Risk factors for ectopic pregnancy in assisted reproductive technology: a 6-year, single-center study

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Objective: To explore factors affecting the incidence of ectopic pregnancy (EP) in assisted reproductive technology (ART).
Design: A retrospective cohort study on the incidence of EPs in IVF/intracytoplasmic sperm injection (ICSI) and IUI cycles from June 2009 to August 2015. Age of patients, tubal factor infertility, type of cycle (fresh or thawed), embryo being transferred (cleavage embryo or blastocyst), and number of embryos transferred were analyzed to explore their relationship with the incidence of EP.
Setting: Teaching hospital.
Patient(s): A total of 18,432 pregnancies resulting from ART treatment were retrospectively analyzed.
Intervention(s): None.
Main Outcome Measure(s): Ectopic pregnancy rate.
Result(s): For IVF/ICSI cycles, the incidence of EP was different between cycles transferred with cleavage embryo and blastocyst (3.45% vs. 2.47%). In multivariate logistic regression analysis, tubal infertility was associated with EP (adjusted odds ratio 1.716, 95% confidence interval 1.444–2.039). For IUI cycles, EP was significantly higher in stimulated cycles compared with natural cycles (2.62% vs. 0.99%). The EP rate in cycles with sperm from donor and husband was 1.08% and 3.54%, respectively. However, when patients were stratified according to tubal infertility, the EP rate increased with level of peak estrogen. In thawed embryo transfer cycles, the EP rate was lower in blastocyst transfer cycles and in cycles transferred with fewer embryos.
Conclusion(s): Irrespective of tubal infertility, for fresh IVF/ICSI cycles the rate of EP is positively associated with ovarian stimulation; for thawed IVF/ICSI cycles, blastocyst transfer or transfer with fewer embryos reduces the EP rate. In IUI cycles, EP is associated with sperm source. (Fertil Steril® 2016;106:90–4. ©2016 by American Society for Reproductive Medicine.)
Key Words: ART, ectopic pregnancy, relative risk, tubal factor

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Ectopic pregnancy (EP) after assisted reproductive technology (ART) procedures is a complication with potentially serious consequences for patients. There are multiple reports that describe a higher incidence of EPs after various methods of ART (2.2% to 4.5%) than in spontaneous pregnancy (1–3).

In previous studies, many factors have been described as being associated with EP (3, 4). Of these, the difference of EP between fresh and thawed embryo transfer cycles and blastocyst transfer have attracted much interest from clinicians. It has been shown that blastocyst transfer may reduce EP in IVF/intracytoplasmic sperm injection (ICSI) cycles (5, 6), even though this conclusion is inconsistent with other studies (7, 8). Meanwhile, many studies demonstrate a lower incidence of EP in thawed embryo transfer cycles compared with fresh embryo transfer in stimulated cycles (9–12), indicating that the tubal–uterine environment after ovarian stimulation has been different from physiologic status, which contributes to abnormal implantation eventually. This point of view has been supported by a recent study with a large sample size (>100,000 pregnancies) from the Society for Assisted Reproductive Technology registry in United States (13).

Because the incidence of EP is relatively low, and many factors are associated with EP, a large sample size is

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needed in such studies to reach a reasonable conclusion. Moreover, several EP-associated factors should be analyzed at the same time. Thus, to explore factors affecting the incidence of EP in ART, and to explore possible ways to reduce EP in different ART procedures, we retrospectively analyzed nearly 50,000 ART cycles during 6 years at our center. We hypothesize that factors associated with EP may be different in multivariate analysis.

MATERIALS AND METHODS

This study was approved by the institutional review board of First Affiliated Hospital of Zhengzhou University. For patients undergoing ART (IVF/ICSI and related technology, IUI) treatment in our center, all have allowed use of their medical record data for research. Written, informed consent was obtained from all patients before IVF treatment. All patients were registered in our data management system, which is used to save all medical information of patients trying to conceive through ART.

