Gynecology and Obstetrics Clinical Medicine 2 (2022) 171–174

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Research Paper

Analysis of factors related to early miscarriage after in vitro fertilization embryo transfer

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ARTICLE INFO

Keywords:
In vitro fertilization and embryo transfer
Early spontaneous miscarriage
Fresh cycle
Frozen cycle

ABSTRACT

Aims: This research aims to explore the factors related to early spontaneous miscarriage after in vitro fertilization and embryo transfer (IVF-ET) pregnancy, and to provide guidance for improving pregnancy outcomes.
Methods: We retrospectively analyzed the data for clinical pregnant women (2591 cycles) undergoing IVF-ET at the reproductive center of Peking University People's Hospital from January 2017 to December 2018; This included 544 ET cycles and 2047 frozen embryo transfer cycles. The analysis of factors associated with early miscarriage in the overall population of IVF/intracytoplasmic sperm injection (ICSI) pregnancies (including fresh and thawing cycles) was performed.
Results: The risk of early miscarriage in the 35–39 age group was 1.35 times higher than that in the <35 age group (OR = 1.35 [1.05,1.73], $p = 0.02$). In addition, the risk of early miscarriage was 3.88 times higher in the group ≥40 years old than in the group <35 years old (OR = 3.88 [2.68,5.62], $p < 0.001$). Endometrial thickness also affected the miscarriage rate; the early miscarriage risk with endometrial thickness ≥8.5 mm was 0.78 times than that of the <8.5 mm group (OR = 0.78 [0.62,0.98], $p = 0.03$). The early miscarriage rate during frozen embryo transfer was 1.48 times higher than that during fresh embryo transfer (OR = 1.48 [1.08,2.02], $p = 0.01$), while in the fresh cycle, the risk of early miscarriage with high-quality embryos was 0.5 times lower than that with non-high quality embryos (OR = 0.5 [0.27,0.9], $p = 0.02$). In the frozen cycle, the risk of early miscarriage in natural cycle transplantation was 0.73 times that in hormone replacement treatment (HRT) cycle transplantation (OR = 0.73 [0.54,0.97], $p = 0.03$) .
Conclusions: Advanced age is an independent risk factor for early miscarriage, while endometrial thickness at the date of transplantation is an independent protective factor. The risk of early miscarriage in fresh-cycle transplanted embryos is significantly lower than that in frozen embryos, and the number of high-quality embryos in the fresh cycle lowers the miscarriage rate significantly. Natural cycle transplantation has a lower rate of early miscarriage than hormone replacement therapy.

1. Introduction

In vitro fertilization and embryo transfer (IVF-ET) has increasingly become an important means of infertility treatment. At present, IVF-ET can achieve a certain clinical pregnancy rate and live birth rate, but miscarriage still occurs at a significant rate. Miscarriage not only increases the number times of assisted reproduction treatment and the economic burden, but also damages the endometrium. The double blow to the body and the mind will cause a poorer outcome for the next pregnancy.

IVF-ET has a similar early miscarriage rate (approximately 15%) compared with natural pregnancy.[1,2] However, for women receiving IVF-ET, there are different causes of infertility, and the effects of ovulation, in vitro fertilization, and the freezing and thawing process on

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Publishing services by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.gocm.2022.09.001
Received 22 February 2021; Received in revised form 21 May 2021; Accepted 23 September 2022
Available online 12 November 2022

miscarriage are not yet clear. Here, a retrospective cohort study was used to analyze the factors related to early miscarriage in all clinical pregnancy women with IVF-ET.

2. Material and methods

2.1. Study design

A total of 2591 cycles were collected in the Reproductive Center of our hospital. The inclusion criteria were as follows; (1) Infertility women undergoing IVF/intracytoplasmic sperm injection (ICSI) who were admitted to our hospital from 2017 to 2018. (2) Clinical pregnant cycles through fresh or frozen embryo transfer, and the exclusion criteria were as follows: (a) donor embryo transfer; (b) having serious systemic diseases and unable to tolerate pregnancy; (c) biochemical pregnancy and ectopic pregnancy. For those women who met the criteria, basic clinical data were collected, including age, body mass index (BMI), years of infertility, basal follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), endometrial thickness, fresh or frozen embryo transfer, number of transferred embryos, type of transferred embryos, and number of previous miscarriages.

2.2. Methods

The factors related to early miscarriage in women with clinical pregnancy were analyzed. Miscarriage (excluding biochemical pregnancy) at< 12 weeks of pregnancy is early miscarriage; the early miscarriage rate = number of early miscarriages/number of clinical pregnancies. According to the embryo scoring system at cleavage, the quality of embryos at 72 h after oocyte retrieval was assessed at four levels, of which grade I and II embryos were considered to be of high quality.

