

# Factors associated with the incidence of ectopic pregnancy in women undergoing assisted reproductive treatment

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## Abstract

**Background:** Ectopic pregnancy (EP) is a common complication in women undergoing assisted reproductive treatment, but the underlying causes for this remain unclear. This study aimed to explore factors affecting the incidence of EP in *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI).

**Methods:** This was a retrospective study on the incidence of EP in IVF/ICSI cycles between January 1, 2013 and December 31, 2017. Patient age, infertility diagnosis (tubal factor or not), primary or secondary infertility, type of cycle (frozen-thawed or fresh), type of embryo(s) transferred (cleavage embryo or blastocyst), number of embryos transferred (one, two, or three), previous history of EP, and endometrial combined thickness were analyzed to explore their relationships with the incidence of EP. Based on clinical testing results, the patients were divided into an EP group or a non-EP group. Categorical variables were analyzed using Chi-squared test or Fisher exact test. Logistic regression analysis was performed to explore their associations with the incidence of EP.

**Results:** The percentage of patients with primary infertility in EP group was significantly lower than that in non-EP group (31.3% vs. 46.7%,  $\chi^2 = 26.032$ ,  $P < 0.001$ ). The percentage of patients with tubal infertility in EP group was also significantly higher than that in non-EP group (89.2% vs. 63.6%,  $\chi^2 = 77.410$ ,  $P < 0.001$ ). The percentages of patients with transfer of cleavage-stage embryo or blastocyst (91.4% vs. 84.4%,  $\chi^2 = 10.132$ ,  $P = 0.001$ ) and different endometrial combined thickness (ECT) ( $\chi^2 = 18.373$ ,  $P < 0.001$ ) differed significantly between EP and non-EP groups. For patients who had a previous history of one to four EPs, the percentage of patients undergoing transfer of a cleavage-stage embryo was significantly higher in EP group than that in non-EP group (92.2% vs. 77.6%,  $\chi^2 = 13.737$ ,  $P < 0.001$ ). In multivariate logistic regression analysis, tubal infertility was strongly associated with EP (adjusted odds ratio: 3.995, 95% confidence interval: 2.706–5.897,  $P < 0.001$ ).

**Conclusions:** In IVF/ICSI cycles, transfer of a blastocyst-stage embryo, especially for patients with a previous history of EP, reduced the rate of EP. Tubal infertility was strongly associated with EP.

**Keywords:** Prognostic factor; Ectopic pregnancy; Tubal factor; *In vitro* fertilization; Intracytoplasmic sperm injection

## Introduction

Ectopic pregnancy (EP) is an obstetric condition potentially associated with maternal death in the first trimester. It is a well-known risk in cases involving the use of assisted reproductive technology (ART). The rate of EP following ART varies widely, ranging from 1.6% to 8.6% and being over four times that reported for natural conceptions.<sup>[1-3]</sup>

EP has, therefore, been well studied and known factors for it have been identified, including endometriosis, inflammatory disease, and smoking. More recently identified prognostic factors for EP associated with ART include ethnicity, fresh embryo transfer (ET), number of embryos transferred, volume of transfer fluid, and transfer depth.<sup>[2]</sup>

Because many factors are associated with EP, several EP-associated factors should be analyzed at the same time. However, there have been few studies involving multivariate analysis with a large sample size on the effects of multiple factors on EP. In 2016, Bu *et al*<sup>[4]</sup> analyzed 18,432 ART cycles and reported that, for fresh *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles, the rate of EP was positively associated with ovarian stimulations, and that thawed cycle blastocyst transfer or transfer with fewer embryos reduced the EP rate. In addition, Cheng *et al*<sup>[5]</sup> analyzed 3006 IVF cycles and found that thawed ET was not associated with a lower incidence of EP than fresh ET; they also found that the

