In vitro fertilization-embryo transfer in patients with unexplained recurrent pregnancy loss

Tai-Yang Li[1], Rong Li[1], Lin Zeng[2], Li Li[1], Jie Qiao[1], Ping Liu[1], Hai-Yan Wang[1]

[1]Center of Reproductive Medicine, Peking University Third Hospital, Beijing 100191, China;
[2]Clinical Epidemiology Research Center, Peking University Third Hospital, Beijing 100191, China.

Abstract

Background: Empiric therapy for patients with unexplained recurrent pregnancy loss (URPL) is not precise. Some patients will ask for assisted reproductive technology due to secondary infertility or advanced maternal age. The clinical outcomes of URPL patients who have undergone *in vitro* fertilization-embryo transfer (IVF-ET) require elucidation. The IVF outcome and influencing factors of URPL patients need further study.

Methods: A retrospective cohort study was designed, and 312 infertile patients with URPL who had been treated during January 2012 to December 2015 in the Reproduction Center of Peking University Third Hospital were included. By comparing clinical outcomes between these patients and those with tubal factor infertility (TFI), the factors affecting the clinical outcomes of URPL patients were analyzed.

Results: The clinical pregnancy rate (35.18% *vs.* 34.52% in fresh ET cycles, $P = 0.877$; 34.48% *vs.* 40.27% in frozen-thawed ET cycles, $P = 0.283$) and live birth rate (LBR) in fresh ET cycles (27.67% *vs.* 26.59%, $P = 0.785$) were not significantly different between URPL group and TFI group. URPL group had lower LBR in frozen-thawed ET cycles than that of TFI group (23.56% *vs.* 33.56%, $P = 0.047$), but the cumulative LBRs (34.69% *vs.* 38.26%, $P = 0.368$) were not significantly different between the two groups. The increased endometrial thickness (EMT) on the human chorionic gonadotropin day (odds ratio [OR]: 0.848, 95% confidence interval [CI]: 0.748–0.962, $P = 0.010$) and the increased number of eggs retrieved (OR: 0.928, 95% CI: 0.887–0.970, $P = 0.001$) were protective factors for clinical pregnancy in stimulated cycles. The increased number of eggs retrieved (OR: 0.875, 95% CI: 0.846–0.906, $P < 0.001$), the increased two-pronucleus rate (OR: 0.151, 95% CI: 0.052–0.437, $P < 0.001$), and increased EMT (OR: 0.876, 95% CI: 0.770–0.997, $P = 0.045$) in ET day were protective factors for the cumulative live birth outcome.

Conclusion: After matching ages, no significant differences in clinical outcomes were found between the patients with URPL and the patients with TFI. A thicker endometrium and more retrieved oocytes increase the probability of pregnancy in fresh transfer cycles, but a better normal fertilization potential will increase the possibility of a live birth.

Keywords: Unexplained recurrent pregnancy loss; Cumulative live birth rate; Tubal factor infertility

Introduction

Recurrent pregnancy loss (RPL) is defined as three consecutive spontaneous pregnancy losses (SPLs) before 20 weeks in the same couple under normal circumstances; after two SPLs, patients often seek clinical help. Therefore, the current international consensus is that a history of two or more SPLs can be diagnosed as RPL. The causes of RPL are complex and can be broadly divided into maternal and fetal factors. Maternal factors include anatomical abnormalities in the genital tract, a hypercoagulable state, autoimmune diseases, parental chromosomal abnormalities, genetic susceptibility, etc; and fetal factors mainly include fetal chromosomal abnormalities.[1] Some RPLs have one or more of the above causes, but approximately 50% of RPLs have no clear causes and are called unexplained recurrent pregnancy loss (URPL).

URPL patients choose *in vitro* fertilization (IVF) because of secondary infertility, concern about declining fertility due to advanced age, and an urgent desire to achieve a live birth. Multiple SPLs are regarded as a pathological condition. The possible pathogenesis includes low levels of serum folic acid due to mutations in folate metabolism-related genes,[2] abnormal maturation and activation of peripheral blood lymphocyte subsets,[3-5] and a lack of factors associated with placental vascularization and vascular endothelial cell function maintenance.[6,7] However, the treatments for these conditions, such as anti-

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001657

Correspondence to: Hai-Yan Wang, Center of Reproductive Medicine, Peking University Third Hospital, Beijing 100191, China
E-Mail: wangquan1991@sina.com

Chinese Medical Journal 2021;134(20)
Received: 12-12-2020 **Edited by:** Jing Ni

coagulation, lymphocyte immunotherapy, intravenous immunoglobulin therapy, and pre-implantation genetic testing for aneuploidy (PGT-A), have not been consistently effective.[8-10] These treatments may increase the incidence of some adverse reactions. What are the outcomes of URPL patients undergoing IVF without PGT-A? URPL patients are known to have a higher risk of miscarriage than non-URPL patients in natural pregnancy. However, whether patients with a history of recurrent miscarriage can be defined as having a poorer assisted reproductive technology prognosis is unclear. This study was designed to answer the above questions.

Methods

Ethical approval

The study was approved by the Ethics Committee of the Reproductive Medicine Center of Peking University Third Hospital (No. 2019SZ-076) and exempted from informed consent.

Study design

The study was designed as a retrospective cohort. The exposure group included infertile patients with URPL. All patients were infertile for >1 year after experiencing more than two times of unexplained miscarriages. The non-exposed group included patients with tubal factor infertility (TFI). The main outcome measures were the clinical pregnancy rate and live birth rate (LBR) during fresh and frozen-thawed embryo transfer (ET) cycles and the cumulative LBR between the two groups.