2.3. Statistical analysis

SPSS 23.0 was used for statistical analysis. The quantitative data were normally distributed, as described by the mean ± standard deviation (x ± s) and Student's t-test; non-normal distributions, expressed by the median (lower quartile-upper quartile) [M (P25-P75)] were analyzed using a non-parametric test comparison. Qualitative data was expressed by the frequency and composition ratio, and the Chi-square test or Fisher's exact probability method was used for inter-group comparison. Univariate and multivariate logistic regression was used to analyze the factors influencing early miscarriage. Variables with $p < 0.05$ in the univariate logistic regression were included in the multivariate logistic regression. In addition, the multivariate logistic regression also included factors that might affect miscarriage based on clinical experience. Multivariate logistic regression independent variables were screened by the input method, and the difference was statistically significant at $p < 0.05$ on both sides.

3. Results

A total of 2591 cycles which met the criteria were divided into an early miscarriage group (n = 381) and an ongoing pregnancy group (n = 2210). The rate of early miscarriage was 14.7% (381/2591). There were significant differences in the distributions of age and endometrial thickness between the early miscarriage group and the ongoing pregnancy group, but there was no significant difference in the distribution of BMI, years of infertility, FSH, LH, and E2 between the two groups ($p > 0.05$). The demographic data for women in the two groups are shown in Table 1.

Age is an independent risk factor for early miscarriage, The risk of early miscarriage after IVF-ET in 35–39 years old group was 1.35 times higher than that in <35 years old group (OR = 1.35 [1.05,1.73], P = 0.02) . The risk in the ≥40 years old group was 3.88 times higher than that in <35 years old group (OR = 3.88 [2.68,5.62], P < 0.001). Endometrial thickness was an independent protective factor (OR = 0.78 [0.62,0.98], P = 0.03). Compared with the fresh embryo transfer cycle, the frozen-thawed embryo transfer cycle was a risk factor (OR = 1.48 [1.08,2.02], P = 0.01). No correlation was found between the number and type of transferred embryos, duration of infertility, number of previous miscarriages, BMI, and occurrence of early miscarriages ($p > 0.05$) (Table 2).

Table 1
Comparison of general data between the early miscarriage group and the ongoing pregnancy group [M (P25-P75)] or n (%).

	Early miscarriage (n = 381)	Ongoing pregnancy (n = 2210)	Z/c2	P
Age (years)	34 (31,37.5)	33 (30,35)	−6.193	< 0.001
Duration of infertility (years)	3 (2,5.5)	3 (2,5)	−1.692	0.091
BMI (kg/m^2)	22.5 (20.5,25.3)	22.2 (20.3,24.9)	−1.317	0.188
Endometrial thickness (mm)	9 (8,10)	9 (8,10.9)	−3.386	0.01
No. of previous miscarriages(n)			4.680	0.197
0	321 (84.3)	1901 (86.0)		
1	43 (11.3)	248 (11.2)		
2	12 (3.1)	50 (2.3)		
≥3	5 (1.3)	11 (0.5)		
Infertility diagnosis(n)			1.267	0.867
Endometriosis	38 (10)	247 (11.2)		
PCOS	44 (11.5)	255 (11.5)		
POI	32 (8.4)	167 (7.6)		
Pelvic cavity and fallopian tube factors	128 (33.6)	776 (35.1)		
others	139 (36.5)	765 (34.6)		
FSH (IU/L)	7.6 (6.1,9.5)	7.4 (6.0,9.0)	−1.468	0.142
LH (IU/L)	4.0 (2.8,5.7)	4 (2.81,5.8)	−0.652	0.514
E2 (pg/ml)	42.7 (29.6,56.1)	42.5 (30.5,57.2)	−0.215	0.830

PCOS:polycystic ovary syndrome**; POI:** primary ovarian insufficiency; **FSH**: follicle-stimulating hormone; **LH**: luteinizing hormone **E2**:estradiol.

Analysis of the factors that influenced early miscarriage in the fresh embryo transfer cycle: the results of univariate analysis showed that there were fewer insemination methods and ovulation-promoting schemes. The number of high-quality embryos was an independent protective factor for early miscarriage (OR = 0.5 [0.27,0.9], $p = 0.02$). After adjusting for other factors by multivariate analysis, we found that there was no correlation between the number of oocytes retrieved, controlled ovarian hyperstimulation protocols, insemination methods, the number of previous miscarriages, and the occurrence of early miscarriages ($p > 0.05$). (Table 3).