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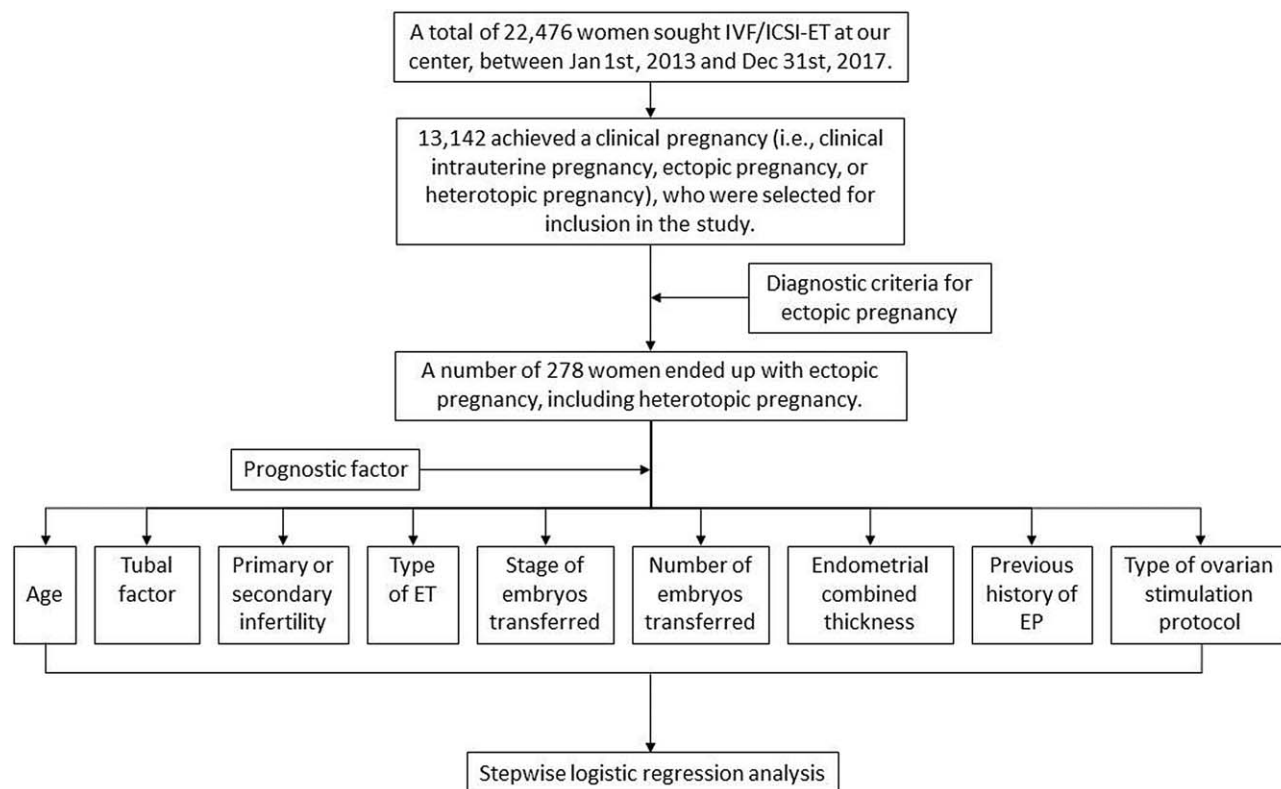
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**Figure 1:** Outline of the selection process for the cycles included in the study. EP: Ectopic pregnancy; ET: Embryo transfer; ICSI: Intracytoplasmic sperm injection; IVF: *In vitro* fertilization.

embryo stage at transfer did not affect the rate of EP. In addition, tubal ET and ET under full bladder distention had significant effects on EP.

Thus, we analyzed the prognostic factors of EP to explore possible ways to reduce the rate of EP in ART. In this retrospective study, we analyzed 13,142 IVF/ICSI cycles during 5 years at our center. We hypothesized that factors associated with EP may be different in multivariate analysis.

## Methods

### Ethical approval

This study was conducted in accordance with the *Declaration of Helsinki* and approved by the Ethics Committee of Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (No. SRRSHRMEC2019001). The requirement to obtain informed consent was waived.

