Risk Factors of Ectopic Pregnancy in Women Undergoing In Vitro Fertilization and Embryo Transfer (IVF-ET)

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Objective: *To identify risk factors associated with ectopic pregnancy (EP) in women undergoing assisted reproductive technology (ART).*

Material and Method: *A retrospective study was conducted among pregnant women after in vitro fertilization and embryo transfer (IVF-ET) in an assisted conception unit of a university hospital between 1994 and 2013. The factors evaluated included the use of donor oocyte, use of intracytoplasmic sperm injection (ICSI), stage of embryo, number of embryos transferred, and fresh or frozen-thawed cycle.*

Results: *One thousand three hundred eighty five pregnancies were identified of which 29 were ectopic pregnancies. Embryo transfer (ET) in fresh cycle significantly increased risk for EP compared with frozen-thawed cycle (p = 0.005). Day-5 ET also significantly increased higher EP risk than day-3 ET (p = 0.041). Three or more embryos transfer (p = 0.085), use of donor oocyte (p = 0.999), and use of ICSI (p = 0.246) did not significantly influence EP risk.*

Conclusion: *Embryo transfer in fresh cycle and day-5 embryo transfer significantly increased EP risk. More patients and embryo characteristics are necessary to be studied to identify more valid predictors.*

Keywords: *Ectopic pregnancy, IVF-ET, Risk factor*

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Ectopic pregnancy (EP) is one of the top serious complication of patients undergoing assisted reproductive technology (ART). Not only the patients will lose their pregnancy but their life may also be threatened if the condition could not be detected in time. The mortality from EP was accounted for 13% of all maternal death[1].

The reported incidences of EP from in vitro fertilization and embryo transfer (IVF-ET) range between 1.38 to 2.10% of clinical pregnancy[2-5], which is increased 2.50 to 5.00 folds compared with its incidence in spontaneous pregnancy[5]. The reasons why risks of EP are increased in pregnancy from ART are still not clear but some explanations have been proposed such as alterations in uterine contractility, hormonal influences on endometrial, and tubal receptivity[5].

However, many factors found in patients undergoing ART have been reported to increase risk for EP such as tubal factor infertility[4], multiple embryos transfer[2], fresh compared with frozen-thawed ET[6,7], cleavage stage ET compared with blastocyst transfer[8], GnRH agonist trigger cycle[9], and non-donor oocyte cycle[2]. An ability to identify high-risk patients for EP is important so that clinicians may start early treatment, avoid radical tubal surgery, and eventually preserve future fertility[10]. The main objective of the present study was to identify risk factors associated with EP in pregnancy from ART, so this information might be used for early detection and appropriate treatment offered to the patients.

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Material and Method

Data were retrospectively collected from ART cycles between January 1994 and December 2013 at the assisted conception unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University which resulted in a clinical intrauterine pregnancy or an ectopic pregnancy. A clinical pregnancy was defined when ultrasonography confirmed the presence of intrauterine gestational sac. If the ultrasonography document was not available, a clinical intrauterine pregnancy was identified by the birth of a fetus or conceptive product, collected from vaginal canal by the patient or from uterine curettage, which revealed chorionic villi. The EP was defined when ultrasonography confirmed the presence of a gestational sac outside the uterine cavity. If the

ultrasonography document was not available, EP was diagnosed by the presence of chorionic villi in tissue collected from peritoneal cavity.

Patient's age and IVF-ET cycle characteristics which might be risk factors for EP were recorded. These factors included use of intracytoplasmic sperm injection (ICSI), number of days in embryo culture, number of embryos transferred, fresh or frozen-thawed cycle, and the use of donor or non-donor oocyte for fertilization.

