Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles

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Objective: To analyze the incidence of ectopic pregnancy (EP) in fresh compared with frozen-thawed cycles.
Design: Retrospective cohort study.
Setting: Teaching hospital.
Patient(s): Thirty-one thousand nine hundred twenty-five women undergoing in vitro fertilization–embryo transfer (IVF-ET) from January 2006 to December 2013.
Intervention(s): Fresh IVF-ET compared with frozen-thawed ET (FET).
Main Outcome Measure(s): Incidence of EP with fresh IVF-ET compared with frozen-thawed ET cycles, clinical pregnancy rate, and rate of EP per clinical pregnancy.
Result(s): For the fresh IVF cycles, 19,173 patients underwent oocyte retrieval; 15,042 had an ET, 6,431 of these patients (42.7%) had a clinical pregnancy, and among these 297 (1.97%) appeared to have an EP. The group of patients undergoing frozen-thawed ET (12,752 patients) included 12,255; there were 5,564 pregnancies (45.4%) and 124 ectopic implants (1.01%). The incidence of an EP per clinical pregnancy was 4.62% for the fresh transfer group compared with 2.22% for the frozen-thawed cycle group; this difference was statistically significant. In addition, the fresh ET cycles had the highest risk of EP, followed by day-3 embryo FET cycles; blastocyst FET cycles had the lowest risk of EP, and the differences were all statistically significant.
Conclusion(s): Frozen-thawed ET cycles were associated with a statistically significantly lower risk of EP when compared with fresh cycles. These findings are consistent with ovarian stimulation being associated with an increased risk of EP. (Fertil Steril® 2014;102:1345–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ectopic pregnancy, embryo cryopreservation, fresh cycle, frozen-thawed ET, ovarian stimulation

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An ectopic pregnancy (EP) is defined as a pregnancy implanted outside of the uterine cavity. Over 98% of EPs implant within the fallopian tubes (1, 2), commonly referred to as a tubal pregnancy. In vitro fertilization–embryo transfer (IVF-ET) is one of the major risk factors for an EP (3, 4). In fact, the first IVF treatment resulted in an EP (5). The reason why this pathology remains a common association of IVF is unclear (6). After IVF, embryos are transferred directly into the uterine cavity, and this process associated with assisted reproduction techniques (ART) would actually seem to reduce the risk of an EP. However, a significant number of ectopic pregnancies still occur. Reported rates of EPs in women undergoing IVF range from 2% to 5% (4, 6), which is higher than the rate among spontaneous pregnancies at 1%–2% (7, 8). Some of the hypotheses reported to explain the increased risk for EP associated with ART are tubal disease (9, 10), increased

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uterine contractions due to ovarian stimulation (11, 12), and dysfunction of the uterine musculature due to high progesterone levels (13).

Recent research has suggested that frozen-thawed ET (FET) is associated with a greatly reduced incidence of EP compared with fresh transfers (14, 15). However, ART data from clinics in Belgium from 2002–2012 reported no statistically significant difference in the incidence of EP in comparisons between fresh IVF cycles and frozen-thawed cycles (16). Therefore, further study is needed to determine whether FET is associated with a different EP risk compared with fresh cycles. If the risk for EP is reduced with FET embryos, cryopreservation might also reduce the risks associated with ovarian hyperstimulation. We retrospectively analyzed the data from an IVF center in the People's Republic of China from January 2006 to December 2013 to determine whether FET was associated with a significantly lower risk for EP compared with fresh cycles.

MATERIALS AND METHODS

This was a noninterventional, retrospective, single-center cohort study of patients in a routine clinical practice. To reflect the broad range of patients typically encountered in this clinical practice, no inclusion/exclusion criteria were applied to the baseline characteristics. Patients were treated at the Reproductive Medicine Center of Tongji Hospital from January 2006 to December 2013. A total of 31,925 patients were enrolled, and all patients gave written informed consent. Institutional review board approval was not required because all patients in this study underwent routine long gonadotropin-releasing hormone (GnRH) agonist IVF-ET clinical treatment at the center, and no additional intervention was performed.