Analysis of the factors related to early miscarriage in the frozen-thawed embryo transfer cycle: the number of transferred embryos was a protective factor for early miscarriage (OR = 0.71 [0.52,0.96], $p = 0.03$). Compared with hormone replacement treatment (HRT) cycle transfer, natural cycle (NC) transfer was a protective factor (OR = 0.73 [0.54,0.97], P = 0.03). There was no significant difference in the risk of early miscarriage between the ovulation cycle group and the HRT cycle group (P = 0.5). After adjusting for other factors by multivariate analysis, there was no correlation between endometrial thickness, type of infertility, and early miscarriage. ($p > 0.05$). (Table 4).

4. Discussion

The results of our study showed that advanced age was an independent risk factor for early miscarriage, which is similar to the results of previous relevant research.[3,4] In the fresh embryo transfer cycle, the early miscarriage rates in the <35-year-old group and ≥35-year-old group

Table 2
Analysis of the correlation factors of early miscarriage.

	Early miscarriage rate (%)	Single-factor analysis		Multivariate -factor analysis	
		OR	*P*	OR	*P*
Age (years)					
<35	12.0				
35–39	16.3	1.417	0.005	1.348	0.018
≥40	35.8	4.110	<0.001	3.883	<0.001
BMI (kg/m^2)					
18.5–23.9	13.9				
<18.5	15.3	1.117	0.611	1.873	0.389
24–27.9	15.2	1.107	0.446	1.391	0.681
≥28	17.9	1.353	0.103	2.067	0.062
Endometrial thickness (mm)					
<8.5	17.3				
≥8.5	13.2	0.724	0.005	0.776	0.032
Cycle type					
Fresh cycle	10.1				
Frozen-thawed cycle	15.9	1.684	0.001	1.477	0.014
Embryo type					
Cleavage-stage embryo	13.8				
Blastocyst-stage embryo	15.6	1.073	0.206	–	–
Embryo number					
1	18.3				
2	14.0	0.729	0.026	0.762	0.065
No. of previous miscarriages					
0	14.4				
1	14.8	1.027	0.88	0.888	0.526
2	19.4	1.421	0.282	1.123	0.735
≥3	31.3	2.692	0.068	1.772	0.308

Table 3
Analysis of factors related to early miscarriage in the fresh embryo transfer cycle.

	Early miscarriage rate (%)	Single-factor analysis		Multivariate -factor analysis	
		OR	*P*	OR	*P*
Ovarian stimulation protocol					
GnRH-ant protocol	10.8				
GnRH-a long protocol	9.1	0.827	0.547	–	–
Other protocol	9.5	0.865	0.757	–	–
Insemination methods					
IVF	10.8				
ICSI	9	0.821	0.513	–	–
No. of high-quality embryos		0.920	0.019	0.932	0.066
0	13.9				
≥1	7.2	0.481	0.012	0.499	0.020

GnRH-a: Gonadotrophin-releasing hormone agonist; GnRH-ant: Gonadotropin-releasing hormone antagonist; IVF-ET: In vitro fertilization and embryo transfer; ICSI: intracytoplasmic sperm injection.

were 8.4% and 13.9%, respectively. Although there was no statistical difference after multivariate analysis ($p > 0.05$), it can still be seen that the early miscarriage rate increased significantly with increasing age. However, there was no significant difference because the sample size of the fresh cycle group was small. The high miscarriage rate in the older patients was mainly related to the aneuploidy of the embryos. Pylyp et al.[5] examined the early miscarriage tissues of ICSI pregnancies using cytogenetic technology and found that the chromosome abnormality rates in the miscarriage tissues of the <35 years-old group, the 36-40 years-old group, and the ≥40 years-old group were 45.32%, 54.7%, and 63.2%, respectively, suggesting that the aneuploidy rate of the

Table 4
Analysis of factors related to early miscarriage in the Frozen-Thawed embryo transfer cycle.

	Early miscarriage rate (%)	Single-factor analysis		Multivariate-factor analysis	
		OR	*P*	OR	OR
Endometrial preparation protocol					
HRT cycle	17.3				
NC cycle	13.1	0.720	0.022	0.73	0.034
OI cycle	15.1	0.849	0.434	0.876	0.538
Embryo type					
Cleavage-stage embryo	16.5				
Blastocyst-stage embryo	15.6	0.968	0.599	–	–
Embryo number					
1	19.6				
2	15.2	0.733	0.039	0.708	0.026

HRT: hormone replacement treatment; NC: natural cycle; OI: ovulation induction.

embryos was the main cause of ICSI pregnancy miscarriage. Gruhn et al.[6] discovered that the relationship between human oocyte aneuploidy and age described a u-curve; that is, the aneuploidy rate of oocytes in women <20 years old and >35 years old was significantly higher, because only 1%–8% of male spermatozoa showed aneuploidy, and this did not increase with the ages of the men. However, about 20%–30% of oocytes in women of childbearing age were aneuploid.[7,8] The aneuploidy rate of oocytes in women aged >35 years old is as high as 70%.[9] Therefore, female age is an independent factor leading to early miscarriage in IVF/ICSI, and the increase in oocyte aneuploidy is the root cause.