Statistical analysis was conducted by using SPSS 17.0 statistics software. Continuous data were presented as mean ± standard variation (SD) and categorical variables were defined as percentages (%). Continuous variables were compared with the student's t-test, while Chi-squared or Fisher's exact tests were performed for comparison of categorical variables where appropriate. Logistic regression analysis was used to calculate odds ratio (OR) and 95% confident interval (CI). Multiple logistic regression was subsequently used to determine independent factors for EP. A value of $p<0.05$ was considered to be statistically significant.

Ethical approval for the study was obtained from the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB No 522/58).

Results

One thousand three hundred eighty five pregnancies were included in the present study. Of these, 1,356 (97.91%) were reported as intrauterine pregnancies and 29 (2.09%) were EP. Patient's age in both EP and intrauterine group were not significantly different (36.17±3.69 years vs. 36.21±4.10 years, $p = 0.95$).

Table 1 showed incidence of EP among all pregnancies conceived by IVF-ET yearly between 1994 and 2013. There was not obvious change of the incidence during the period of the present study.

Table 2 demonstrated patient and IVF cycle characteristics which may be associated with risks for EP. In the unadjusted analysis, use of donor or non-donor oocytes (unadjusted OR 1.87, 95% CI 0.25-13.92) and use of ICSI or not use (unadjusted OR 3.98, 95% CI 0.54-29.49) were not found to modify the risk for EP significantly. However, the number of replaced embryos, the length of embryo culture, and the fresh cycle ET were shown to increase the EP risk. Stage of embryo was demonstrated to influence the EP risk. Replacement of day-4 embryo compared with day-3 embryo significantly increased risk for EP (unadjusted OR 3.06, 95% CI 1.27-7.33) but not for day-5 embryo vs. day-3 embryo (unadjusted OR 2.25, 95% CI 0.57-8.84). Regarding the number of replaced embryos, the EP risk among pregnancies in which three or more embryos were transferred was significantly higher than those of which two or fewer embryos replacement group (unadjusted OR 2.65, 95% CI 1.01-7.00). Considering the transfer cycle, the significantly higher risk occurred when embryos were transferred in fresh cycle compared with frozen-thawed ones (unadjusted OR 3.72, 95% CI 1.57-8.76).

In order to find the most predictive risk factors, multivariate analysis was performed to independently assess associations between the significant risk factors from unadjusted analysis and the occurrence of EP. The results of multivariate analysis revealed that fresh embryo transfer and day-5 embryo transfer were independent factors which showed significantly increased risk for EP. Embryo transfer in fresh cycles increased a significantly higher

Table 1. Incidence of ectopic pregnancy among pregnancy conceived after IVF-ET between 1994 and 2013

Year	No. of ectopic pregnancy	No. of all pregnancy after IVF-ET	Incidence (%)
1994	0	31	0
1995	0	13	0
1996	0	24	0
1997	3	30	10.00
1998	1	56	1.78
1999	3	62	4.83
2000	1	66	1.51
2001	0	76	0
2002	1	75	1.33
2003	4	73	5.47
2004	1	75	1.33
2005	5	84	5.95
2006	2	74	2.70
2007	1	75	1.33
2008	2	94	2.12
2009	0	89	0
2010	0	97	0
2011	0	98	0
2012	5	105	4.76
2013	0	88	0
Total	29	1,385	2.09