### Study design

This was a retrospective study carried out at the Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, China, between January 1, 2013 and December 31, 2017. During the study period, of a total of 22,476 women seeking IVF/ICSI-ET at our reproductive center, 13,142 achieved a clinical pregnancy (ie, clinical intrauterine pregnancy, EP, or heterotopic pregnancy) and were included in the final analysis in this study. Figure 1 shows the outline of the selection process for the cycles included in the study.

## Definitions

In accordance with the definitions and guidelines established by the Society for Assisted Reproductive Technology, an intrauterine gestation is defined as the presence of one or more gestational sacs recorded by ultrasound or by documentation of a birth, spontaneous abortion, or therapeutic abortion in cases of missing ultrasound data. A biochemical pregnancy is defined in the registry as the occurrence of a positive pregnancy test without a visible gestational sac and no clinical diagnosis of pregnancy. An EP is reported when there is a gestational sac observed by ultrasound outside the uterine cavity, and a heterotopic pregnancy is defined by the coexistence of a clinical intrauterine gestation and an EP. The incidence of EP was calculated as the number of EPs per hundred positive pregnancy tests after ART.

For the ART follow-up procedure at our center, all patients were asked to come back for a blood  $\beta$ -human chorionic gonadotropin ( $\beta$ -hCG) test 12 days after ET.  $\beta$ -hCG levels below 5 IU/L were considered negative, those between 5 and 15 IU/L were considered indeterminate, and those above 15 IU/L were considered positive. Patients with indeterminate  $\beta$ -hCG levels were considered positive if their  $\beta$ -hCG increased after 48 h. Those patients with increasing  $\beta$ -hCG were followed sonographically 22 days after ET for the first time, especially for those with a history of EP or previous tubectomy to rule out EP earlier. In addition, 35 days after ET, they underwent a second ultrasound scan to confirm the presence of a gestational sac with fetal heart in the uterus (especially transvaginal). Only

a small number of the diagnoses of ectopic/heterotopic pregnancy were made in other hospitals; for those cases, we confirmed that the diagnostic criteria of EP or heterotopic pregnancy were the same as those in our hospital. As the main purpose of our study was to investigate factors associated with the occurrence of EP, heterotopic pregnancy was also considered to be an EP in our analysis.

### Ovarian stimulation protocol

Two ovarian stimulation protocols, namely, a long gonadotrophin-releasing hormone (GnRH) agonist protocol and a short GnRH agonist protocol, were used. Daily injections of recombinant follicle-stimulating hormone (Gonal-F, Serono Laboratories, Aubonne, Switzerland; or Puregon, N.V. Organon, Oss, the Netherlands) (150–300 IU, daily) started from the third or fifth day of the menstrual cycle, and the dose was adjusted according to follicle development. When a cohort of three or more follicles reached a mean diameter of 16–18 mm or more, human chorionic gonadotropin (HCG) (6500–10,000 IU; Serono Laboratories, Modugno, Italy) was given to trigger ovulation. Embryos were transferred on day 3 or day 5 after oocyte retrieval during the study period. Excess available embryos were cryopreserved for subsequent frozen-thawed embryo transfer cycles.

### Statistical analysis

Categorical variables were presented as numbers (percentages) and analyzed using Chi-squared test or Fisher exact test. Univariate and multivariate logistic regression analysis were adopted to determine factors associated with EP. All variables from the univariate analysis with a  $P$  value  $< 0.1$  were entered into a forward-stepwise multivariate logistic regression analysis. Using EP or not (heterotopic pregnancy was also considered to be EP) as the dependent variable, and the following factors as independent variables: age, tubal factor (yes/no), primary or secondary infertility, type of ET (frozen-thawed or fresh), type of embryo(s) transferred (cleavage embryo or blastocyst), number of embryos transferred (one, two, or three), endometrial combined thickness (ECT), previous history of EP, and type of ovarian stimulation protocol. SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Prognostic factors were evaluated using odds ratio (OR) and its 95% confidence interval (CI) with logistic regression. A two-sided  $P$  value of  $< 0.05$  was considered statistically significant.