According to previous studies, URPL patients had a miscarriage rate of 33% to 50% (median, 34%) after IVF pregnancy,[9,11,12] and patients with TFI had a miscarriage rate of 8.70% to 21.30% (median, 12.37%) after IVF pregnancy.[13-16] This study is a retrospective cohort study. According to previous literature, the miscarriage rate in the exposed group was as low as 33%, while that in the non-exposed group was as high as 21.30% ($\alpha = 0.05$ [bilateral], $\beta = 0.01$). The sample sizes ($N1 = N2 = 302$) for the exposed group and non-exposed group were calculated using PASS 11 software (NCSS, Kaysville, USA), with a statistical power of 99%.

Based on the sample size calculation, the study population was limited to patients who met the diagnostic criteria and entered a stimulated cycle at the Reproduction Center of Peking University Third Hospital from January 2012 to December 2015.

Considering that a maternal age has a considerable influence on clinical outcomes, the ages of the women in the two groups were matched such that no difference existed between the two groups. The patients in the two groups were matched 1:1 according to age.

Selection and description of the participants

The database of the Reproduction Center of Peking University Third Hospital was searched for patients with a clinical diagnosis of RPL. Patients conforming to this diagnosis underwent a total of 835 stimulated cycles from January 2012 to December 2015, and 53 stimulated cycles were submitted to PGT-A. The remaining patients underwent a total of 782 stimulated cycles. According to routine clinical practice, the remaining RPL patients had accepted the following examinations: two-dimensional or three-dimensional ultrasound for uterine factors, cervical cytology, endocrine evaluation: basal prolactin (PRL), testosterone (T), androstenedione (A), thyroid-stimulating hormone (TSH), free thyroxine (FT4), anti-thyroid antibodies (anti-thyroid peroxidase and thyroid receptor antibody); screening of the couple's chromosome karyotype; immune evaluation: antiphospholipid antibody, including lupus anticoagulant, anticardiolipin antibody, anti-β2 glycoprotein antibody; and thrombogenic screening: protein C, protein S, serum homocysteine, serum fibrinogen, and D-dimer. A total of 230 stimulated cycles with parental chromosomal abnormalities, and 145 stimulated cycles with other known factors causing RPL, were identified. Patients with normal results were defined as URPL patients, and the first stimulated cycles (312 stimulated cycles) of these patients (407 stimulated cycles) were included. The screening process is shown in Figure 1.

The same database was searched for TFI patients with the following clinical diagnoses: bilateral tubal obstruction and bilateral tubal resection. The stimulated cycles of these patients from January 2012 to December 2015 were selected. Patients with any of the following conditions were excluded: unilateral or bilateral hydrosalpinx confirmed by surgery or hydrosalpinx suspected by hysterosalpingogram or ultrasound; abnormal basal PRL, T, A, TSH, and FT4 levels; an abnormal chromosome karyotype; positive autoimmune antibody: antiphospholipid antibody, lupus anticoagulant, anticardiolipin antibody, anti-β2 glycoprotein antibody; abnormal levels of protein C, protein S, serum homocysteine, serum fibrinogen, and D-dimer; and two or more spontaneous abortions. According to the examination results of patients without the above conditions, only patients diagnosed by hysterosalpingogram and laparoscopy with bilateral fallopian tube obstruction or patients with a history of bilateral salpingectomy were included. According to the results of the routine examination of male semen, couples suffering from infertility caused by male azoospermatism and severe oligospermia were excluded in both groups.

The stimulated cycles of patients with TFI were screened out to match the stimulated cycles in the URPL group at a ratio of 1:1, thus forming the TFI group. The screening criteria were as follows: In the TFI group, no repeat stimulated cycles were performed for the same couple, and screening was conducted in the following four steps: (1) Each stimulated cycle in the TFI group was matched according to the date of egg retrieval for each cycle of the URPL group (<10 days); (2) the cycles were screened for those with matching female ages (±3 years or less); (3) the cycles were screened for those with close male ages; and (4) the cycles were screened for those with close egg retrieval times. If multiple cycles met the screening criteria, computer software selected the cycles at random.

