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Live birth rate and perinatal outcomes following sequential embryo transfer in women with recurrent implantation failure undergoing frozen-thawed embryo transfer cycles

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Abstract

Background Sequential embryo transfer (ET) has been used to improve clinical outcomes in patients with recurrent implantation failure (RIF). This study aimed to evaluate whether sequential ET influence the live birth rate and perinatal outcomes of women with RIF.

Methods A cohort study of RIF patients who underwent sequential ET (Seq-ET) during frozen-thawed embryo transfer (FET) cycles between January 2020 and June 2023 was performed. FET patients who underwent double cleavage-stage ET (D3-dET) and double blastocyst ET (D5/6-dET) during the same period composed the control group. The live birth rate and perinatal outcomes of the groups were analyzed and compared.

Results The Seq-ET group had a significantly greater live birth rate (42.9%) than the D3-dET group (33.1%), and the live birth rate of the Seq-ET group was comparable to that of the D5/6-dET group (35.7%). Female BMI (aOR 0.96, 95%CI 0.92-1.00), stimulation for endometrial preparation (aOR 0.47, 95%CI 0.26–0.84), and endometrial thickness (aOR 1.08, 95%CI 1.00-1.16) were contributing factors to the live birth rate. No statistically significant differences were found in the rate of healthy birth or twins among the Seq-ET, D3-dET and D5/6-dET groups. There was no statistically significant difference in the rates of preterm delivery, birth weight or length, low birthweight, macrosomia, small for gestational age (SGA), gestational diabetes mellitus (GDM), gestational hypertensive disease (GHD), or the sex ratio among the three groups. The infants born in D5/6-dET group had less gestational weeks than in the Seq-ET and D3-dET groups (38.05 ± 1.88 vs. 38.43 ± 2.09 and 38.45 ± 1.80, $P = 0.013$) and had a higher risk of large for gestational age (LGA) (aOR 2.15, 95%CI 1.00-4.62) compared to infants born in the D3-dET group.

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Conclusion The live birth rate was significantly higher in the Seq-ET group compared with the D3-dET group, and slightly higher compared to the D5/6-dET group. Our results suggested sequential ET did not affect the perinatal outcomes.

Keywords ART, Sequential embryo transfer, Live birth rate, Perinatal outcomes, RIF

Background

Assisted reproductive technology (ART) has been applied effectively to enable patients with infertility to have healthy offspring. However, some patients continue to experience implantation failures after undergoing treatment with various in vitro fertilization (IVF) techniques. If patients do not become pregnant after three or more fresh embryo transfer (ET) or frozen–thawed embryo transfer (FET) cycles, despite the transfer of no fewer than four good-quality embryos or two blastocysts, they are considered to have repeated implantation failure (RIF) [1]. The estimated incidence of RIF varies from 5 to 10% worldwide [2, 3]. RIF poses a challenge in ART because it increases not only the financial and psychological burdens on patients and their families but also the technical burden on health providers.

Successful implantation relies on the embryo, the endometrium, and favorable cross-talk between them, and is facilitated by several factors, such as growth factors, cytokines, adhesion molecules, and transcription factors [4]. Many endeavors have been made to improve the clinical outcomes of patients with RIF, such as performing preimplantation genetic testing for aneuploidy (PGT-A) [5], endometrial receptivity arrays for the window of implantation (WOI) [6], and endometrial injury [7]. In addition, the intrauterine administration of several factors (human chorionic gonadotropin, peripheral blood mononuclear cells, platelet-rich plasma, granulocyte colony-stimulating factor, growth hormones, etc.) could improve reproductive outcomes in women with RIF [8, 9].

Sequential ET, in which the transfer of a day 3 cleavage-stage embryo is followed by the transfer of a day 5/6 blastocyst in one cycle, has been used to improve clinical outcomes in patients with RIF [10–15] or poor ovarian response (POR) [16]. Most studies and meta-analyses have shown that sequential ET has positive effects on clinical pregnancy rates during ART treatment [10, 11, 14–16]. Our previous study revealed that RIF patients who underwent sequential ET in FET cycles had a greater implantation rate and clinical pregnancy rate and a lower multiple pregnancy rate than patients who underwent double cleavage-stage ET, which is comparable to double blastocyst transfer [17].

To date, there are few reports on whether sequential ET has an impact on the live birth rate, and there are no reports evaluating the effect of sequential ET on the perinatal health of mothers and offspring. Therefore, the objective of this study was to further investigate the live birth rate and perinatal outcomes after sequential ET in women with RIF based on our previous data. The primary outcomes were the live birth rate and the healthy live birth rate. The secondary outcomes were the live birth rate of twins and perinatal outcomes, including gestational age, birth weight and length, and the rates of preterm delivery, low birthweight, macrosomia, small for gestational age (SGA), large for gestational age (LGA), gestational hypertensive disease (GHD), gestational diabetes mellitus (GDM), and newborn sex.