## Results

### Analysis of the incidence of EP

In total, 13,142 cycles ending in pregnancy for the patients undergoing assisted reproductive treatment (IVF/ICSI-ET) between January 1, 2013 and December 31, 2017, were selected for inclusion in the study. Among these 13,142 pregnancies, the incidence of EP was 2.12% (278 of 13,142). The incidence of heterotopic pregnancy was 0.27% (35 of 13,142).

The results of subgroup analysis of the incidence of EP in women undergoing IVF/ICSI-ET treatment are shown in Table 1. Overall, there was no significant difference in age distribution between EP and non-EP group ( $\chi^2 = 0.672$ ,  $P = 0.715$ ). The percentage of patients diagnosed with secondary infertility was significantly higher in EP group than that in non-EP group (68.7% vs. 53.3%,  $\chi^2 = 26.032$ ,  $P < 0.001$ ). In addition, the percentage of patients with tubal infertility was also significantly higher in EP group than that in non-EP group (89.2% vs. 63.6%,  $\chi^2 = 77.410$ ,  $P < 0.001$ ). The percentage of cycles involving transfer with a cleavage embryo was significantly higher in EP group than that in non-EP group (91.4% vs. 84.4%,  $\chi^2 = 10.132$ ,  $P = 0.001$ ). There was also a significant difference in ECT ( $\chi^2 = 18.373$ ,  $P < 0.001$ ) and previous history of EP ( $\chi^2 = 89.563$ ,  $P < 0.001$ ) between EP and non-EP group. However, the results showed no significant difference in type of transfer ( $\chi^2 = 3.329$ ,  $P = 0.068$ ), or number of embryo transferred ( $\chi^2 = 2.896$ ,  $P = 0.235$ ) between EP and non-EP group [Table 1].

### The effect of a history of EP on the occurrence of EP

For patients who had not previously experienced an EP, there was no significant difference in the stage of embryo transferred between EP and non-EP group ( $\chi^2 = 3.015$ ,  $P = 0.083$ ). Meanwhile, for patients with a previous history of one to four EPs, the percentage of patients with transfer of a cleavage-stage embryo was significantly higher in EP group than that in non-EP group (92.2% vs. 77.6%,  $\chi^2 = 13.737$ ,  $P < 0.001$ ) [Table 2].

### EP occurrence in cycles stratified by tubal infertility in IVF/ICSI cycles

For patients with or without tubal infertility, there was no significant difference in the percentage of patients with fresh embryo transfer between the EP and non-EP groups (5.2% vs. 8.5%,  $\chi^2 = 3.323$ ,  $P = 0.068$ ; 10.0% vs. 9.6%,  $\chi^2 = 0.006$ ,  $P = 0.937$ ). Moreover, there was no significant difference in the number of embryo transferred between EP and non-EP group with ( $\chi^2 = 2.053$ ,  $P = 0.358$ ) or without tubal factor ( $\chi^2 = 1.340$ ,  $P = 0.512$ ) [Table 3].

### Factors associated with EP by stepwise logistic regression analysis

To further investigate the factors associated with the occurrence of EP, logistic regression analysis was performed. The regression model selected the following variables in decreasing order of importance: tubal factor (yes/no) (OR: 3.995; 95% CI: 2.706–5.897;  $P < 0.001$ ), stage of embryo (cleavage/blastocyst) (OR: 2.355; 95% CI: 1.540–3.600;  $P < 0.001$ ), previous history of EP (OR: 1.465; 95% CI: 1.275–1.683;  $P < 0.001$ ), and ECT (OR: 0.658; 95% CI: 0.534–0.811;  $P < 0.001$ ) [Table 4].

