largest reported sample sizes. Second, the authors took into account other factors that affected the EP recurrence rate and other IVF/ICSI outcomes, including hydrosalpinx and tubal ligation or salpingectomy. They excluded patients who underwent tubal ligation after failure of Fre-ET. All of the patients had intact tubes, although some had untreated hydrosalpinx. Third, the authors focused on the special type of patients who had EP before IVF/ICSI. Although most of the previous studies discussed the differences between Fre and Fro ET in patients undergoing IVF, few focused on the special patients who had a previous EP before IVF.

Some limitations of the present research should be noted. This is a retrospective study, which can include selection bias. For example, the authors excluded those who had tubal ligation or salpingectomy to compare the difference in EP recurrence rate. They did not analyze the Fro-ET and Fre-ET outcomes in patients who had a previous EP with intact tubes. They also admit that there is significant bias in a retrospective cohort study when comparing cohorts (Fre-ET *vs.* Fro-ET) with different ages, diagnoses, and differences in clinical parameters such as endometrial thickness. The age in the two groups was not balanced. The mean age was significantly lower in the frozen group. However, there was almost no clinical significance for the differences in age between the Fro-ET group and the Fre-ET group because the mean difference value was less than one year. Rombauts *et al.* reported that thin endometrial thickness measured prior to embryo transfer is associated with higher ectopic pregnancy rate [19]. PCOS was associated with an increased risk of EP after controlled ovarian hyperstimulation (COH) in Fre-ET cycles, but not in Fro-ET cycles [20]. Therefore, both endometrial thickness and PCOS were risk factors for EP. However, the present results showed that despite those risk factors, the EP recurrence rate for Fro-ET was still lower than Fre-ET. Data were collected from one IVF center. Many of the present patients who underwent Fro-ET had previous failures with Fre-ET which may also cause bias. Moreover, follow-up of pregnancy outcomes was mainly achieved by telephone which might cause recall bias. However, in this retrospective study, the authors only included patients with full medical records.

A well-designed randomized controlled trial on this issue is needed to generate the best evidence while overcoming the pitfalls of observational studies.

Conclusions

The present results suggest that women who have an EP history have a lower recurrence risk of EP and a better pregnancy outcome, including delivery rate, during Fro-D5 cycles than Fre-D3 cycles. It could be suggested that infertile women with an EP history use single or double blastocyst Fro-ET directly. This study suggested a potential benefit for future clinical consideration. Because these patients carry a higher incidence of recurrent EP and fresh transfer may carry a higher incidence of EP than cryopreserved cycles, the authors propose the hypothesis that infertile women with an EP history should have better IVF outcomes in Fro-D5 than Fre-D3 and Fro-D5 could be directly suggested.

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