From June 2009 to August 2015, 18,432 cycles resulted in a reported clinical intrauterine, ectopic, or heterotopic pregnancy, and were included in this study. For the ART follow-up procedure in our center, all patients are asked to come back for a blood hCG test 14 and 18 days after ET or IUI. Those with hCG elevation return for ultrasound examination 35 days after ET or IUI. Thus, [1] most of the EP cases are diagnosed with ultrasound examination (ultrasound scan, especially transvaginal, showing a gestational sac with fetal heart in the adnexa area). [2] Pregnancy of unknown location, which indicates there is a positive pregnancy test result but no pregnancy has been visualized using transvaginal ultrasound examination, is also included. In this case, we usually suggest that patients stay in hospital, and we administer mifepristone or methotrexate when necessary. [3] Sometimes laparoscopy or laparotomy are also used to visually confirm an EP. In this case, EP is also confirmed by pathology. However, most of these patients are treated in local hospitals and inform us by telephone. [4] In rare cases dilation and curettage is also performed to diagnose EP. The clinical intrauterine pregnancy was defined as documentation of one or more gestational sacs visible by ultrasound examination. Heterotopic pregnancy was defined as a pregnancy that met the criteria for both ectopic and clinical intrauterine pregnancy. Heterotopic pregnancy was also grouped into EP in this study.

The parameters evaluated were patient age, infertility diagnosis (tubal infertility or not), type of cycle (fresh or frozen–thawed), type of embryos transferred (cleavage embryo or blastocyst), and number of embryos transferred (one, two, or three). For IUI cycles the impact of IUI cycle type (natural or stimulated) and sperm source (husband or donor) on incidence of EP was also evaluated.

Multivariate logistic regression analysis was used to identify the prognostic variables mentioned before and to evaluate the association between the variables and EP. For IVF/ICSI cycles, all patients were stratified by tubal infertility, and risk factors for EP were re-evaluated. Tubal infertility mainly includes [1] surgery history on fallopian tubes: previous EP, salpingostomy, and tube reconstruction surgery for infertility treatment; [2] hysterosalpingography examination: hydrosalpinx, salpingitis (however, saline salpingogram or clamydia antibody titers were not used to diagnose tubal factor); [3] salpingectomy and ligation are not included in this study. In fact, for patients with hydrosalpinx, we sometimes suggest tube ligation and a microcoil device to perform hysteroscopic tubal occlusion for treating hydrosalpinx. A P value of $<.05$ was considered statistically significant.

RESULT

In the 18,432 pregnancies, the incidence of EP was 3.17% (585 of 18,432). The incidence of EP in IVF/ICSI and IUI cycles was 3.33% (538 of 16,139) and 2.05% (47 of 2,293), respectively.

Overall, EP had no difference in the three age groups (<28, 29–37, and 38 years). For patients diagnosed with secondary infertility, the EP rate was significantly higher than that in patients with primary infertility (3.92% vs. 2.61%, $P=.000$). In addition, EP in patients with tubal infertility was also higher when compared with that in patients without tubal infertility (4.36% vs. 2.50%, $P=.000$) (Table 1).

For IVF/ICSI cycles, the incidence of EP was different between cycles transferred with cleavage embryo and blastocyst (3.45% vs. 2.47%, $P=.0265$). However, EP had no difference

TABLE 1

Ectopic pregnancy rate in different groups.

Cycles	EP rate, % (n)	χ^2	*P* value
All ART cycles			
Age (y)		0.7647	.3819
<28	2.87 (168/5855)		
29–37	3.38 (381/11257)		
≥38	2.73 (36/1320)		
Infertility diagnosis		24.9325	.000
Primary infertility	2.61 (275/10518)		
Secondary infertility	3.92 (310/7914)		
Tubal factor existed		48.1318	.000
Yes	4.36 (292/6698)		
No	2.50 (293/11734)		
Type of ART		10.7682	.001
IVF/ICSI	3.33 (538/16139)		
IUI	2.05 (47/2293)		
IVF/ICSI cycles			
Type of transfer		1.0559	.304
Fresh embryo	3.22 (327/10149)		
Thawed embryo	3.52 (211/5990)		
Stage of embryo		4.9232	.0265
Cleavage stage	3.45 (491/14240)		
Blastocyst stage	2.47 (47/1899)		
No. of embryos transferred		3.3802	.0660
1	3.51 (53/1512)		
2	3.09 (346/11214)		
3	4.07 (139/3413)		
IUI cycles			
Type of cycle		6.8892	.0087
Natural cycle	0.99 (8/805)		
Stimulated cycle	2.62 (39/1488)		
Sperm source		16.5046	.000
Husband	3.54 (32/904)		
Donor	1.08 (15/1389)		

Bu. Risk factor for ectopic pregnancy in ART. Fertil Steril 2016.