## Discussion

EP is a well-known risk of IVF. A number of factors have been shown to be related to EP in natural pregnancy or when undergoing treatment with ART. Factors associated

**Table 1: Comparison of the differences between EP and non-EP group undergoing IVF/ICSI-ET treatment.**

Parameters	EP group (n = 278)	Non-EP group (n = 12,864)	$\chi^2$	P
Age (years)			0.672	0.715
<28	77 (27.7)	3666 (28.5)		
29–37	179 (64.4)	8337 (64.8)		
≥38	22 (7.9)	861 (6.7)		
Infertility diagnosis			26.032	<0.001
Primary infertility	87 (31.3)	6010 (46.7)		
Secondary infertility	191 (68.7)	6854 (53.3)		
Tubal factor existed			77.410	<0.001
Yes	248 (89.2)	8186 (63.6)		
No	30 (10.8)	4678 (36.4)		
Type of transfer			3.329	0.068
Fresh embryo	16 (5.8)	1144 (8.9)		
Thawed embryo	262 (94.2)	11720 (91.1)		
Stage of embryo			10.132	0.001
Cleavage stage	254 (91.4)	10856 (84.4)		
Blastocyst stage	24 (8.6)	2008 (15.6)		
No. of embryo transferred			2.896	0.235
1	41 (14.7)	2074 (16.1)		
2	231 (83.1)	10646 (82.8)		
3	6 (2.2)	144 (1.1)		
ECT (mm)			18.373	<0.001
<9	165 (59.4)	6113 (47.5)		
9–12	104 (37.4)	5764 (44.8)		
>12	9 (3.2)	987 (7.7)		
Previous history of EP			89.563	<0.001
0	163 (58.6)	10316 (81.2)		
1	74 (26.6)	1672 (13.0)		
2	32 (11.5)	734 (5.7)		
3	7 (2.5)	128 (1.0)		
4	2 (0.7)	9 (0.1)		

Values are presented as n (%). EP: Ectopic pregnancy; IVF/ICSI-ET: *In vitro* fertilization/intracytoplasmic sperm injection-embryo transfer; ECT: Endometrial combined thickness.

**Table 2: The effect of stage of embryo on the occurrence of EP in patients with or without a history of EP.**

Stage of embryo	Previous EP = 0		$\chi^2$	P	Previous EP = 1–4		$\chi^2$	P
	EP group (n = 163)	Non-EP group (n = 10,316)			EP group (n = 115)	Non-EP group (n = 2548)		
Blastocyst stage	15 (9.2)	1438 (13.9)	3.015	0.083	9 (7.8)	571 (22.4)	13.737	<0.001
Cleavage stage	148 (90.8)	8878 (86.1)			106 (92.2)	1977 (77.6)		

Values are presented as n (%). EP: Ectopic pregnancy.

with EP after natural conception, including previous EP, pelvic inflammatory disease, tubal disease or surgery, and smoking, have been well described.<sup>[6]</sup> Data on the risk factors for developing EP after IVF are inconsistent; this may be due to differences in the hormonal milieu, the reproductive health of the infertile women studied, the technical aspects of the IVF procedures, and the estimated embryo implantation potential among previous studies.<sup>[7,8]</sup>

Tubal factor is well known to be the most important factor for EP. In selected groups with tubal factor infertility, the rate of EP is as high as 11%.<sup>[9]</sup> As expected, in this study the univariate analysis identified tubal infertility as an

independent prognostic factor for EP. This is in line with previous findings, in which it was identified as a prognostic factor for EP in both natural conceptions and following ART.<sup>[3]</sup> Similarly, in our dataset, it remained significant in both univariate and multivariate analyses. Tubal factor infertility was defined as the presence of any of the following: tubal scarring including occlusion, hydro-salpinx, previous salpingectomy, or previous EP.<sup>[10]</sup> Previous EP can cause tubal infertility. Weigert *et al*<sup>[11]</sup> investigated the influence of the condition of the fallopian tubes in women who had experienced a tubal EP in a previous pregnancy on the incidence of repeated tubal pregnancy in an IVF-ET cycle. Women with a prior tubal EP had a significantly increased risk of a further EP, even in