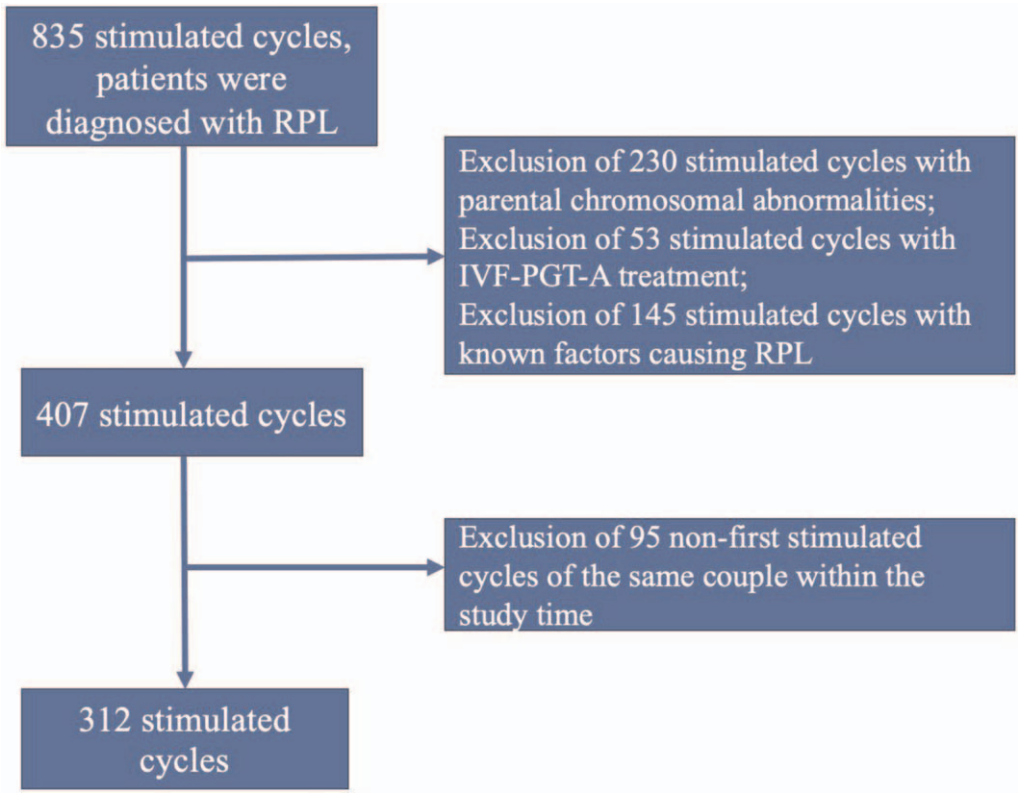

Figure 1: Screening process of the first stimulated cycles for unexplained recurrent pregnancy loss patients. IVF-PGT-A: *In vitro* fertilization-preimplantation genetic testing for aneuploidy; RPL: Recurrent pregnancy loss.

Laboratory screening criteria

All patients underwent controlled ovarian hyperstimulation in accordance with the conventional ovulation induction program of the Reproduction Center of Peking University Third Hospital. Transvaginal oocyte retrieval was performed 36 h after human chorionic gonadotropin (HCG) injection. The clinician determined the method of insemination based on the results of the semen test and the patient's personal situation. Metaphase II (M II) oocytes were selected for intracytoplasmic sperm injection. The prokaryotes of the fertilized oocytes were observed 16 to 18 h after insemination. Two-pronucleate (2PN) embryos were considered normal fertilized embryos. The embryos were classified into four grades[17]: Grades I and II referred to high-quality embryos, Grades I–III applied to transferable embryos, and Grade IV represented non-transferable embryos. Blastocyst culture was determined on a case-by-case basis. All embryos that had not been transferred in stimulated cycle were frozen. The selection between natural and artificial frozen-thawed ET cycles was based on the center's routine, and corpus luteum support after transfer was provided depending on the patient's individual condition.

Clinical analysis factors and the follow-up process

The following patients' information were recorded: the ages of the couple, female body mass index (BMI), basic sexual hormone levels of the female, sexual hormone levels on the HCG day, the total dose of gonadotropin, sperm density, percentage of forward motility sperm, the number of retrieved eggs, the 2PN oocytes rate, the insemination method, the number of high-quality embryos, the number of transferable embryos, and the number of frozen embryos. Endometrial thickness (EMT) values on HCG day were recorded in fresh transfer cycles. EMT values on transfer day were recorded in frozen-thawed transfer cycles. The data of frozen-thawed ET cycles included the initial number of frozen embryos, the number of embryos after thawing, the number of embryos transferred, and the number of remaining embryos. If not all the embryos retrieved during the corresponding stimulated cycle were transferred during the follow-up period, the stimulated cycle and subsequent frozen-thawed ET cycle were removed from calculation of the cumulative pregnancy rate and cumulative LBR. The ratio of the number of live birth cycles to the number of transfer cycles is the LBR.

The patients were followed up three times after the fresh cycle and the frozen-thawed ET cycle, that is, 14 days after ET, 12 weeks after pregnancy confirmation, and after full-term pregnancy. At 14 days after ET, serum HCG was measured, and biochemical pregnancy was confirmed if the serum HCG was >25 IU/L. Early pregnancy results were obtained at the follow-up before 12 weeks of pregnancy in our hospital or in other hospitals, and intrauterine pregnancy was recorded if ultrasound examination confirmed intrauterine fetal heartbeats. During the follow-up visit after full-term pregnancy, pregnancy outcomes,

delivery methods, gestational age, neonatal conditions, and pregnancy complications were recorded. If adverse pregnancy outcomes occurred, such as pregnancy loss (PL), premature delivery, or mid-trimester induction of labor, the gestational age at termination of the pregnancy, the causes of the adverse outcomes, and examination results were recorded in detail.

Statistical analysis

All data were calculated using SPSS 23.0 software (IBM Corporation, Armonk, NY, USA). Continuous data with a normal distribution are reported as the mean value with the standard deviation. Continuous variables with a non-normal distribution are reported as the median with interquartile range. A two independent-samples *t*-test was used to analyze differences between means. Categorical data are presented as percentages, and the Chi-square test was used to test differences between the two groups. A P value < 0.050 was considered statistically significant.