IVF-ET = in vitro fertilization and embryo transfer

Table 2. Factors associated with EP among pregnancies conceived by IVF-ET

Factors	Ectopic pregnancy n (%)	Intrauterine pregnancy n (%)	Crude odds ratio (95% CI)	*p*-value	Adjusted odds ratio (95% CI)	*p*-value
Type of cycle						
Donor	1 (1.16)	85 (98.84)	1			
Non-donor	28 (2.15)	1,271 (97.85)	1.87 (0.25-13.92)	0.999	-	-
Use of ICSI						
No	1 (0.59)	169 (99.41)	1			
Yes	28 (2.30)	1,187 (97.70)	3.98 (0.54-29.49)	0.246	-	-
Stage of embryo						
Day 3 or less	7 (1.06)	653 (98.94)	1			
Day 4	19 (3.17)	579 (96.83)	3.06 (1.27-7.33)	0.009	1.22 (0.34-4.39)	0.757
Day 5 or more	3 (2.36)	124 (97.64)	2.25 (0.57-8.84)	0.209	2.51 (1.03-6.09)	0.041
No. of embryo						
2 or fewer	5 (1.02)	483 (98.98)	1			
3 or more	24 (2.67)	873 (97.33)	2.65 (1.01-7.00)	0.048	2.41 (0.88-6.59)	0.085
Cycle transfer						
Frozen-thawed	7 (0.94)	735 (99.06)	1			
Fresh	22 (3.42)	621 (96.58)	3.72 (1.57-8.76)	0.001	3.50 (1.47-8.33)	0.005

risk for EP compared with frozen-thawed cycle (adjusted OR 3.50, 95% CI 1.47-8.33). Interestingly, day-5 embryo transfer significantly increased the EP rate compared with day-3 embryo transfer (adjusted OR 2.51, 95% CI 1.03-6.09). However, day-4 embryo transfer did not significantly increase higher risk for EP compared with day-3 embryo transfer after multivariate analysis (adjusted OR 1.22, 95% CI 0.34-4.39). Regarding the number of the replaced embryos, although the univariate analysis showed significantly higher risk for EP in three or more embryos transfer compared with two or fewer embryos transferred (unadjusted OR 2.65, 95% CI 1.01-7.00). However, multivariate analysis did not find statistical significant difference between the two groups (adjusted OR 2.41, 95% CI 0.88-6.59).

Discussion

The rate of EP in the present study was 2.09% which was higher than that of spontaneous conception (0.80%)[11]. An earlier study[12] also demonstrated that infertility was a risk factor of EP from natural conception. Many risk factors have been studied in many countries but there are still conflicting results. In the present study, five IVF cycle characteristics were recorded and analyzed. By using multiple logistic regression analysis, fresh cycle embryo transfer (p = 0.005), and day-5 embryo transfer (p = 0.041) were found significantly increase EP risk.

There were many hypotheses which might explain the negative effect on implantation capacity of embryo transfer in fresh cycle. High dose of gonadotropins, peritoneal irritation caused by ovarian enlargement, ovarian trauma from ovum pick up were proposed to be responsible for uterine motility and adversely affect intrauterine embryo implantation capacity[13]. Findings from the present study also supported previous studies in which embryo transfer in fresh cycle could increase risk for EP[3,14].

Pregnancy from blastocyst transfer was less likely to be associated with EP compared with early stage embryo. It has been proposed that a larger size of blastocyst makes it more difficult for blastocyst to pass tubal ostium. Moreover, timing of blastocyst transfer usually takes place in late luteal phase when the uterine contractility decreases[15]. Conversely, at least two studies demonstrated that blastocyst transfer increased a higher chance of EP occurrence compared to cleavage stage embryo[16,17]. The present study showed that only day-5 embryo transfer significantly increased risk for EP (p = 0.041) after the multiple logistic regression analysis was used. Perhaps, the plausible supporting evidence for this finding was the high potential capacity of day-5 embryo to implant either intrauterine or extrauterine cavity[15].

Generally, the use of donated oocytes in ART is proposed to be protective strategy against EP. Firstly, donated oocytes usually come from young and fertile women. Therefore, EP risk in this group is proposed to be as low as the spontaneous conception[6]. Secondly, embryo derived from donated oocytes

usually are transferred in non-hyperstimulated cycles in which the risk of abnormally located implantation is decreased[3]. Clayton et al[4] reported EP incidence from IVF donor cycle was significantly lower than non-donor group (1.40% vs. 2.20%). Subsequently, Londra et al[3] confirmed the same finding (0.93% vs. 1.50%, $p<0.001$). However, the present study showed the same trend but the difference was not statistically significant (1.16% vs. 2.15%, $p = 0.99$).

