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Frozen–Thawed Embryo Transfer Cycles Have a Lower Incidence of Ectopic Pregnancy Compared With Fresh Embryo Transfer Cycles

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Abstract

Objective: To evaluate the risk of ectopic pregnancy of embryo transfer. **Design:** A retrospective cohort study on the incidence of ectopic pregnancy in fresh and frozen–thawed embryo transfer cycles from January 1st, 2010, to January 1st, 2015. **Patients:** Infertile women undergoing frozen–thawed transfer cycles or fresh transfer cycles. **Intervention:** In-vitro fertilization, fresh embryo transfer, frozen–thawed embryo transfer, ectopic pregnancy. **Main Outcome Measures:** Ectopic pregnancy rate and clinical pregnancy rate. **Result:** A total of 69 756 in vitro fertilization–embryo transfer cycles from 2010 to 2015 were analyzed, including 45 960 (65.9%) fresh and 23 796 (34.1%) frozen–thawed embryo transfer cycles. The clinical pregnancy rate per embryo transfer was slightly lower in fresh embryo transfer cycles compared with frozen–thawed embryo transfer cycles (40.8% vs 43.1%, $P < .001$). Frozen–thawed embryo transfer is associated with a lower incidence of ectopic pregnancy per clinical pregnancy, compared with fresh embryo transfers (odds ratio = 0.31; 95% confidence interval = 0.24-0.39). Female age and body mass index have no influence on ectopic pregnancy. In the frozen–thawed embryo transfer cycles, blastocyst transfer shows a significantly lower incidence of ectopic pregnancy (0.8% vs 1.8%, $P = .002$) in comparison with day 3 cleavage embryo transfer. **Conclusion:** The risk of ectopic pregnancy is lower in frozen–thawed embryo transfer cycles than fresh embryo transfer cycles, and blastocyst transfer could further decrease the ectopic pregnancy rate in frozen–thawed embryo transfer cycles.

Keywords

ectopic pregnancy, in vitro fertilization–embryo transfer, frozen–thawed embryo transfer, fresh embryo transfer

Background

Infertility has been considered to be an essential risk factor for ectopic pregnancy and undesirable pregnancy outcomes.[1] In in vitro fertilization–embryo transfer (IVF-ET), the embryos are directly transferred into the uterine cavity, but a significant number of ectopic localizations of gravidity occurred.[2] A recent review describes that the incidence of ectopic pregnancy occurring after IVF ranges from 2.1% to 8.6% of all clinical pregnancies.[3] The possible reasons are complicated, including tubal disease,[4] impaired endometrial receptivity,[5] ethnic difference,[6] and obstetric history. Other hypothesized causes include the use of ovarian stimulation, resulting in increased uterine contractions secondary to elevated estradiol (E2) levels, or ciliary dysfunction in fallopian tubes secondary to elevated progesterone (P) levels.

In recent studies, some researches indicated that frozen–thawed embryo transfer had a lower incidence of ectopic pregnancy, compared with fresh embryo transfer.[7] However, some other researches held the opposite opinion.[8] Most of these researches collected the embryo transfer cycles performed between 2000 and 2013. Meanwhile, in this decade with the rapid development of assisted reproductive technology (ART) technology, the conclusion might have deviation as a result of long period of data collection.

So we retrospectively analyzed single-center data of more than 60 000 IVF-ET cycles in 5 years and compared the

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outcomes of fresh embryo transfer or frozen–thawed embryo transfer to investigate the relationship between the types of embryo transfer and ectopic pregnancy rate.