**Table 3: Ectopic pregnancy in cycles stratified by tubal infertility in IVF/ICSI cycles.**

Parameters	Tubal factor		$\chi^2$	P	Non-tubal factor		$\chi^2$	P
	EP group (n = 248)	Non-EP group (n = 8186)			EP group (n = 30)	Non-EP group (n = 4678)		
Cycles type of transfer			3.323	0.068			0.006	0.937
Fresh embryo	13 (5.2)	696 (8.5)			3 (10.0)	448 (9.6)		
Thawed embryo	235 (94.8)	7490 (91.5)			27 (90.0)	4230 (90.4)		
No. of embryo transferred			2.053	0.358			1.340	0.512
1	37 (14.9)	1328 (16.2)			4 (13.3)	746 (15.9)		
2	206 (83.1)	6768 (82.7)			25 (83.3)	3878 (82.9)		
3	5 (2.0)	90 (1.1)			1 (3.3)	54 (1.2)		

Values are presented as n (%). IVF/ICSI: *In vitro* fertilization/intracytoplasmic sperm injection; EP: Ectopic pregnancy.

**Table 4: Factors associated with EP by stepwise logistic regression analysis.**

Factors	Unadjusted		Adjusted	
	OR (95% CI)	P	OR (95% CI)	P
Tubal factor (yes/no)	4.724 (3.229–6.912)	<0.001	3.995 (2.706–5.897)	<0.001
Previous history of EP	1.707 (1.500–1.943)	<0.001	1.465 (1.275–1.683)	<0.001
Stage of embryo (cleavage/blastocyst)	1.959 (1.285–2.985)	<0.001	2.355 (1.540–3.600)	<0.001
ECT	0.636 (0.517–0.784)	<0.001	0.658 (0.534–0.811)	<0.001

EP: Ectopic pregnancy; OR: Odds ratio; CI: Confidence interval; ECT: Endometrial combined thickness.

IVF-ET (8.95% *vs.* 0.75%,  $P < 0.001$ ). The findings showed that a greater number of previous EPs was associated with poorer quality of the fallopian tubes. Likewise, with our dataset, both univariate and multivariate analyses revealed that a previous history of a greater number of EPs was associated with a higher possibility of EP after ET.

There is a possible explanation for the observation that a significant lower percentage of patients with blastocyst ETs in EP group compared to that in non-EP group, that is, the speculated decreased uterine contractility during late luteal phase and much bigger size of the embryo replaced. When not restricted by the previous history of EP in a patient, we found that the EP rates were higher following Day 3 ETs. Previous studies indicated that the EP rate in blastocyst-transfer cycles is significantly lower than that in cleavage-transfer cycles during IVF or ICSI.<sup>[12-15]</sup> A meta-analysis by Zhang *et al*<sup>[16]</sup> in 2016 also indicated that Day 5 ET reduces the risk for EP in cycles that use IVF or ICSI, compared with Day 3 ET. An implantation potential per embryo that is higher at the blastocyst stage than at the cleavage stage may adjust these effects. Meanwhile, another large study, which adjusted for the number of fertilized embryos, found no statistically significant difference in EP rates between Day 3 and Day 5 ETs.<sup>[17]</sup>

However, in our study, when we analyzed the differences in stage of embryo between EP and non-EP group according to the patients' previous history of EP, we found no statistically significant difference in the group of patients with no previous EP history. Meanwhile, in the group of patients with a previous history of one to four EPs, we found that the percentage of patients with Day 5

ETs was significantly lower in EP group. From these results, we strongly recommend that patients choose Day 5 ET especially when they have a history of one or more EPs.