Among infertile couples undergoing IVF-ET, those who need ICSI are proposed to have low risk for EP. They are likely to have male factor, not tubal disease which is commonly associated with EP, as an indication to undergo ART[4]. Nevertheless, it was found that EP risk in non-ICSI pregnancy was not significantly different from ICSI pregnancy (adjusted OR 0.91, 95% CI 0.81-1.02)[4]. In the present study, ICSI was not shown to have protective effect for EP. EP risk in ICSI pregnancy was not statistical significant difference from non-ICSI pregnancy group (unadjusted OR 3.98, 95% CI 0.54-29.49). The reason for the protective effect of ICSI against EP risk could not be noticed in the present study may arise from the fact that ICSI has currently been used for fertilization in the majority of infertile couples, not restricted only to couples with male factor.

The higher number of embryo transfer might increase implantation rate both intra or extrauterine cavity. It has been shown that transfer of two embryos or less was a protective measure to decrease EP risk. Transfer of three and four embryos increased EP risk (unadjusted OR 1.30, 95% CI 1.14-1.48 and unadjusted OR 1.51, 95% CI 1.33-1.72 respectively) compared with transfer of one or two embryos[4]. However, other study did not support this finding. Ribic-Pucelj et al[18] studied in 1,059 EP from ART and reported that the EP rate did not correlate with the number of transferred embryos. The EP rate of one, two, and three embryos transferred were 4.76%, 5.95%, and 3.39% respectively[18]. In the present study, the EP rate of three or more embryos transferred group was higher than that of two or fewer embryo transferred group in univariate analysis (unadjusted OR 2.65, 95% CI 1.01-7.00). Nevertheless, subsequent multivariate analysis failed to show the statistically significant difference (adjusted OR 2.41, 95% CI 0.88-6.59). If other characteristics, e.g., embryo quality, had been collected in the present study, more solid evidence would have been identified.

Recently, Bu et al[19] conducted a six-year study, between 2009 and 2015, with 18,432 pregnancies resulting from ART and intrauterine insemination (IUI) to determine risk factors for EP. In ART cycles, it was demonstrated that only tubal infertility significantly increased risk of EP whereas type of cycle (fresh/frozen-thawed), stage of embryo (cleavage/blastocyst) and number of embryos transferred did not show statistically significant difference. Remarkably, only one risk factor, i.e., number of embryos transferred was not shown to be a significant risk factor of EP which supported the present study. In the present study, cause of infertility was not included for study because of medical record problem. Moreover, the difference of duration of study between Bu et al[19] and the present study (6 and 20 years respectively) during which there have been such many changes and progress in ART field, e.g., embryo selection tools, embryo culture system, might be responsible for different outcomes.

The strengths of the present study were sample size and long duration of study. Almost 1,400 pregnancies have been studied and the duration of the study was 20-year long. Certain variations of time and subjects could be minimized. The weakness of the study was retrospective study. Some factors could not be completely collected and analyzed, e.g., cause of infertility, use of GnRH agonist to trigger ovulation. Therefore, only some risk factors could be determined. Further study in which more comprehensive characteristics are collected and analyzed may show more informative conclusions.

Conclusion

The present study showed that embryo transfer in fresh cycle and day-5 embryo transfer significantly increased EP risk in pregnancy following IVF-ET. Type of oocyte (donor or non-donor), ICSI use and number of embryo transferred did not show significantly increased EP risk. More patients and IVF cycle characteristics are needed to be studied to get more precise conclusion.

What is already known on this topic?

There are many patient and IVF-ET cycle characteristics which have been shown to be associated with EP risk, e.g., history of tubal disease, fresh cycle transfer, high number of embryos replacement, use of non-donor oocyte. These risk factors might be useful information for counseling and making a plan for each couple. However, there are still conflicting evidence in many studies.

What this study adds?