Materials and Methods

A total of 69 756 embryo transfer cycles performed at the Reproductive Medicine Center of Peking University Third Hospital were analyzed. All embryo transfer cycles between January 1st, 2010, and January 1st, 2015, were included. This study was approved by the institutional review board of Peking University Third Hospital. A total of 39 306 patients received 45 960 fresh embryo transfers in group A, and a total of 19 498 patients received 23 796 frozen–thawed embryo transfers in group B. In group A, long agonist, antagonist, and short agonist protocols for ovarian stimulation were performed as described elsewhere.[9] Thirty-six hours before oocyte retrieval, human chorionic gonadotropin (hCG) was administered when more than 2 follicles reached 18 mm. After a holistic evaluation of patient characteristics, experienced doctors would decide whether patients receive fresh embryo transfer or frozen–thawed embryo transfer. In fresh embryo transfer cycles, most embryos were transferred on day 3 (D3) after insemination, except for patients with a history of repeated miscarriage or high risk of ovarian hyperstimulation syndrome or abnormal uterine cavity. In group B, the frozen–thawed embryos were transferred in natural cycles or artificial autologous cycles. For patients without ovulation, E2 valerate (Progynova) was given (2 mg twice daily orally from D3 to D5 and 3 mg twice daily orally from D6 of menstrual cycle). Progesterone was administered when a 9-mm-thick endometrium (Em) was visualized. The method of freezing and thawing protocol and the evaluation of embryo and blastocyst were described elsewhere.[10] The endometrial thickness was measured by ultrasound on the day of hCG trigger in fresh embryo transfer cycles, on the day before progesterone in artificial autologous cycles, or on the day before ovulation in natural cycles.

Data collected included age, obstetric history, body mass index (BMI), duration of infertility, and indication for IVF. To confirm the early pregnancy, serum hCG was measured 13 days after the cleavage embryo transfer and 11 days after the blastocyst transfer. Ultrasound scan was performed 30 to 35 days after embryo transfer. An intrauterine clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity in the uterine cavity. An ectopic pregnancy was defined when a gestational sac with fetal heart activity was determined outside the uterine cavity or as an empty uterine cavity with increasing hCG levels which was confirmed by surgery and pathology. The cases with heterotopic pregnancy were included as ectopic pregnancy.

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software, version 23.0 (IBM, Armonk, New York, USA). Univariate analysis was used to compare baseline characteristics. Pearson χ^2 test was applied to detect the difference between the fresh embryo transfer cycle and frozen–thawed transfer cycle in terms of ectopic pregnancy rate. A multivariate logistic regression model was used to estimate the risk factors on ectopic pregnancy. All reported *P* values were 2 tailed, and $P < .05$ was established as the level of significance.

Table 1. Baseline Characteristics of Fresh and Frozen–Thawed Embryo Transfer Cycles.

	Group A (n = 45 960)	Group B (n = 23 796)
Female age at transfer, years, mean (SD)	33.02 (4.95)	32.31 (4.54)
BMI, kg/m^2, mean (SD)	22.51 (3.33)	22.22 (3.27)
Duration of infertility, years, mean (SD)	5.17 (3.91)	4.71 (3.42)
Type of infertility, no. (%)		
Primary	24 632 (53.6)	13 842 (58.2)
Secondary	21 328 (46.4)	9902 (41.6)
Causes of infertility		
N (number of patients)	39 306	19 498
Tubal (per patient%)	18 325 (46.6)	8643 (44.3)
Endometriosis (per patient%)	2238 (5.7)	749 (3.8)
PCOS (per patient%)	3636 (9.3)	2433 (12.5)
Uterine issues (per patient%)	1891 (4.8)	821 (4.2)
Diminished ovarian reserve (per patient%)	2804 (7.1)	443 (2.3)
Male factors (per patient%)	17 530 (44.6)	7882 (40.4)
Unexplained (per patient%)	4434 (11.3)	2566 (13.2)
Others (per patient%)	3071 (7.8)	274 (1.4)

Abbreviations: BMI, body mass index; PCOS, polycystic ovary syndrome.

Results

A total of 45 960 fresh (group A) and 23 796 frozen–thawed embryo transfer cycles (group B) were included in our study (Tables 1 and 2). We compared the baseline characteristics of women undergoing fresh or frozen–thawed embryo transfer and the causes of infertility. The overall clinical pregnancy rate was 40.8% in fresh embryo transfer, slightly lower than that in frozen–thawed transfer cycle (43.1%). Among all the embryo transfer cycles, 856 ectopic pregnancies occurred. The ectopic pregnancy rate per embryo transfer was 1.2%, while the ectopic pregnancy rate was 2.9% per clinical pregnancy. Compared with fresh embryo transfer cycles, the ectopic pregnancy rates is statistically lower in frozen–thawed embryo transfer cycles.