Because EP represents a failure of endometrial implantation,<sup>[18]</sup> differences in the hormonal or biochemical environment within the uterus at the time of ET and implantation may account for the higher rate of EP reported with the use of ART.<sup>[19]</sup>

The reported incidence of EP after the transfer of frozen-thawed embryos also varies among different studies. A meta-analysis by Jee *et al*<sup>[20]</sup> concluded that the rate of EP was 2.31% (49/2125 pregnancies) for frozen ET and 1.48% (162/10,934 pregnancies) for fresh ET, but the difference was not statistically significant. Moreover, Jun and Milki<sup>[21]</sup> reported that the rate of EP was not significantly increased after the transfer of frozen-thawed blastocysts compared with fresh blastocyst transfer (2.8% *vs.* 1.8%). A large retrospective cohort study published in 2014 by Decleer *et al*<sup>[22]</sup> also found no significant difference in EP risk between fresh and frozen cycles (1.92% fresh *vs.* 1.28% frozen,  $P = 0.23$ ). In our dataset, we found that the percentage of fresh ETs was higher in the non-EP group, although the difference was not statistically significant. It is possible that the difference between the two sets of data is too small due to the small number of samples, so additional samples may be needed to identify a significant difference. Considering previous data in the literature and theoretical mechanisms, it may be helpful to use ultrasound guidance with an emphasis on the distance from the fundus. However, we currently lack data about the risk of EP comparing transfer with and without trans-abdominal ultrasound guidance and the data of mock ET.

Rombauts *et al*<sup>[10]</sup> reported that the risk of EP is increased fourfold in women with an ECT of <9 mm compared with that of women with an ECT of >12 mm. ECT has been reported as a statistically significant independent prognostic factor for EP. With reference to their data classification, we analyzed our data in the univariate analysis and found that, in women with an ECT of >12 mm, the risk of EP was lowest (9/996, 0.9%); with a thinner ECT, the risk of EP was statistically significantly higher. This factor also remained significant in the multivariate analysis of our dataset. One possible explanation for why women with thinner endometrium are more likely to suffer from EP is that the endometrial receptivity is compromised under this condition, and might be less friendly to the coming embryo, leading to the implantation of embryo outside the uterus cavity.

Some studies found an association between the number of embryos transferred and the likelihood of an EP. Clayton *et al*<sup>[11]</sup> reported that the transfer of embryos with an indication of high implantation potential was associated with decreased ectopic risk when two or fewer embryos were transferred (OR: 0.7, 95% CI: 0.5–0.9), but not when three or more embryos were transferred. In addition, Perkins *et al*<sup>[2]</sup> reported that the rate of EP increased with an increasing number of embryos transferred per cycle; the rate of EP was 1.6% (95% CI: 1.4%–1.7%) when one embryo was transferred compared with 1.7% (95% CI: 1.7%–1.8%), 2.2% (95% CI: 2.1%–2.3%), and 2.5% (95% CI: 2.4%–2.6%) when two, three, or four or more embryos were transferred, respectively. The highest risk occurred when four or more embryos were transferred compared with only one embryo transferred (adjusted risk ratio: 1.49, 95% CI: 1.25–1.78). Our study also found that the percentage of patients with less embryos transferred was significantly higher in the non-EP group as compared with EP groups. However, the difference was not statistically significant between the two groups. Therefore, we usually recommend transferring one or two embryos per cycle.

Our study has a number of limitations. First, given that the study was retrospective, it is possible that there was a recall bias. As such, the data relating to the incidence of EP should be considered preliminary, pending confirmation by prospectively planned studies. Second, our work was based on data from a single center in a defined geographical area in which the incidence of tubal disease in women undergoing IVF treatment appeared to be high. It would be interesting to compare the obtained data with those from other centers to determine whether the same pattern can be observed elsewhere. Additionally, a history of previous EP is currently reported as part of the diagnosis of tubal infertility, and it is not possible to know which patients assigned a diagnosis of tubal infertility had a previous EP or another tubal abnormality.

In conclusion, our study showed that tubal infertility is still the most important factor affecting the occurrence of EP in ART cycles. Secondary infertility, a history of previous EP, and ECT are all been considered as factors associated with the occurrence of the EP. Especially for patients who have previously undergone EP, we recommend blastocyst

transfer and restriction of the number of embryos transferred in order to decrease the EP rate.

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### Conflicts of interest

None.

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