All patients participating in fresh cycles underwent controlled ovarian stimulation according to the routine long GnRH agonist protocol. Pituitary suppression was achieved by daily subcutaneous injection of triptorelin acetate (Decapeptyl; Ferring) starting at the midluteal phase of the preceding cycle. When complete pituitary desensitization was confirmed by a low plasma estradiol (E_2) level of ~30 pg/mL and a luteinizing hormone (LH) level of ~2 mIU/mL, ovarian stimulation was started with the administration of recombinant follicle-stimulating hormone (FSH) (Gonal F, Serono; or Puregon, Schering-Plough). Recombinant human chorionic gonadotropin (hCG) (250 mg; Ovidrel; Serono) was given to trigger ovulation when two leading follicles reached a mean diameter of 18 mm. Oocytes were retrieved transvaginally 34 to 36 hours after hCG administration.

Fertilization of the oocytes took place either by IVF or intracytoplasmic sperm injection (ICSI), according to the sperm quality. The methods for sperm preparation, IVF, and embryo culture have been described previously elsewhere (17). Briefly, semen was collected in sterile containers by masturbation after 3 to 5 days of sexual abstinence and then was maintained at 37°C for 30 minutes. After liquefaction, the samples were analyzed for sperm concentration, motility, and morphology according to World Health Organization criteria (18). The oocytes were incubated in G-IVF medium (Vitrolife) and fertilized 3 to 4 hours after retrieval. Normally, fertilized oocytes were continuously cultured in G1 medium for 2 more days. Usually fewer than two best-quality embryos were transferred on day 3 after oocyte retrieval, according to the protocol developed by Chinese legislation. The additional good-quality embryos or blastocysts were cryopreserved for subsequent FET cycles (by slow freezing). From 2009 onward, vitrification was used for embryo cryopreservation at this center.

The FET cycles were from both natural cycles after spontaneous ovulation and hormone replacement treatment (HT) cycles. For the natural cycles, transvaginal ultrasound scans and measurement of the serum progesterone levels was initiated from cycle days 10–12 to assess endometrial thickness, follicle growth, and ovulation. Frozen-thawed ET was planned for 3 days after ovulation. Progesterone administration was started for luteal support from 1 day after ovulation. For the HT cycles, oral estradiol (Progynova; Bayer) was provided, 2 mg/day from cycle days 1–4, 4 mg/day from days 5–8, and 6 mg/day from days 9–12. Transvaginal ultrasound scanning was performed to assess the endometrial thickness and ovulation from day 13; the estradiol dosage was adjusted based on the endometrial thickness. We administered 40 mg of progesterone intramuscularly when the endometrium reached a thickness of 8 mm or maximum. Administration of 60, 80, or 80 mg of progesterone was provided for the following 3 days. Embryo transfer was performed on day 4, after 3 days of progesterone administration.

Serum hCG was measured to diagnose a pregnancy 2 weeks after ET and then was tested serially to monitor rising titers. A clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity observed on ultrasound examination 5 weeks after oocyte retrieval (19). An EP was defined when a pregnancy was determined and accompanied by sonographic visualization of an extrauterine gestational sac (including any heterotopic gestations) or with an empty uterine cavity and increasing hCG levels (20).

All data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 13.0 (IBM). The data were analyzed to compare fresh cycles with FET cycles. For the FET group, the data from day-3 ET were compared with the blastocyst transfer group and analyzed. For the day-3 FET cycles, the difference between the two methods of ET was also analyzed. The differences in outcomes between the two groups were analyzed using chi-square tests. $P < .05$ was considered statistically significant.

RESULTS

From January 1, 2006, to December 2013, 19,173 fresh cycles were included in the study, and 258,625 oocytes were retrieved. The overall clinical pregnancy rate (PR) per oocyte retrieval for this 8-year period was 33.5%, and the PR per transfer was 42.7% (Table 1). The etiology of infertility for these pregnancies included tubal factors (61.5%), ovarian factors (4.2%), endometriosis (2.4%), male-factor infertility (23.8%), and others. There were 297 EPs (1.97%) that occurred after the transfer cycles. During the same period, a total of 12,752 FET cycles were enrolled. The PR of the FET cycles was 45.4% per ET, which was statistically significantly higher

TABLE 1

Outcomes for the two study groups (2006–2013).

Outcome	Fresh	Frozen	Total	*P* value
Retrieval	19,173		19,173	—
Retrieval with ET	15,042		15,042	—
Cryopreservation		12,752	12,752	—
Frozen-thawed with ET		12,255	12,255	—
Clinical pregnancy (% per ET)	6,431 (42.7)	5,564 (45.4)	11,995	<.001
Ectopic pregnancy				
Number	297	124	421	—
Per ET (%)	1.97	1.01	1.54	<.001
Per clinical pregnancy (%)	4.62	2.22	3.51	<.001
Tubal factor				
In clinical pregnancy (%)	3,952 (61.5)[a]	3,362 (60.4)	7,314 (61.0)	.307
In EP (%)	212 (71.4)	81 (65.3)	293 (69.6)	.245

Note: ET = embryo transfer; EP = ectopic pregnancy.
[a] Compared with tubal factor in EP, *P*< .001.

