Study on the incidence and influences on heterotopic pregnancy from embryo transfer of fresh cycles and frozen-thawed cycles

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Abstract

Purpose The purpose of this study was to determine the heterotopic pregnancy rate using fresh versus frozen-thawed embryo transfers and factors associated with heterotopic pregnancy (HP). Management and clinical outcomes after heterotopic pregnancy were also evaluated.

Methods In this retrospective cohort study, we included 12,484 women who had clinical pregnancies after in vitro fertilization treatment at our fertility center between 2012 and 2017. Patients received fresh day 3 embryos (F-D3 group), fresh day 5 blastocysts (F-D5 group), frozen-thawed day 3 embryos (T-D3 group), or frozen-thawed day 5 or 6 blastocysts (T-D5/6 groups) transfers. The primary outcome measure was the occurrence of heterotopic pregnancy. Factors associated with heterotopic pregnancy were analyzed using logistic regression.

Results The heterotopic pregnancy rates were 0.58% in the F-D3, 0.39% in F-D5, 0.56% in T-D3, and 0.33% in T-D5/6 groups, but no differences were found between groups. The risk factors of HP included a history of previous ectopic pregnancy (odds ratio [OR] 5.805, 95% CI 4.578–9.553, $P=0.016$) and pelvic inflammation diseases (OR 1.129, 95% CI 1.021–3.178, $P=0.047$). Salpingectomy was performed in 62.9% patients either through laparoscopy or through laparotomy. The early abortion rate and late abortion rate were 29.03% and 1.61%, respectively. In total, 66.13% of the patients had a live birth, either a singleton (90.24%) or twins (9.76%).

Conclusion No significant difference in the incidence of heterotopic pregnancy in fresh IVF cycles vs. frozen-thawed cycles could be demonstrated in a large cohort of patients. The risk factors of HP included history of ectopic pregnancy and PID. The clinical outcome after heterotopic pregnancy appears to be favorable.

Keywords Heterotopic pregnancy · Ectopic pregnancy · IVF · Risk factors · Management

Introduction

Widespread use of assisted reproductive technology (ART) has substantially increased the incidence of heterotopic pregnancy (HP)—a simultaneous intrauterine and ectopic pregnancy [1]. Despite the fact that IVF allows the direct transfer of embryos into the uterine cavity with the complete bypass of the fallopian tubes, heterotopic pregnancies are more common

following IVF than natural conception and are a well-recognized complication of IVF [1–4].

The increased incidence of heterotopic pregnancies following IVF remains inadequately explained. It has been reported that 72% of HP patients had a history of pelvic inflammation disease (PID) [5]. In addition, multiple embryos are transferred into the uterus during in vitro fertilization, thereby increasing the potential for multiple embryo implantations (both in the uterus and other places) [6–8].

Strategies to decrease the risk of heterotopic pregnancy are limited; heterotopic pregnancies are similar to ectopic pregnancies to some degree [7]. It has been reported that the risk of ectopic pregnancy is lower with the transfer of single frozen blastocyst [9, 10]. Whether the rate of HP is lower when choosing frozen-thawed blastocyst transfer cycles is unknown.

Heterotopic pregnancy is a complex obstetric complication with potentially serious consequences, and the experience of this

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complicated complication is confined to several case reports [11–14]. Additionally, the management of heterotopic pregnancy is particularly challenging because the goal is not only to remove the ectopic pregnancy, but also to be as minimally invasive as possible to continue the intrauterine pregnancy.

The purpose of this study was to analyze the occurrence of heterotopic pregnancy with fresh and frozen-thawed IVF cycles and the risk factors associated with heterotopic pregnancy. Furthermore, the clinical outcomes after heterotopic pregnancy were evaluated.