The present study revealed two risk factors for EP conceived by IVF-ET among Thai patients. They were fresh cycle transferred and day-5 embryo transferred. These findings would be more useful for the clinician to discuss a plan or option of treatment with the infertile couple in Southeast Asia to decrease EP risk. To date, there have been very few studies on this topic in the region.

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Potential conflicts of interest

None.

References

1. Lawson HW, Atrash HK, Saftlas AF, Finch EL. Ectopic pregnancy in the United States, 1970-1986. MMWR CDC Surveill Summ 1989; 38: 1-10.
2. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001-2011. Obstet Gynecol 2015; 125: 70-8.
3. Londra L, Moreau C, Strobino D, Garcia J, Zacur H, Zhao Y. Ectopic pregnancy after in vitro fertilization: differences between fresh and frozen-thawed cycles. Fertil Steril 2015; 104: 110-8.
4. Clayton HB, Schieve LA, Peterson HB, Jamieson DJ, Reynolds MA, Wright VC. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol 2006; 107: 595-604.
5. Smith LP, Oskowitz SP, Dodge LE, Hacker MR. Risk of ectopic pregnancy following day-5 embryo transfer compared with day-3 transfer. Reprod Biomed Online 2013; 27: 407-13.
6. Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil Steril 2015; 103: 655-61.
7. Huang B, Hu D, Qian K, Ai J, Li Y, Jin L, et al. Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles. Fertil Steril 2014; 102: 1345-9.
8. Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil Steril 2011; 95: 1966-9.
9. Sahin S, Ozay A, Ergin E, Turkgeldi L, Kurum E, Ozornek H. The risk of ectopic pregnancy following GnRH agonist triggering compared with hCG triggering in GnRH antagonist IVF cycles. Arch Gynecol Obstet 2015; 291: 185-91.
10. Weigert M, Gruber D, Pernicka E, Bauer P, Feichtinger W. Previous tubal ectopic pregnancy raises the incidence of repeated ectopic pregnancies in in vitro fertilization-embryo transfer patients. J Assist Reprod Genet 2009; 26: 13-7.
11. King Chulalongkorn Memorial Hospital. Annual statistical report. Bangkok: King Chulalongkorn Memorial Hospital, Thai Red Cross Society; 2011.
12. Bunyavejchevin S, Havanond P, Wisawasuk-mongchol W. Risk factors of ectopic pregnancy. J Med Assoc Thai 2003; 86 (Suppl 2): S417-21.
13. Decleer W, Osmanagaoglu K, Meganck G, Devroey P. Slightly lower incidence of ectopic pregnancies in frozen embryo transfer cycles versus fresh in vitro fertilization-embryo transfer cycles: a retrospective cohort study. Fertil Steril 2014; 101: 162-5.
14. Wang J, Wei Y, Diao F, Cui Y, Mao Y, Wang W, et al. The association between polycystic ovary syndrome and ectopic pregnancy after in vitro fertilization and embryo transfer. Am J Obstet Gynecol 2013; 209: 139.e1-9.
15. Chang HJ, Suh CS. Ectopic pregnancy after assisted reproductive technology: what are the risk factors? Curr Opin Obstet Gynecol 2010; 22: 202-7.
16. Keegan DA, Morelli SS, Noyes N, Flisser ED, Berkeley AS, Grifo JA. Low ectopic pregnancy rates after in vitro fertilization: do practice habits matter? Fertil Steril 2007; 88: 734-6.
17. Rosman ER, Keegan DA, Krey L, Liu M, Licciardi F, Grifo JA. Ectopic pregnancy rates after in vitro fertilization: a look at the donor egg population. Fertil Steril 2009; 92: 1791-3.
18. Ribic-Pucelj M, Tomazevic T, Vogler A, Meden-Vrtovec H. Risk factors for ectopic pregnancy after in vitro fertilization and embryo transfer. J Assist Reprod Genet 1995; 12: 594-8.
19. Bu Z, Xiong Y, Wang K, Sun Y. Risk factors for ectopic pregnancy in assisted reproductive technology: a 6-year, single-center study. Fertil Steril 2016; 106: 90-4.