Huang. FET associated with lower risk of EP. Fertil Steril 2014.

than for the fresh cycles (*P*< .001). The etiology of infertility for FET pregnancies included tubal factors (60.4%), ovarian factors (5.6%), endometriosis (2.1%), male-factor infertility (21.9%), and others.

There were 124 patients found to have extrauterine pregnancies. The incidence of EP for the FET cycles was 1.01%. These results suggest that FET cycles were associated with a statistically significantly lower incidence of EP (*P*< .001). The proportion of patients with a clinical pregnancy who had tubal factor infertility was similar in the fresh and FET groups (*P*= .307). However, in both the fresh and FET groups, the proportion of patients with an EP and tubal factor infertility was higher; for those patients with a clinical pregnancy, in the fresh group, this difference was statistically significant (*P*< .001).

Among 12,752 FET cycles, patients could be divided into two groups: a day-3 embryo FET group and a blastocyst FET group. Clinical outcomes of all FET cycles in each group were evaluated (Table 2). The results showed that the blastocyst FET group obtained a statistically significantly higher PR (63.3%) compared with the day-3 embryos FET group (*P*< .001). In addition, there was a statistically significant difference between the two groups with regard to the risk of a pregnancy being localized outside of the uterus. To investigate the EP risk among fresh ET cycles, day-3 embryo FET cycles, and blastocyst FET cycles, the incidence of EP was compared among these three groups. As shown in Figure 1, the results demonstrated that fresh ET cycles had the highest risk for an EP, followed by day-3 embryo FET cycles; blastocyst FET cycles had the lowest risk of EP. These differences were all statistically significant.

For the FET cycles, there were two frozen embryo methods used in this study: slow freezing and vitrification (Table 3). There were no statistically significant differences in the PR and the risk for EP between these two frozen embryo methods. With regard to the EP risk for achieved pregnancies, the risk associated with FET cycles with vitrification (4.68%) was higher than that associated with slow freezing cycles (3.36%). However, these differences did not reach statistical significance (*P*= .126).

TABLE 2

Outcomes for the frozen-thawed groups (2006–2013).

Outcome	Day-3 embryo	Blastocyst	Total	*P* value
Cryopreservation	7,708	5,044	12,752	—
Frozen-thawed with ET	7,302	4,953	12,255	—
Clinical pregnancy (n)	2,430 (33.3)	3,134 (63.3)	5,564	<.001
Ectopic pregnancy				
Number	91	33	124	—
Per ET (%)	1.25	0.67	1.01	.002
Per clinical pregnancy (%)	3.74	1.05	2.22	<.001

Note: ET = embryo transfer.

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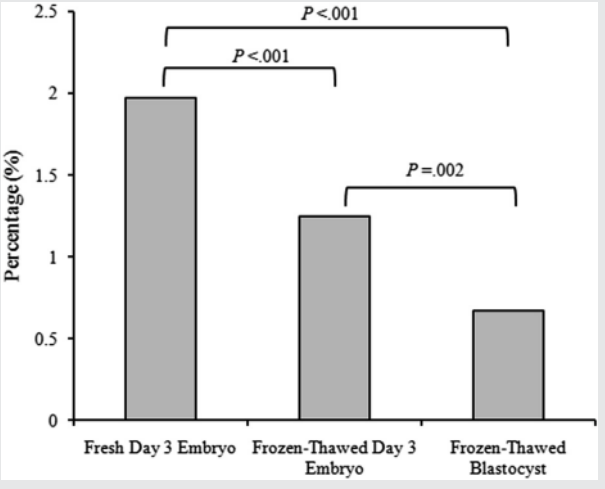
DISCUSSION

This retrospective study including more than 30,000 cycles revealed that the FET cycles were associated with a statistically significantly lower incidence of ectopic pregnancies. The rate of EP after IVF remains higher (approximately 2%–5%) than the rate of EP associated with spontaneous pregnancies (21). The reason for the increased incidence of tubal ectopic pregnancy after IVF remains unclear (4). However, the results of this study showed that ovarian stimulation with fresh cycle transfers was associated with an increased risk for EP.