Materials and methods

This study was a retrospective cohort analysis of women who achieved clinical pregnancy and heterotopic pregnancy after IVF-ET (in vitro fertilization–embryo transfer) in the Clinical Center for Reproductive Medicine, Shenzhen Zhongshan Urology Hospital, between January 2012 and January 2017. Data for individuals who started IVF treatment in our center have been captured routinely in the database of the Clinical Center of Reproductive Medicine. The database includes detailed information on patients' demographic and baseline characteristics, medical history, data of IVF procedures, and follow-up pregnancy outcomes. This study was approved by the Institutional Review Board of the Shenzhen Urology Hospital, and owing to the retrospective nature of the study, the requirement of informed consent was waived.

For this study, we selected procedures performed from January 1, 2012, through January 1, 2017, that resulted in either an intrauterine-only pregnancy or a heterotopic pregnancy. An intrauterine-only pregnancy (IUP) was defined as one or more gestational sacs that were visible by ultrasound and located solely within the uterus. Heterotopic pregnancy was defined as one or more gestational sacs in the uterus and one or more gestational sacs outside the uterus.

The primary outcome measure was the occurrence of HP, and the HP rate was calculated by dividing HPs by the total number of clinical pregnancies (sum of ectopic and clinical intrauterine pregnancies).

In addition, the pregnancy outcomes after heterotopic pregnancies were evaluated. Early abortion was defined as a pregnancy ending in the spontaneous loss of the embryo or fetus before 12 weeks of gestation. Late abortion was defined as a pregnancy ending in the spontaneous loss of the embryo or fetus between 12 and 28 weeks of gestation. In addition, live birth was defined as the delivery of one or more infants with any signs of life.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences Version 22.0 (IBM Corp, Armonk,

NY, USA). Measurement data were compared using Student's *t* test; chi-square test was used for qualitative data. We performed logistic regression analysis to evaluate the risk factors of heterotopic pregnancy. A value of $P < 0.05$ was considered statistically significant.

Results

A total of 12,484 women who achieved clinical pregnancies were recruited in this study; among whom, 62 women (0.50%) had HPs and 12,195 women had intrauterine-only pregnancies (IUP). Among the women, 5691 women conceived after fresh ET, and 6793 women conceived after frozen-thawed ET. The clinical characteristics after fresh or frozen embryo transfer are shown in Table 1. The age and BMI were comparable between fresh and frozen embryo transfer, while the gravidity, parity, previous ectopic pregnancies, maternal infertility diagnosis, fertilization method, rate of assisted hatching, culture day, and number of embryo transferred were significantly different between the fresh and frozen embryo transfer groups.

The HP rates were 0.58% in fresh D3 embryo transfer, 0.37% in the fresh D5 embryo transfer, 0.56% in frozen-thawed D3 embryo transfer, and 0.33% in frozen-thawed blastocyst transfer cycles. The rate of heterotopic pregnancy was the lowest in the frozen-thawed blastocyst ET cycles (Table 2). However, there was no significant difference between the groups.

The risk factors of HP included a history of previous ectopic pregnancy (odds ratio [OR] 5.805, 95% CI 4.578–9.553, $P = 0.016$) and PID (OR 1.129, 95% CI 1.021–3.178, $P = 0.047$) (Table 3).

The sites of ectopic pregnancy were the following: fallopian tubes ($n = 52$), interstitial ($n = 4$), and cornual ($n = 6$). The management of heterotopic pregnancies is shown in Table 4. All patients were diagnosed by ultrasound, and four patients had rupture of ectopic pregnancy before the surgery. Salpingectomy was performed in 62.9% patients either through laparoscopy or through laparotomy. There were 25.81% patients who received conservative surgery to remove the ectopic mass, with no methotrexate or potassium chloride injection. Ultrasound-guided fetal reduction was performed for a case of cornual pregnancy ($n = 1$, 1.61%). And, 8.06% patients received expectant management; none of them suffered rupture of ectopic pregnancy during hospitalization.

The clinical outcome after heterotopic pregnancy is shown in Table 5. The early abortion rate and late abortion rate were 29.03% and 1.61%, respectively. In total, 66.13% of the patients had a live birth, either a singleton 90.24% or twins 9.76%. The clinical outcome of four patients 3.23% was unknown.