In this study, the number of patients was the same as the number of stimulated cycles. A binary logistic regression model was used to analyze the influencing factors of the clinical pregnancy outcome and live birth outcome of the fresh transfer cycle. When calculating the cumulative LBR, only one live birth was recorded for each couple in the fresh ET cycle and subsequent frozen-thawed ET cycle. The ratio of the number of patients with a live birth to the total number of patients at the beginning was the cumulative LBR. Similarly, when calculating the cumulative pregnancy rate, only one clinical pregnancy could be recorded for each couple in the fresh ET cycle and subsequent frozen-thawed ET cycle. The ratio of the number of patients who had a clinical pregnancy to the total number of patients at the beginning was the cumulative pregnancy rate. In the URPL group and the TFI group, the patients who did not reach the follow-up endpoints and whose remaining embryos were not transferred during the follow-up were excluded when calculating the cumulative pregnancy rate and cumulative LBR. Since the URPL patients were at risk of PL, binary logistic regression analysis of the outcome of cumulative live birth was conducted, and the patients who did not reach the follow-up endpoints and their cycles were excluded from the statistical analysis. A history of RPL was used as the independent variable, and the average EMT on all ET days of each ET cycle was taken as the EMT on the ET day. Because the ages of males and females were matched, to avoid the influence of confounding factors, age was not included as an independent variable in all multivariate analyses.

Results

The URPL group and TFI group each included 312 patients (312 stimulation cycles). The average ages of the women in the URPL group and TFI group were 35.60 ± 4.80 and 35.51 ± 4.69 years, respectively, and women >35 years accounted for 48.72% (152/312) of the overall sample in both groups. The baseline characteristics of the patients are shown in Table 1.

After matching, no significant differences in age were noted between the two groups, but the differences in EMT on the HCG day and sperm density were statistically significant. The patients in the two groups underwent 312 stimulation

Table 1: Baseline characteristics of the unexplained recurrent pregnancy loss (URPL) and tubal factor infertility (TFI) groups.

Items	URPL group	TFI group	*P* value
Number of stimulated cycles	312	312	–
Initial number of patients	312	312	–
Female age, years	35.60 ± 4.80	35.51 ± 4.69	0.800
Male age, years	37.08 ± 5.43	36.70 ± 5.73	0.394
Number of SPLs	2 (2–3)	1 (0–1)	<0.001
ICSI	65/309	74/308	0.374
Routine-IVF	244/309	234/308	0.374
Female BMI, kg/m^2			
≥25	73/310 (23.55)	71/307 (23.13)	0.902
18–25	228/310 (73.55)	221/307 (71.99)	0.663
<18	9/310 (2.90)	15/307 (4.89)	0.203
Basal follicle-stimulating hormone (FSH) ≤10 IU/L	233/283 (82.33)	253/292 (86.64)	0.153
Serum E_2 on HCG day (pmol/L)	6519.50 (4106.25–11,989.75)	7841.00 (4242.00–12,863.00)	0.153
Serum luteinizing hormone (LH) on HCG day, IU/L	1.07 (0.5–2.52)	0.99 (0.5–2.36)	0.440
Serum P on HCG day (≤2 ng/mL)	299/312 (95.83)	291/307 (94.79)	0.538
EMT on HCG day (cm)	1.0 (0.9–1.1)	1.1 (1.0–1.2)	<0.001
Total amount of Gn	2850.00 (1987.50–3825.00)	2850.00 (2025.00–4106.25)	0.174
Percentage of forward motility sperm (Grade A and B), %	28.20 (12.69–42.26)	31.99 (19.44–45.27)	0.190
Sperm density, million/mL	47.39 (25.11–71.06)	52.25 (28.43–94.96)	0.016

Data are presented as mean ± standard deviation, *n* (range) or *n*/*N* (%). BMI: Body mass index; EMT: Endometrial thickness; E_2: Estradiol; FSH: Follicle-stimulating hormone; Gn: Gonadotropin; HCG: Human chorionic gonadotropin; ICSI: Intracytoplasmic sperm injection; IVF: *In vitro* fertilization; LH: Luteinizing hormone; P: Progesterone; SPLs: Spontaneous pregnancy losses; TFI: Tubal factor infertility; URPL: Unexplained recurrent pregnancy loss.

Table 2: Outcomes of fresh cycles and frozen-thawed ET cycles in the URPL and TFI groups.

Items	URPL group	TFI group	*P* value
Number of retrieved eggs	10 (6, 16)	10 (5, 15)	0.447
Rate of mature M II eggs	694/855 (81.17)	719/878 (81.89)	0.699
2PN rate	2115/3413 (61.67)	2110/3389 (62.26)	0.804
Number of high-quality embryos	4 (1–7)	3 (1–7)	0.470
ET rate in fresh cycles	253/312 (81.09)	252/312 (80.77)	0.919
ET rate of high-quality embryos in fresh cycles	432/528 (81.82)	442/530 (83.40)	0.498
Transferred embryos on day 5/day 6	7/253 (2.77)	10/252 (3.97)	0.454
Proportion of cycles without egg retrieval	3/312 (1.00)	4/312 (1.28)	0.704
Proportion of cycles without available embryos to transfer	29/312 (9.29)	21/312 (6.73)	0.238
Pregnancy outcome of fresh ET cycles			
Intrauterine pregnancy rate per fresh ET cycle	89/253 (35.18)	87/252 (34.52)	0.877
LBR per fresh ET cycle	70/253 (27.67)	67/252 (26.59)	0.785
Miscarriage rate in fresh ET cycles	19/89 (21.35)	20/87 (22.99)	0.793
Ectopic pregnancy rate per fresh ET cycle	1/253 (0.40)	1/252 (0.40)	0.998
Pregnancy outcome of frozen-thawed ET cycles			
Number of frozen-thawed ET cycles	174	149	–
Intrauterine pregnancy rate per frozen–thawed ET cycle	60/174 (34.48)	60/149 (40.27)	0.283
LBR per frozen-thawed ET cycle	41/174 (23.56)	50/149 (33.56)	0.047
Miscarriage rate in frozen-thawed ET cycles	18/60 (30.00)*	10/60 (16.67)	0.084
Rate of high-quality embryos in frozen-thawed ET cycles	239/321 (74.45)	207/291 (71.13)	0.356
Loss rate of the frozen-thawed embryos	240/562 (42.70)	261/552 (47.28)	0.125