TABLE 2

Factors associated with EP by logistic regression analysis.

	IVF/ICSI		IUI	
Factor	**Adjusted OR (95% CI)**	***P* value**	**Adjusted OR (95% CI)**	***P* value**
Tubal factor (yes/no)	1.716 (1.444–2.039)	.000	1.018 (0.420–2.467)	.968
Type of transfer (fresh/thawed)	1.111 (0.922–1.338)	.268	–	–
Stage of embryo (cleavage/blastocyst)	0.715 (0.511–1.001)	.051	–	–
No. of embryos transferred	1.044 (0.871–1.250)	.643	–	–
Type of cycle (natural/stimulated)	–	–	2.162 (0.995–5.698)	.052
Sperm source (husband/donor)	–	–	0.335 (0.177–0.632)	.001

Bu. Risk factor for ectopic pregnancy in ART. Fertil Steril 2016.

in fresh embryo transfer cycles and thawed embryo transfer cycles, or in cycles transferred with one, two, or three embryos (Table 1). In addition, in multivariate logistic regression analysis, only tubal infertility was associated with increased EP when adjusted for the factors mentioned above (adjusted odds ratio [OR] 1.716, 95% confidence interval [CI] 1.444–2.039; $P=.000$) (Table 2).

For IUI cycles, EP was significantly higher in stimulated cycles compared with natural cycles (2.62% vs. 0.99%, $P=.0087$). Meanwhile, patients inseminated with sperm from donors had lower EP compared with patients inseminated with husband's sperm (1.08% vs. 3.54%, $P=.000$). However, in multivariate logistic regression analysis, only sperm source was associated with EP (adjusted OR 0.335, 95% CI 0.177–0.632; $P=.001$) (Table 2).

Table 3 shows the incidence of EP in different groups when patients were stratified according to tubal infertility. In fresh embryo transfer cycles, for patients with or without tubal infertility, the EP rate was comparable in patients transferred with cleavage embryo or blastocyst and was also comparable when a different number of embryos (one, two, or three) was transferred. Interestingly, the incidence of EP increased with level of peak estrogen in both the tubal infertility group and the non–tubal infertility group.

In thawed embryo transfer cycles, the EP rate was not different between natural cycle and artificial cycle in patients with or without tubal infertility. However, for all patients included in this study, the EP rate in blastocyst transfer cycles was significantly lower compared with that in cleavage transfer cycles. Moreover, the incidence of EP also increased with

TABLE 3

Ectopic pregnancy rate in cycles stratified by tubal infertility in IVF/ICSI cycles.

	Tubal factor			Non-tubal factor		
Cycle	**EP rate, % (n)**	**χ^2**	***P* value**	**EP rate, % (n)**	**χ^2**	***P* value**
Fresh embryo transfer	3.82 (154/4034)			2.83 (173/6115)		
Stage of embryo		0.0777	.7804		0.6411	.4233
Cleavage stage	3.84 (142/3695)			2.78 (157/5647)		
Blastocyst stage	3.54 (12/339)			3.42 (16/468)		
No. of embryos transferred						
1	3.85 (17/442)	0.9138	.3391	3.81 (23/604)	1.6921	.1933
2	4.01 (119/2969)			2.75 (131/4769)		
3	2.89 (18/623)			2.56 (19/742)		
Peak E_2 level (pg/mL)		6.6861	.0097		7.8347	.0051
<2,500	1.90 (16/840)			2.09 (23/1103)		
2,500–5000	3.95 (63/1594)			2.20 (51/2314)		
5,000–7,500	4.26 (36/846)			3.22 (43/1336)		
≥7,500	4.49 (22/490)			3.77 (31/823)		
Thawed embryo transfer	5.38 (132/2453)			2.23 (79/3537)		
Type of protocol		1.7084	.1912		0.042	.8375
Natural cycle	6.25 (49/784)			2.32 (21/905)		
Artificial cycle	4.97 (83/1669)			2.20 (58/2632)		
Stage of embryo		7.7059	.0055		4.0903	.0431
Cleavage stage	5.96 (121/2031)			2.48 (71/2867)		
Blastocyst stage	2.61 (11/422)			1.19 (8/670)		
No. of embryos transferred		7.2298	.0072		7.4210	.0064
1	4.57 (9/197)			1.49 (4/269)		
2	4.30 (59/1373)			1.76 (37/2103)		
3	7.25 (64/883)			3.26 (38/1165)		

Bu. Risk factor for ectopic pregnancy in ART. Fertil Steril 2016.

number of embryos transferred in both the tubal infertility group and the non–tubal infertility group.