Table 1 Clinical characteristics for patients having fresh or frozen embryo transfer

Variable	Fresh (<i>n</i> = 5691)	Frozen (<i>n</i> = 6793)	<i>P</i>
Age	31.53 ± 3.99	32.65 ± 4.02	0.083
BMI	21.41 ± 2.78	21.85 ± 2.80	0.889
Gravidity, <i>n</i> (%)			0.000
0	2858 (50.22%)	2787 (41.03%)	
≥ 1	2833 (49.78%)	4006 (58.97%)	
Parity, <i>n</i> (%)			0.004
0	4402 (77.35%)	5400 (79.49%)	
≥ 1	1289 (22.65%)	1393 (20.51%)	
Previous ectopic pregnancies, <i>n</i> (%)			0.000
0	4510 (79.25%)	5588 (82.26%)	
≥ 1	1181 (20.75%)	1205 (17.74%)	
Maternal infertility diagnosis, <i>n</i> (%)			0.305
Tubal factor	2865 (50.34%)	3327 (48.98%)	
Ovulation dysfunction	523 (9.19%)	683 (10.05%)	
Endometriosis	477 (8.38%)	648 (9.54%)	
Unexplained infertility	1826 (32.09%)	2135 (31.43%)	
Fertilization method			0.000
IVF	4738 (83.25%)	4962 (73.05%)	
ICSI	953 (16.75%)	1831 (26.95%)	
Assisted hatching (<i>n</i> , %)			0.000
0	5476 (96.22%)	0	
1	215 (3.78%)	6793 (100%)	
Culture day			0.000
Cleavage stage	5146 (90.42%)	3206 (47.20%)	
Blastocyst stage	545 (9.58%)	3587 (52.80%)	
Number of embryos transferred			0.000
1	115 (2.02%)	623 (9.17%)	
2	3780 (66.42%)	4070 (59.91%)	
3	1796 (31.56%)	2094 (30.83%)	

Discussion

IVF is used with increasing frequency as a useful procedure in women suffering from infertility. Despite the fact that IVF allows direct transfer of embryos into the uterine cavity with the complete bypass of the fallopian tubes, heterotopic pregnancies are more common following IVF than natural

conception and have been a well-recognized complication of IVF [1–4].

The increased incidence of heterotopic pregnancies following IVF remains inadequately explained, and strategies to decrease the risk are limited. Choosing a day 5 embryo transfer in a frozen embryo transfer cycle was suggested as a method to reduce the incidence of ectopic pregnancy [9, 10]. In our

Table 2 Intrauterine and heterotopic pregnancy rates (*n*, %)

	HP (%)	IUP (%)	<i>P</i>
Fresh embryo transfer			> 0.05
F-D3	0.58% (30/5146)	97.24% (5004/5146)	
F-D5	0.37% (2/545)	99.08% (540/545)	
Frozen-thawed embryo transfer			
T-D3	0.56 (18/3206)	97.38 (3122/3206)	
T-D5/6	0.33 (12/3587)	98.40 (3529/3587)	

HP heterotopic pregnancy, IUP intrauterine-only pregnancy, F-D3 fresh D3 embryos, T-D3 frozen-thawed day 3 embryos, T-D5/6 frozen-thawed day 5/6 blastocysts day

Table 3 The risk factors of heterotopic pregnancy in IVF-ET patients

Risk factors	Odds ratio (95% CI)	P value
Age	0.953 (0.839–1.082)	0.340
BMI	0.985 (0.802–1.210)	0.707
Previous birth	1.351 (0.876–2.480)	0.309
Previous ectopic pregnancy	5.805 (4.578–9.553)	0.016*
PID	1.129 (1.021–3.178)	0.047*
Culture day	0.976 (0.551–1.730)	0.934
ART method (fresh or frozen)	1.225 (0.587–2.556)	0.589
No. of embryos transferred	1.671 (0.978–2.855)	0.457