Data are presented as *n* (P_{25}, P_{75}), *n*/*N* (%) or n (range). *One case of induced labor in the second trimester occurred due to fetal malformations in URPL patients, which was not included in miscarriage cycles. 2PN: Two-pronucleate; ET: Embryo transfer; LBR: Live birth rate; M II: Metaphase II; TFI: Tubal factor infertility; URPL: Unexplained recurrent pregnancy loss.

cycles. The numbers of stimulation cycles resulting in no transferable embryos in the URPL group and the TFI group were 32 and 25, respectively ($P = 0.331$). The outcomes of fresh ET cycles in the URPL and TFI groups are shown in Table 2.

No significant differences in the outcomes of fresh ET cycles were observed between the two groups. Binary logistic regression analysis was performed on factors that might affect ET outcomes in fresh transfer cycles. We defined 0 = clinical pregnancy or a live birth in the fresh cycle and 1 = no clinical pregnancy or live birth in the fresh cycle. RPL history was a binary variable. Female BMI were categorized as <18.0, 18 to 25, and ≥25 kg/m^2, respectively. The other variables were continuous variables [Table 3]. The increased EMT on HCG day (odds ratio [OR] = 0.848, 95% confidence interval [CI]: 0.748–0.962, $P = 0.010$) and the increased number of eggs retrieved (OR = 0.928, 95% CI: 0.887–0.970, $P = 0.001$) were protective factors for the clinical pregnancy rate in fresh ET cycles; no factors affecting the LBR in fresh ET cycles were found among the included factors.

Results obtained in the frozen-thawed cycles

By the end of the follow-up (January 2020), the two groups had undergone 174 and 149 frozen-thawed ET cycles. In the URPL group, one case of induced labor in the second trimester occurred due to fetal malformations. No fetal malformations were found in the TFI group. The pregnancy rate per frozen-thawed ET cycle and LBR per frozen-thawed ET cycle were calculated. The loss rate of frozen-thawed embryos was calculated using the number of embryos as the unit. The ET results in the frozen-thawed ET cycle are described in Table 2. The LBR in frozen-thawed transfer cycle of URPL patients was lower than that of TFI patients (23.56% *vs.* 33.56%, $P = 0.047$).

After the case of induced labor in the second trimester was removed, no significant differences in the outcomes of frozen-thawed ET cycles were identified between the two groups. Among the included study subjects, the number of fetal malformations was small and not representative; thus, the malformation rates of the two groups were not compared.

Cumulative pregnancy rate

Although this study had a long follow-up period, some patients in both groups did not reach the follow-up endpoints, that is, no clinical pregnancy or live birth was achieved, and some embryos remained; therefore, these patients were excluded from the statistical analysis. The cumulative pregnancy rate and LBR were calculated using the number of patients as the unit, that is, the percentage of patients who achieved clinical pregnancy/live birth within a cycle among the patients at the beginning [Table 4].

No significant difference in the proportion of patients who did not reach an endpoint was found between the two groups, and both proportions were <6%. Most of the patients in the two groups completed a full cycle, and no significant difference in the cumulative pregnancy rate or LBR was found between these patients.

Table 3: Binary logistic regression analysis of the outcome of clinical pregnancy and live birth in the fresh cycles.

Variables	OR (95% CI)	
	Clinical pregnancy rate in fresh transfer cycles	LBR in fresh cycles
History of RPL		
Yes	1	1
No	1.081 (0.697–1.678)	0.945 (0.414–2.156)
Female BMI, kg/m^2		
<18	2.016 (0.475–8.564)	0.000 (0.000–0.000)
18–25	0.920 (0.549–1.542)	0.597 (0.228–1.563)
≥25	1	1
Basal FSH	0.975 (0.912–1.043)	1.042 (0.937–1.158)
E_2 on HCG day	1.000 (1.000–1.000)	1.000 (1.000–1.000)
LH on HCG day	0.965 (0.887–1.051)	1.012 (0.893–1.148)
P on HCG day	1.202 (0.704–2.052)	1.041 (0.347–3.126)
EMT on HCG day	0.848 (0.748–0.962)*	1.215 (0.963–1.534)
Total amount of Gn	1.000 (1.000–1.000)	1.000 (1.000–1.000)
Percentage of forward motility sperm, %	0.998 (0.987–1.009)	0.982 (0.960–1.004)
Sperm density	1.002 (0.998, 1.005)	1.000 (0.992–1.009)
Number of retrieved eggs	0.928 (0.887–0.970)*	0.970 (0.887–1.061)
2PN rate	0.369 (0.135–1.004)	0.170 (0.020–1.469)

*P value < 0.05. 2PN: Two-pronucleate; BMI: Body mass index; CI: Confidence interval; EMT: Endometrial thickness; FSH: Follicle-stimulating hormone; Gn: Gonadotropin; HCG: Human chorionic gonadotropin; LH: Luteinizing hormone; LBR: Live birth rate; OR: Odds ratio; RPL: Recurrent pregnancy loss.