ปัจจัยเสี่ยงของการตั้งครรภ์นอกมดลูกในสตรีที่ได้รับการช่วยการเจริญพันธุ์ด้วยวิธีการปฏิสนธินอกร่างกายและการใส่กลับตัวอ่อน

เจริญ ทวีผลเจริญ

วัตถุประสงค์: *เพื่อศึกษาหาปัจจัยเสี่ยงต่อการเกิดการตั้งครรภ์นอกมดลูกในสตรีที่ตั้งครรภ์จากการช่วยการเจริญพันธุ์ด้วยวิธีการปฏิสนธินอกร่างกายและใส่กลับตัวอ่อนทางช่องคลอด*

วัสดุและวิธีการ: *เป็นการศึกษาแบบย้อนหลัง ผู้นิพนธ์ได้ศึกษาข้อมูลของสตรีที่มารับการช่วยการเจริญพันธุ์ด้วยวิธีการปฏิสนธินอกร่างกายและใส่กลับตัวอ่อนทางช่องคลอด ที่หน่วยชีววิทยาช่วยการเจริญพันธุ์ ระหว่าง พ.ศ. 2537 ถึง พ.ศ. 2556 ปัจจัยที่ทำการศึกษา ได้แก่ การใช้ไข่จากผู้บริจาคหรือไข่ของผู้รับบริการ การฉีดอสุจิเข้าเซลล์ไข่เพื่อการปฏิสนธิ อายุของตัวอ่อนที่ใส่กลับ จำนวนตัวอ่อนที่ใส่กลับ และรอบที่ใส่กลับตัวอ่อนเป็นรอบที่กระตุ้นไข่หรือรอบแช่แข็ง*

ผลการศึกษา: *มีสตรีตั้งครรภ์จากการปฏิสนธินอกร่างกายและใส่กลับตัวอ่อนในช่วงเวลาการศึกษาจำนวน 1,385 ราย ซึ่งเป็นการตั้งครรภ์นอกมดลูกจำนวน 29 ราย ปัจจัยเสี่ยงที่เพิ่มโอกาสการตั้งครรภ์นอกมดลูกอย่างมีนัยสำคัญคือ การใส่กลับตัวอ่อนในรอบที่กระตุ้นไข่ ($p = 0.005$) การใส่กลับตัวอ่อนที่มีอายุ 5 วัน ($p = 0.041$) ส่วนการใส่ตัวอ่อนตั้งแต่ 3 ใบขึ้นไป ($p = 0.085$) การใช้ไข่ของผู้บริจาค ($p = 0.999$) และการใช้วิธีการช่วยการปฏิสนธิด้วยการฉีดอสุจิเข้าเซลล์ไข่ ($p = 0.246$) ไม่ได้เพิ่มความเสี่ยงของการเกิดการตั้งครรภ์นอกมดลูกอย่างมีนัยสำคัญ*

สรุป: *ปัจจัยที่เพิ่มความเสี่ยงของการตั้งครรภ์นอกมดลูกในสตรีที่ตั้งครรภ์ภายหลังการใช้วิธีช่วยการเจริญพันธุ์ด้วยวิธีปฏิสนธินอกร่างกายและใส่กลับตัวอ่อนอย่างมีนัยสำคัญ ได้แก่ การใส่กลับตัวอ่อนในรอบการรักษาที่มีการกระตุ้นไข่ และการใส่ตัวอ่อนที่มีอายุ 5 วัน ในอนาคต การศึกษาปัจจัยอื่น ๆ ของสตรีผู้รับบริการและลักษณะของตัวอ่อนน่าจะทำให้ได้ข้อมูลที่ใช้ทำนายความเสี่ยงของการเกิดการตั้งครรภ์นอกมดลูกได้แม่นยำขึ้น*