In order to explore the potential influence factors, a multivariate logistic regression analysis was performed in the 29 030 transfer cycles that were confirmed clinical pregnancy as mentioned before. The results showed that age, BMI, and types of embryo were not responsible for ectopic pregnancy. Instead, fresh embryo transfer was an independent risk factor for ectopic pregnancy (odds ratio [OR] = 0.31; 95% confidence interval [CI] = 0.24-0.39; Table 3). Secondary infertility had a higher incidence of ectopic pregnancy compared with primary infertility. The thickness of Em is a possible protective factor of ectopic pregnancy.

In fresh embryo transfer cycles, 96.4% were D3 embryo transfer, while only 3.6% were transferred in blastocyst stage. By contrast, about 18.3% of embryos transferred in frozen–

Table 2. Clinical Pregnancy and Ectopic Pregnancy Rates.

	Group A (n = 45 960)	Group B (n = 23 796)	*P* Value
Clinical pregnancy (per ET%)	18 765 (40.8)	10 265 (43.1)	<.001
Ectopic pregnancy no.	694	162	
Per ET%	1.5	0.7	<.001
Per clinical pregnancy%	3.7	1.6	<.001

Abbreviation: ET, embryo transfer.

Table 3. Multivariate Logistic Regression Analyses of Potential Confounding Factors Influencing Pregnancy Outcomes.

	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Female age	1.03 (1.01-1.04)	.002	1.01 (0.99-1.03)	.201
BMI	1.00 (0.98-1.02)	.724	0.99 (0.97-1.01)	.481
Secondary infertility	1.35 (1.18-1.55)	<.001	1.19 (1.03-1.38)	.021
Thickness of Em	0.86 (0.83-0.90)	<.001	0.78 (0.74-0.81)	<.001
D5 embryo	0.49 (0.36-0.66)	<.001	0.79 (0.56-1.10)	.163
Frozen transfer cycle	0.42 (0.35-0.50)	<.001	0.31 (0.24-0.39)	<.001

Abbreviations: BMI, body mass index; CI, confidence interval; D5, day 5; Em, endometrium; OR, odds ratio.

Table 4. The Different Outcomes Between D3 Cleavage and Blastocyst Transfer.

	Group A			Group B		
Outcome	D3 (n = 44 323, 96.4%)	Blastocyst (n = 1636, 3.6%)	*P* Value	D3 (n = 19 425, 81.6%)	Blastocyst (n = 4360, 18.3%)	*P* Value
Clinical pregnancy (per ET%)	18 148 (40.9)	617 (37.7)	.009	7966 (41.0)	2297 (52.8)	<.001
Ectopic pregnancy no.	669	25		142	20	
Per ET (%)	1.5	1.5	.951	0.7	0.5	.048
Per clinical pregnancy (%)	3.7	4.1	.636	1.8	0.8	.002

Abbreviations: D3, day 3; ET, embryo transfer.

thawed transfer cycles were blastocysts. In fresh transfer cycles, there was no significant difference between D3 embryo and blastocyst transfer in terms of ectopic pregnancy. On the contrary, in the frozen–thawed transfer cycles, compared with D3 embryo transfer, blastocyst transfer was associated with a lower incidence of ectopic pregnancy (0.8% vs 1.8%, $P = .002$) and a higher pregnancy rate (Table 4). We further performed a multivariate logistic regression analysis to investigate which indication of IVF accompanies with higher risk of ectopic pregnancy in fresh embryo transfer cycles. Among tubal factors, polycystic ovary syndrome, uterine issues, diminished ovarian reserve, male factors, unexplained causes, tubal factors, and diminished ovarian reserve are independent risk factors for ectopic pregnancy (OR = 1.34, 95% CI = 1.13-1.58 and OR = 1.62, 95% CI = 1.19-2.22).

Discussion

This study revealed that frozen–thawed embryo transfer was associated with a lower incidence of ectopic pregnancy compared with fresh embryo transfer, which reached statistical significance. Furthermore, the study analyzed the influence of baseline characteristics, day of transplantation, and causes of infertility, which displayed that blastocyst transfer could decrease ectopic pregnancy rate in frozen–thawed embryo transfer cycle.