Table 4: Cumulative pregnancy rate of URPL group and TFI group.

Items	URPL group	TFI group	*P* value
Initial number of cycles	312	312	–
Initial number of patients	312	312	–
Patients without one live birth and with remaining embryos	18/312 (5.77)	14/312 (4.49)	0.468
Patients without one pregnancy and with remaining embryos	13/312 (4.17)	12/312 (3.85)	0.838
Number of patients with a live birth	102	114	–
First live birth in a fresh ET cycle	70/102 (68.63)	67/114 (58.77)	0.133
First live birth in a frozen-thawed ET cycle	32/102 (31.37)	47/114 (41.23)	0.133
Number of patients with clinical pregnancy	133	134	–
Cumulative pregnancy rate	133/298 (44.63)	134/299 (44.82)	0.964
Cumulative LBR	102/294 (34.69)	114/298 (38.26)	0.368

Data are presented as *n*/*N* (%). ET: Embryo transfer; LBR: Live birth rate; TFI: Tubal factor infertility; URPL: Unexplained recurrent pregnancy loss.

No significant difference in the cumulative LBR was observed between the two groups. A regression analysis was run to identify factors affecting the cumulative LBR. Each patient who completed one complete cycle in both the URPL and TFI groups was an observation subject. The outcome of each observation subject was at least one live birth or no live birth, that is, 0 = at least one live birth, 1 = no live birth; and RPL history was a binary variable. We analyzed the influence of various factors on the unobtained live birth outcome. The average EMT on ET days in all transfer cycles was taken as the EMT for the patient. Except for RPL history, all the variables were continuous variables [Table 5]. More eggs, a higher 2PN rate, and thicker endometrium on transfer day were associated with an increased cumulative LBR. No risk factors that lowered the cumulative LBR were identified among the included factors. The number of retrieved eggs (OR = 0.875, 95% CI: 0.846–0.906, $P < 0.001$), the 2PN rate (OR = 0.151, 95% CI: 0.052–0.437, $P < 0.001$), and EMT on ET day (OR = 0.876, 95% CI: 0.770–0.997, $P = 0.045$) were protective factors.

Discussion

We did not find different clinical outcomes between the URPL population and TFI patients. Although the LBR of TFI patients was higher than that of URPL patients during the frozen-thawed transfer cycle (33.56% *vs.* 23.56%, $P = 0.047$), the cumulative LBR of the two groups was eliminated. It indicates that patients who experience multiple spontaneous abortions do not have a higher risk of adverse outcomes when choosing IVF, which is contrary to the usual perception[18,19] and to results showing different abortion rates between these two populations in previous literature. We observed a lower miscarriage rate among URPL patients than that reported

Table 5: Binary logistic regression analysis of the cumulative LBR.

Variable	OR (95% CI)	*P* value
History of RPL		
Yes	1	–
No	1.122 (0.720–1.749)	0.610
Basal FSH	1.004 (0.936–1.077)	0.921
Basal LH	1.017 (0.976–1.059)	0.419
Basal E_2	1.000 (0.999–1.002)	0.791
Number of retrieved eggs	0.875 (0.846–0.906)	<0.001
2PN rate	0.151 (0.052–0.437)	<0.001
Average number of embryos transferred per cycle	0.780 (0.529–1.149)	0.208
EMT on ET day	0.876 (0.770–0.997)	0.045
Sperm density	1.000 (0.996–1.004)	0.976
Percentage of forward motility sperm	0.997 (0.986–1.008)	0.619

2PN: Two-pronucleate; CI: Confidence interval; E_2: Estradiol; ET: Embryo transfer; EMT: Endometrial thickness; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; LBR: Live birth rate; OR: Odds ratio; RPL: Recurrent pregnancy loss.

in the study of Christiansen *et al* and Stef *et al*.[9,11] The numbers of previous miscarriages in these two articles were higher than that in our study, with median numbers of miscarriages of five and three, respectively. Between the two studies, patients with a higher median number of miscarriages were also found to have a higher recurrence rate than patients with a lower median miscarriage number. Patients with three or more miscarriages may have significant differences in clinical outcomes compared to other patients. A total of 101 patients with three or more abortions were included in our study, but the sample size did not allow sufficient statistical power.

Considering that female age affects the pregnancy rate after IVF treatment,[20] this study matched the ages of males and females in the initial design. Among the observed results, the pregnancy rate, miscarriage rate, and LBR in fresh ET cycles did not significantly differ between the two groups. In the regression analysis of the clinical outcomes of the fresh ET cycles, EMT on the ET day and the number of eggs retrieved were positively correlated with the probability of achieving clinical pregnancy in a fresh ET cycle. Retrieval of a larger number of eggs and a better intrauterine environment correspond to a higher probability of pregnancy. However, no factors affecting the live birth outcomes of fresh ET cycles were found.