A randomized controlled trial showed a significant increase in the live birth rate of frozen-thawed embryo transfer cycle compared with fresh cycle,[10] but there was no significant difference in the miscarriage rate. However, we found that the frozen-thawed embryo transfer cycle was an independent risk factor for early miscarriage in IVF-ET, which may be due to the fact that embryo freezing technology affects the developmental potential of embryos. Although vitrification freezing technology is currently recognized as being economical and safe, embryos still have to undergo the process of freezing and thawing, and are exposed to physical and chemical media such as cryoprotectants, which may affect the structure of cells and the integrity of the genome. In addition, differences in the skills of laboratory operators may have different effects on the embryos.[11] Because this paper reports a retrospective study, the general situation of the infertile population is heterogeneous, so the results have some limitations.

The results of this study show that compared with the HRT cycle, the NC cycle is a protective factor for early miscarriage, and studies by Cerrillo[12] and Hatoum[13] et al. have reached similar conclusions. The use of a large amount of estrogen in the HRT cycle, and the high level of estrogen and the imbalance of estrogen and progesterone, have adverse effects on the receptivity of the endometrium. Morozov et al.,[14] found that the E2 level in the HRT cycle was higher than in the NC cycle. The high serum E2 level affects endometrial receptivity by regulating the expression of endometrial genes. Therefore, high estradiol levels in the HRT cycle may interfere with embryo implantation and thus affect the pregnancy outcome.[15] Also, in the HRT cycle, the dose and timing of progesterone administration will also affect the pregnancy outcome. Generally, the endometrium will have histological changes on the sixth day of progesterone administration. However, Gomaa and Díaz-Gimeno et al.[16,17] found that about 25% of the endometrium had no histological changes and presented a delayed state. The time of progesterone administration and transfer in the HRT cycle were closely related to the synchronicity of the embryo and endometrium, while NC cycle transplantation did not involve this problem. Therefore, endometrial biopsy can be used to determine whether there are histological changes in the endometrium, so as to adjust the number of days after progesterone

administration before embryo transfer and to correct the non-synchronization of endometrial and embryonic development, which may reduce the occurrence of miscarriage in the HRT cycle. There is no doubt that endometrial thickness affects the embryo implantation rate. Previous studies in our center have found that when the endometrium is < 6 mm thick, the embryo implantation rate, clinical pregnancy rate, and live birth rate significantly decrease.[18] In this study, we found that endometrial thickness is an independent factor that influences early miscarriage (OR = 0.78, $p < 0.05$), and the rate of early miscarriage was lower when the endometrium was ≥8.5 mm thick. Gallos et al.[19] reached a similar conclusion, which have a certain guiding significance for clinical treatment.

In this study, we found that the early miscarriage rate of those women with high-quality embryos in the fresh cycle (7.2%) was significantly lower than that in women without high-quality embryos (13.9%, $p < 0.05$). Chang et al.[20] found that the high-quality embryo rate and miscarriage rate were lower in women with ovarian dysfunction. Therefore, the number of high-quality embryos, to a certain extent, reflects the quality of ovarian reserve function, which thus affects the outcome of the pregnancy.

5. Limitations

Because this study is a single-center retrospective study, the general factors between the study group and the control group cannot be strictly controlled, resulting in some bias. Therefore, the results have certain limitations, and a large sample prospective randomized controlled study is still needed to analyze the factors related to early miscarriage.

6. Conclusion

We conclude that female age, endometrial thickness, cycle type, and the freeze-thaw protocol are independent factors that influence early miscarriage. Early miscarriage is the main complication in IVF-ET pregnancy. Finding and avoiding possible risk factors and further providing a basis for clinical intervention measures to reduce the miscarriage rate are urgently needed at present. Due to fact that this study is a single-center retrospective study, the results have some limitations, and a large sample of prospective randomized controlled trials are still needed to analyze the effects of factors related to early miscarriage in IVF-ET.

Ethics statement

The study was approved by the Ethics Committee of Peking University People's University (2021PHB083) and all women involved in the study provided informed consent.

Author contributions

Liying Zuo and Li Tian conceived and designed the experiments. Liying Zuo collected and analyzed the data. Li Tian reviewed the draft. All authors contributed to the article and approved the submitted version.

Funding

No.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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