Some hypotheses have been offered to explain the increased risk of EP after ovarian stimulation; these include

FIGURE 1



The ectopic pregnancy rate for fresh day-3 embryos, frozen-thawed day-3 embryos, and frozen-thawed blastocysts.

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TABLE 3

Results of the two frozen embryo methods from day-3 embryo frozen-thawed transfer cycles (2006–2013).

Outcome	Vitrification	Slow freezing	Total	*P* value
Cryopreservation	2,196	5,512	7,708	—
Frozen-thawed with ET	2,132	5,170	7,302	—
Clinical pregnancy (n)	705 (33.1)	1,725 (33.4)	2,430	.827
Ectopic pregnancy				
Number	33	58	91	—
Per ET (%)	1.55	1.12	1.25	.163
Per clinical pregnancy (%)	4.68	3.36	3.74	.126

Note: ET = embryo transfer.

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an altered endocrine environment that may affect embryo-tubal transport, uterine motility, and contractions (22–24). In addition, Revel et al. (6) compared the expression of E-cadherin at tubal implantation sites of spontaneous tubal pregnancies and tubal pregnancies following IVF using immunohistochemistry. The E-cadherin protein was found to be more strongly expressed at the tubal implantation sites in women with an IVF pregnancy compared with those with a spontaneous pregnancy. The results of this study demonstrated a difference in the EP rate between embryos transferred during fresh cycles compared with embryos transferred during FET cycles; although the etiology of infertility in the two groups was similar, especially the proportion of patients with tubal factor infertility ($P=.307$). These findings may support the suggestion that a supraphysiologic hormonal milieu increases the risk of EP, as noted by Clayton et al. (10) and Shapiro et al. (14). In addition, these findings support the results of Ishihara et al. (15) that showed a significantly higher EP rate associated with fresh cycles among 10,312 clinical pregnancies; this national registry study during 2008 indicated that ovarian stimulation had a negative effect on endometrial receptivity. In addition, among the 297 patients with EP after fresh cycles, 184 had FET from the same oocyte retrieval. There were 103 patients with an intrauterine pregnancy and 3 with an EP. This might support that a supraphysiologic hormonal milieu increases the risk of an EP. However, the data are limited by the one-child policy in the People's Republic of China and must be further evaluated by more extensive studies.

Some researchers have hypothesized that tubal factor infertility leads to an increased risk for EP (9, 10, 25). The results of our study showed that for both the fresh and FET groups, the proportion of patients with an EP and tubal factor infertility was higher, and for those patients who achieved a clinical pregnancy the difference was statistically significant for the fresh ET group ($P<.001$). This result is consistent with tubal disease increasing the risk for an EP.

Of note was that the blastocyst FET cycles had the lowest risk for an EP (Fig. 1) and the highest PR (Table 2) among all fresh and FET cycles. These findings support the conclusion of Clayton et al. (10) and Shapiro et al. (14) that a high implantation potential was protective against an EP.

In addition, as shown in Figure 1, fresh day-3 embryo ET cycles had a significantly higher risk for an EP than day-3 embryo FET cycles. This finding suggests that the implantation potential that protects against an EP requires favorable endometrial receptivity and that endometrial receptivity might be impaired by ovarian stimulation (26–29). Furthermore, in our center, most fresh ET cycles were transferred on day 3 after oocyte retrieval, and fresh blastocyst ET was not a routine clinical practice. We do not have data for fresh blastocyst transfer, so we could not compare the prevalence of EP on day-3 and day-5 ET for both fresh ET and FET.

With regard to the comparison of the EP risk associated with FET cycles and embryos cryopreserved by slow-freezing and vitrification, currently there are no data from other reproductive centers showing an EP risk associated with either of these freezing methods. In our study, the risk associated with vitrification cycles and FET was higher than that associated with slow freezing cycles; however, the difference was not statistically significant. These findings need to be further evaluated by more extensive studies. Some reports (16, 30) have found no significant difference in the EP rate between fresh and FET cycles.

CONCLUSION

The results of our study clearly illustrate that FET cycles are associated with a statistically significantly lower risk of an EP. A decreased risk of EP could be an important outcome in ART. Freezing embryos during the fresh cycle and transferring them during a natural cycle or HT cycle would support the freezing of all embryos. Our findings have shown that embryo cryopreservation could reduce the risk of EP without losing the chance of obtaining an intact intrauterine pregnancy.

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