Most studies have shown that a thinner endometrium is associated with worse IVF outcomes and the beneficial effect of EMT on clinical pregnancy in fresh cycles. When EMT increased from 5 to 10 mm, the clinical pregnancy rate, biochemical pregnancy rate, and LBR showed significant increasing trends. The miscarriage rate increased from 15.60% to 33.10% at the same time. It is mainly due to biochemical pregnancies; the decrease in EMT is unrelated to abortion after clinical pregnancy.[21] Another study found that the average EMT of clinically pregnant patients was significantly higher than that of non-pregnant patients. The researchers also found that EMT (OR = 1.097; $P < 0.001$) and the number of eggs retrieved (OR = 1.011; $P = 0.012$) are positively correlated with the clinical pregnancy rate.[22]

Multivariate analysis showed that the number of eggs retrieved was positively correlated with the cumulative LBR. System analysis also revealed that when embryos in all transfer cycles were considered, the cumulative LBR increases with the number of eggs retrieved.[23] A large-scale multicenter retrospective study reported that the number of eggs retrieved was significantly higher in patients who successfully delivered live births than in those who did not give birth after all embryos were used ($P < 0.001$) and that the cumulative LBRs increased steadily with the number of eggs retrieved.[23]

The 2PN rate reflects the potential of normal oocyte fertilization. The frequency of abnormally fertilized embryos, such as three-pronucleate (3PN) embryos, can increase with age.[24] Fertilized 3PN embryos cannot complete the second meiosis stage, resulting in an abnormal number of chromosomes.[25] In many studies, older women (age >35 years) have been observed to have a high rate of aneuploidy.[26,27] Normally fertilized embryos have a lower risk of abnormal karyotypes and a reduced risk of early miscarriage, which could increase the probability of achieving a live birth, which can also explain the positive impact of the 2PN rate on cumulative live birth outcomes.

Regarding URPL, male factors are rarely mentioned. Early studies have found that male age is not significantly related to sperm density, the sperm survival rate, or abnormal sperm morphology.[28,29] Although the sperm density of the male subjects in the two groups differed, multivariate regression did not show any influence of male factors. Routine semen examination results have a limited ability to reflect male fertility. Compared with male RPL patients with known causes and normal fertility, URPL male sperm have been found to have a higher rate of nuclear chromatin decondensation and a higher percentage of fragmented DNA.[30,31] In future

analyses, researchers should include more comprehensive factors, such as the sperm DNA fragmentation rate and nuclear chromatin decondensation rate, to correctly reflect the influence of men on pregnancy outcomes. Similarly, the evaluation parameters of ovarian function are not sufficiently comprehensive.

Conclusions

After matching ages, no significant differences in clinical outcomes were found between the patients with URPL and the patients with TFI. A thicker endometrium and more retrieved oocytes increase the probability of pregnancy in fresh ET cycles, but a better normal fertilization potential will increase the possibility of a live birth.

Funding

This study was supported by a grant from the National Science and Technology Major Project of China (No. 2017ZX09304012-012).

Conflicts of interest

None.