* $P < 0.05$

current study, frozen-thawed blastocyst transfer was associated with the lowest incidence of heterotopic pregnancy, although we did not observe any significant difference between groups. Possible reasons are that blastocyst transfer is closer to the natural physiologic state and that the endometrium is in a natural or near-natural state during the frozen-thawed cycles [10, 15]. Previous studies suggested that blastocyst transfer significantly reduced the risk of ectopic pregnancies as a blastocyst has high endometrium receptivity [9, 10, 16]. Furthermore, it is possible that ovarian stimulation in a fresh cycle may have a negative effect on endometrial receptivity [17–19]. Some authors have suggested that increased uterine contractility in stimulated cycles is responsible for pushing the embryo into the tube and may affect endometrium receptivity [17, 20, 21]. Controlled ovarian stimulation induces morphological, biochemical, and functional genomic modifications of the human endometrium during the window of implantation, and it may also have a negative effect on the endometrium and on preferential implantation into the fallopian tube [22, 23].

The incidence of heterotopic pregnancy in IVF patients has increased in recent year, likely due to the widespread use of assisted reproductive technology (ART), pelvic surgery, and a history of pelvic inflammatory disease (PID). Multiple studies have demonstrated that women with tubal factor infertility have an increased risk of ectopic pregnancy after ART compared with those with other types of infertility diagnoses. Tubal

Table 4 Management of heterotopic pregnancy

Variable	N (%)
Laparoscopic salpingectomy	33 (53.23%)
Laparotomy salpingectomy	6 (9.68%)
Laparotomy conservative surgery	4 (6.45%)
Laparoscopic conservative surgery	12 (19.35%)
Ultrasound-guided fetal reduction	1 (1.61%)
Expectant management	5 (8.06%)
Missing	1 (1.61%)

Table 5 Clinical outcome of heterotopic pregnancy

Variable	N (%)
Early abortion	18 (29.03%)
Late abortion	1 (1.61%)
Live birth	41 (66.13%)
Singletons	37 (90.24%)
Twins	4 (9.76%)
Missing	2 (3.23%)

infertility has been shown to be a risk factor for HP cases among women treated with IVF [3, 8]. In our current study, PID and previous ectopic pregnancy were found to be risk factors, which were consistent with the findings of previous studies [3, 24].

The primary goals for treating HP are removing the ectopic mass and sustaining the intrauterine pregnancy. It is reported that approximately 41.2% of HPs ended with natural spontaneous abortion or induced abortion. In our research, the total abortion rate was 30.65% (19/62) in all HP patients, which is lower than what was previously reported, and the possible reasons may be that the diagnosis and management are more timely and positive in these years. Clayton noted out that 63.3% of IUP kept on living when HP cases were treated properly, and that the miscarriage rate for HP patient who underwent surgery was up to 31.25% (25/80) [5]. Although survival of the IUP cannot always be assured in cases of HP, 66.13% of patients had a live birth in our study, and the prognosis for women with HP is generally fairly good.

Due to the rarity of HP, it is difficult to conduct a randomized controlled trial. The limitation of our retrospective study is that the patients enrolled in each group are indeed not comparable in some basal clinical characteristics in a fresh or frozen embryo transfer. It is difficult to determine which method is better for decreasing the risk of HP. In addition, single embryo transfer was rare during the study period, and we cannot know whether single embryo transfer in a frozen-thawed cycle is effective in reducing the HP rate.

In conclusion, the incidence of heterotopic pregnancy after fresh embryo transfer or frozen-thawed embryo transfer is comparable. It is to be expected that with the introduction of selective single blastocyst transfer, the incidence will diminish. Despite the large sample size, there was very minimal power to detect a difference given the low incidence of heterotopic pregnancy. Larger studies are needed given the potentially important clinical implications of whether frozen blastocyst transfer is associated with the risk of ectopic pregnancy following IVF treatment. However, to date, the clinical outcome after heterotopic pregnancy is acceptable.

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Compliance with ethical standards

Disclosure statement The authors have nothing to disclose.

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