The ectopic pregnancy rate in the present study is similar to the data reported perviously, which ranged from 1.5% to 2.4% for fresh embryo transfer cycle and from 0.8% to 1.8% for frozen–thawed embryo transfer cycle.[5,6,8,11] Shapiro et al revealed the rate of visualized ectopic pregnancy was 1.5% in pregnancies in fresh autologous cycles, which was evidently higher than the rate of 0% with autologous post-thaw extended culture. The rates of treated persistent pregnancy of unknown location were 2.5% and 0.3% in these 2 groups, which reached statistically significance (OR = 7.3, 95% CI = 1.7-31.0).[5] Ishihara et al reported the odds ratio of an ectopic pregnancy after frozen–thawed transfer was 0.60 (95% CI = 0.45-0.81) times lower than fresh transfer and 0.61 (95% CI = 0.46-0.82) times lower in blastocyst transfer than in cleaved embryo transfer.[11] In our study, we analyzed all the transfer cycles in the period, and the difference in ectopic pregnancy rate between fresh and frozen–thawed embryo transfer cycle was corresponded with previous researches.[5,8,11] Recently, a retrospective cohort study of 509 938 cycles indicated that fresh embryo transfers had a higher risk of ectopic/heterotopic pregnancy but a lower risk of abnormal implantation. The incidence of biochemical pregnancy and pregnancy loss in first trimester is higher in frozen–thawed embryo transfer.[12] Whether "freeze-all" cycles should be advised or not remain controversial, further researches need to be carried out to provide patients with individual treatment plan. We further analyze the risk factors for ectopic pregnancy in fresh embryo transfer cycles, which indicated that patients with tubal factors or diminished ovarian reserve are more likely to suffer ectopic pregnancy.

Most embryos are transferred into the uterine cavity in cleavage stage. But during natural conception, most of embryos in this stage are still located in the fallopian until developing into blastocysts, which means that during this period cleavage embryo transfer may be a risk factor for ectopic pregnancy. Huang et al revealed that fresh D3 embryo transfer cycles had a significantly higher risk of ectopic pregnancy than D3 embryo frozen–thawed embryo transfer cycles; furthermore, the blastocyst frozen–thawed embryo transfer cycles had the lowest risk of an ectopic pregnancy.[6] Other studies have provided similar results.[13] In our study, we found that there was no significant difference between blastocyst and D3 cleavage embryo in terms of ectopic pregnancy in fresh embryo transfer cycles. But in the frozen–thawed transfer cycles, compared with D3 cleavage embryos, blastocyst transfer had a lower incidence of ectopic pregnancy (0.8% vs 1.8%, $P = .002$). The conclusion may indicate that in fresh embryo transfer cycles, the endocrine environment may be the main factor to elevate the ectopic pregnancy rate but not the quality of embryo.

Ishihara et al indicated that ovarian stimulation had a negative effect on endometrial receptivity.[11] Excessive ovarian response and GnRH agonist trigger were found to be independent risk factors for ectopic pregnancy.[14] Some hypotheses have been proposed to explain the increased risk of ectopic pregnancy after ovarian stimulation including an altered endocrine environment that may affect embryo–tubal transport, uterine motility, and contractions.[15,16] Elevated P levels on the day of trigger during the initial fresh cycle were negatively related to live birth in the fresh transfer cycles but not in subsequent frozen–thawed embryo transfer cycles,[17] which may prove that the endocrine environment of fresh transfer cycle is not favorable for embryo implantation. Furthermore, the process of freezing and thawing may sift the embryos of poor quality and further improve the outcome of embryo transfer. We found that the thickness of Em is a possible protective factor for ectopic pregnancy (OR = 0.78, 95% CI = 0.74-0.81). It has been reported that the risk of ectopic pregnancy is 4-fold increased in women with an endometrial thickness of <9 mm compared with women with an endometrial thickness of >12 mm.[18]

There are limitations to the present study. Although the study enrolled more than 69 000 cycles, it is limited by the retrospective nature of the analysis. Additional, preferably prospective, cohort study will be needed. More work need to be done to analyze the ectopic pregnancy rate of women with different causes of infertility and various protocols and dose for ovarian stimulation.

Conclusion

Compared with fresh embryo transfer, frozen–thawed embryo transfer has a lower incidence of ectopic pregnancy. Frozen–thawed embryo transfer may be a better choice for patients with potential risks of ectopic pregnancy. Blastocyst transfer could reduce ectopic pregnancy rate in frozen–thawed embryo transfer.

Authors' Note

Xinyu Zhang and Caihong Ma equally contributed to the work.

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Declaration of Conflicting Interests

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