References

1. Woolner AMF, Raja EA, Bhattacharya S, Danielian P, Bhattacharya S. Inherited susceptibility to miscarriage: a nested case-control study of 31,565 women from an intergenerational cohort. Am J Obstet Gynecol 2020;222:168.e1–168.e8. doi: 10.1016/j.ajog.2019.08.013.
2. Luo L, Chen Y, Wang L, Zhuo G, Qiu C, Tu Q, *et al*. Polymorphisms of genes involved in the folate metabolic pathway impact the occurrence of unexplained recurrent pregnancy loss. Reprod Sci 2015;22:845–851. doi: 10.1177/1933719114565033.
3. Carbone J, Sarmiento E, Gallego A, Lanio N, Navarro J, Garcia S, *et al*. Peripheral blood T- and B-cell immunophenotypic abnormalities in selected women with unexplained recurrent miscarriage. J Reprod Immunol 2016;113:50–53. doi: 10.1016/j.jri.2015.11.003.
4. Ebina Y, Nishino Y, Deguchi M, Maesawa Y, Nakashima Y, Yamada H. Natural killer cell activity in women with recurrent miscarriage: aetiology and pregnancy outcome. J Reprod Immunol 2017;120:42–47. doi: 10.1016/j.jri.2017.04.005.
5. Yuan J, Li J, Huang SY, Sun X. Characterization of the subsets of human NKT-like cells and the expression of Th1/Th2 cytokines in patients with unexplained recurrent spontaneous abortion. J Reprod Immunol 2015;110:81–88. doi: 10.1016/j.jri.2015.05.001.
6. Bagheri A, Kumar P, Kamath A, Rao P. Association of angiogenic cytokines (VEGF-A and VEGF-C) and clinical characteristic in women with unexplained recurrent miscarriage. Bratisl Lek Listy 2017;118:258–264. doi: 10.4149/Bll_2017_051.
7. Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E. Alteration in the expression of proteins in unexplained recurrent pregnancy loss compared with in the normal placenta. J Reprod Dev 2014;60:261–267. doi: 10.1262/jrd.2013-096.
8. Ober C, Karrison T, Odem RR, Barnes RB, Branch DW, Stephenson MD, *et al*. Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial. Lancet 1999;354:365–369. doi: 10.1016/S0140-6736(98)12055-X.
9. Christiansen OB, Larsen EC, Egerup P, Lunoee L, Egestad L, Nielsen HS. Intravenous immunoglobulin treatment for secondary recurrent miscarriage: a randomised, double-blind, placebo-controlled trial. BJOG 2015;122:500–508. doi: 10.1111/1471-0528.13192.
10. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update 2011;17:454–466. doi: 10.1093/humupd/dmr003.
11. Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyak K, *et al*. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med 2010;362:1586–1596. doi: 10.1056/NEJMoa1000641.
12. Yuksel H, Kayatas S, Boza AT, Api M, Ertekin AA, Cam C. Low molecular weight heparin use in unexplained recurrent miscarriage. Pak J Med Sci 2014;30:1232–1237. doi: 10.12669/pjms.306.5477.
13. Jiang YY, Xu WM, Guo JQ, Zhang SY, Huang XY. Effects of salpingectomy on ovarian reserve and IVF-ET outcome in young women. Chin J Birth Health Hered 2018;26:108–111. doi: 10.13404/j.cnki.cjbhh.2018.02.043.
14. Sun WF, Huang YH, Zhang Y, Xie BG, Lu WY. The impact of paternal age on IVF pregnancy outcome in non-male factor infertility couples. Chin J Birth Health Hered 2017;25:115–117. doi: 10.13404/j.cnki.cjbhh.2017.09.045.
15. Yildirim G, Ficicioglu C, Attar R, Akcin O, Tecellioglu N. Comparision of reproductive outcome of the women with hypogonadotropic hypogonadism and tubal factor infertility. Clin Exp Obstet Gynecol 2010;37:120–122. doi: 10.1016/j.fertnstert.2008.07.150.
16. Nejad EST, Saedi T, Saedi S, Rashidi BH, Nekoo ZA, Jahangiri N. Comparison of in vitro fertilisation success in patients with polycystic ovary syndrome and tubal factor. Gynecol Endocrinol 2011;27:117–120. doi: 10.3109/09513590.2010.501872.
17. Smith GD, Serafini PC, Fioravanti J, Yadid I, Coslovsky M, Hassun P, *et al*. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril 2010;94:2088–2095. doi: 10.1016/j.fertnstert.2009.12.065.
18. Kupka MS, Dorn C, Montag M, Felberbaum RE, van der Ven H, Kulczycki A, *et al*. Previous miscarriages influence IVF and intracytoplasmatic sperm injection pregnancy outcome. Reprod Biomed Online 2004;8:349–357. doi: 10.1016/s1472-6483(10)60916-9.
19. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage: aetiology, management and prognosis. Hum Reprod Update 2002;8:463–481. doi: 10.1093/humupd/8.5.463.
20. Sharma V, Allgar V, Rajkhowa M. Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil Steril 2002;78:40–46. doi: 10.1016/S0015-0282(02)03160-6.
21. Gallos ID, Khairy M, Chu J, Rajkhowa M, Tobias A, Campbell A, *et al*. Optimal endometrial thickness to maximize live births and minimize pregnancy losses: analysis of 25,767 fresh embryo transfers. Reprod Biomed Online 2018;37:542–548. doi: 10.1016/j.rbmo.2018.08.025.
22. Yuan X, Saravelos SH, Wang Q, Xu Y, Li TC, Zhou C. Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF-ICSI cycles. Reprod Biomed Online 2016;33:197–205. doi: 10.1016/j.rbmo.2016.05.002.
23. Drakopoulos P, Errazuriz J, Santos-Ribeiro S, Tournaye H, Vaiarelli A, Pluchino N, *et al*. Cumulative live birth rates in vitro fertilization. Minerva Ginecol 2019;71:207–210. doi: 10.23736/S0026-4784.18.04347-2.
24. Grondahl ML, Christiansen SL, Kesmodel US, Agerholm IE, Lemmen JG, Lundstrom P, *et al*. Effect of women's age on embryo morphology, cleavage rate and competence-a multicenter cohort study. PLoS One 2017;12:e0172456. doi: 10.1371/journal.pone.0172456.
25. Grondahl ML, Andersen CY, Bogstad J, Nielsen FC, Meinertz H, Borup R. Gene expression profiles of single human mature oocytes in relation to age. Hum Reprod 2010;25:957–968. doi: 10.1093/humrep/deq014.
26. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, *et al*. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101:656–663.e1. doi: 10.1016/j.fertnstert.2013.11.004.
27. Capalbo A, Hoffmann ER, Cimadomo D, Ubaldi FM, Rienzi L. Human female meiosis revised: new insights into the mechanisms of chromosome segregation and aneuploidies from advanced genomics and time-lapse imaging. Hum Reprod Update 2017;23:706–722. doi: 10.1093/humupd/dmx026.
28. Sbracia M, Cozza G, Grasso JA, Mastrone M, Scarpellini F. Semen parameters and sperm morphology in men in unexplained recurrent spontaneous abortion, before and during a 3 year follow-up period.

Hum Reprod 1996;11:117–120. doi: 10.1093/oxfordjournals.humrep.a019002.
29. Hill JA, Abbott AF, Politch JA. Sperm morphology and recurrent abortion. Fertil Steril 1994;61:776–778. doi: 10.1016/S0015-0282(16)56661-8.
30. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, Kammoun M, Meniaoui I, Sallem A, *et al*. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. Fertil Steril 2016;105:58–64. doi: 10.1016/j.fertnstert.2015.09.041.
31. Leach M, Aitken RJ, Sacks G. Sperm DNA fragmentation abnormalities in men from couples with a history of recurrent miscarriage. Aust N Z J Obstet Gynaecol 2015;55:379–383. doi: 10.1111/ajo.12373.

How to cite this article: Li TY, Li R, Zeng L, Li L, Qiao J, Liu P, Wang HY. *In vitro* fertilization-embryo transfer in patients with unexplained recurrent pregnancy loss. Chin Med J 2021;134:2421–2429. doi: 10.1097/CM9.0000000000001657