DISCUSSION

Nowadays, ART has been a common technique for infertility treatment worldwide. However, the complications that come along with the new technology, such as ovarian hyperstimulation syndrome, EP, and multiple pregnancy, have drawn more and more attention. Even though the incidence of EP is rare during IVF (approximately 2%–11%) (2), rupture with internal bleeding that may lead to hypovolemic shock is one of the most common complications of EP and is still the leading cause of death during early pregnancy.

As we all know there are a number of risk factors for EP in natural pregnancy. Among these, tubal inflammation is identified as a widely accepted one by scholars (3, 14). During IVF treatment, embryos are transferred directly into the uterus cavity. Theoretically speaking, the EP rate in pregnancies after IVF treatment should be lower than that in natural pregnancies. However, because quite a lot couples seeking IVF treatment have tubal infertility, it is reasonable that the rate of EP is reported to be much higher in IVF cycles when compared with natural conception.

For EP during ART treatment, the reasons are also numerous. First, EP is believed by many scholars to be associated with ovarian stimulation. In 2013 a large retrospective study showed that for patients without polycystic ovary syndrome, the rate of EP was 3.4% in the high E_2 group (>4,085 pg/mL), which was significantly higher than that in the low E_2 group (2.0% for ≤4,085 pg/mL group) (15). Recently, data from 91,504 autologous cycles from the 2008–2010 SART registry found that increasing oocyte yield is also correlated with a significantly increased EP rate (16). Afterward, EP rate was also found to be higher in fresh cycles than thawed cycles during IVF treatment, and to be higher in natural protocol cycles than artificial protocol cycles during thawed embryo transfer (10, 17). All these studies supported the view that an increased rate is mainly due in part to the supraphysiologic hormonal milieu resulting from ovarian stimulation during ART. On the other hand, blastocyst transfer, high P level, and number of embryos transferred were also shown to be associated with EP (5,18–20).

Thus, the incidence of EP, which is quite similar with pregnancy rate in ART cycles, is affected by many factors at the same time and should not be analyzed in single-factor study. In the present study, overall our data also showed that for IVF/ICSI cycles, blastocyst transfer and number of embryos transferred were both associated with EP, even though EP rates were comparable between fresh and thawed embryo transfer cycles. For IUI cycles, stimulated cycle and sperm source also had an impact on the EP rate. However, interestingly, when all factors mentioned above were included into multivariate logistic regression analysis, only tubal infertility was associated with EP in IVF/ICSI cycles, and sperm source was associated with EP in IUI cycles.

Furthermore, for IVF/ICSI cycles after stratifying by tubal infertility, the EP rate increased with peak E_2 level in both the tubal infertility group and the non–tubal infertility group during fresh cycles, which is consistent with previous studies indicating ovarian stimulation increases the risk of EP. In thawed embryo transfer cycles, the EP rate was not impacted by artificial protocol, in which only small dosages of extra-hormone medicine were used. However, patients indeed could benefit from blastocyst transfer and transfer with fewer embryos.

The primary strength of the present study is the ability to explore potential EP risk factors from a single center with large sample size, and also to show the importance of multivariate analysis in such studies. Indeed, with the increasing of ART cycles every year, each center has accumulated more and more precious data. It is easy to conduct univariate retrospective studies; however, in most cases, subgroup analysis is needed because an interaction effect from several factors may exist.

Taken together, our study showed that tubal infertility is still the primary factor impacting EP rate in ART cycles. Irrespective of tubal infertility, for fresh IVF/ICSI cycles, EP rate is positively associated with ovarian stimulation; for thawed IVF/ICSI cycles, blastocyst transfer, or transfer with fewer embryos reduces EP rate. In IUI cycles, EP is associated with sperm source.

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