Methods

Patients and experimental design

This retrospective cohort study was performed at the ART center of a university-affiliated hospital. The data were collected from January 2020 to June 2023. All the patients had two or more implantation failure cycles and were undergoing FET cycles. The exclusion criteria were as follows: (1) incomplete or wrongly recorded data; (2) PGT-SR or PGT-M cycles; (3) egg donor cycles; (4) autoimmune diseases. Then 1740 cycles were included in the analysis and three groups were established according to the ET protocol: sequential ET (Seq-ET) ($n = 268$ cycles), double cleavage-stage ET (D3-dET) ($n = 979$ cycles), and double blastocyst ET (D5/6-dET) ($n = 493$ cycles). The study was approved by the local Institutional Review Board. All patients signed a written informed consent form.

Endometrial preparation and ET procedures

All patients included underwent FET cycles. The preparation of the endometrium was carried out in accordance with routine clinical protocol. Depending on the patient's individual condition, there were three different methods for endometrial preparation: natural cycle, artificial cycle, or stimulation cycle. The natural cycle was adopted for women with regular menstrual cycles. Transvaginal ultrasound examination started from Days 8–10 of the menstrual cycle. When the dominant follicle reached 16–20 mm, serum samples were collected to measure the levels of estradiol, progesterone, and LH. The artificial (hormone replacement) cycle or a stimulation cycle was adopted for women with irregular menstruation and a history of ovulation disorders. In the artificial cycle, oral estradiol (Progynova, Bayer) was administered starting from Day 2 of the menstrual cycle. Transvaginal ultrasound examination was performed 10–14 days later, and

serum samples were collected to measure levels of progesterone. For the stimulation cycle, 50–100 mg of clomiphene citrate (Livzon Pharmaceutical) or 2.5–7.5 mg of letrozole (Hengrui Medicine) was taken orally for 5 days, with or without 75–150 IU of human menopausal gonadotropin (Lizhu Pharmaceutical) injection. Three different procedures of embryo transfer were performed: sequential ET, double cleavage-stage ET, and double blastocyst-stage ET. Sequential ET refers to the transfer of one Day 3 cleavage-stage embryo followed by one Day 5/6 blastocyst-stage embryo after two days in the same FET cycle. Double cleavage-stage ET refers to the simultaneous transfer of two Day 3 embryos on the third day after ovulation. Double blastocyst-stage ET refers to the simultaneous transfer of two Day 5/6 blastocysts on the fifth day after ovulation.

Outcome measures and analysis

The live birth rate, healthy live birth rate, and twin birth rate were analyzed first. A healthy birth was defined as the live birth of a singleton infant at no less than 37 weeks gestation, with a birth weight between 2,500 and 4,000 g and without congenital malformations.

The demographic characteristics of patients who delivered live-born infants in the three groups were compared regarding parental age (years), female body mass index (BMI, kg/m^2), infertility duration (years), primary infertility, number of previous failed cycles, fertilization method, endometrial preparation method, and endometrial thickness (mm).

The following data were included in the analysis of perinatal outcomes among the three groups: mean gestational week at birth, preterm delivery status (defined as a baby born before < 37 weeks amenorrhea), mean birth length (cm), mean birth weight (g), low birthweight (defined as an infant birth weight < 2500 g), macrosomia (defined as an infant birth weight ≥ 4000 g), SGA and LGA, defined as birth weight percentiles as less than 10th percentile and greater than 90th percentile, respectively [18, 19], GHD status (defined as a blood pressure > 140/90 mmHg after pregnancy), GDM (defined as abnormal maternal glucose metabolism with onset or first recognition during pregnancy), and female sex proportion.

Corrections for possible confounders, such as female age and BMI, infertility duration, primary infertility, number of previous failed cycles, fertilization method, endometrial preparation method, and endometrial thickness, were included to determine the quantitative relationships between the explanatory variables and perinatal outcomes.

Statistical analysis

All analyses were performed using SPSS version 23 (IBM). Continuous variables are presented as the means ± standard deviations (SDs), and categorical variables are presented as counts and percentages. For continuous variables, differences among groups were evaluated using one-way ANOVA when the data were normally distributed (Shapiro–Wilk test) or the Kruskal–Wallis test when the data were not normally distributed. For categorical variables, the chi-square test was applied. A binary logistic regression analysis was carried out to correct for possible confounders. Adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were reported. A P value less than 0.05 was considered to indicate statistical significance for all the tests.

Results

The flowchart depicting the included cycles and the distribution of cycles based on the ET protocol is presented in Fig. 1. There were 268 cycles, 979 cycles, and 493 cycles included in the Seq-ET group, D3-dET group, and D5/6-dET group, respectively, and a total of 115 cycles, 324 cycles, and 176 cycles resulting in live births were included in the three groups, respectively. Compared with D3-dET, Seq-ET resulted in a significantly greater live birth rate (42.9% vs. 33.1%, $P = 0.003$). The live birth rate in the Seq-ET group was higher but not significant than in the D5/6-dET group (42.9% vs. 35.7%, $P = 0.051$). No statistically significant differences were found in the twin live birth rate or healthy birth rate among the Seq-ET, D3-dET, and D5/6-dET groups (Fig. 2). Logistic regression analyses showed that the live birth rate in the D3-dET group was lower compared to the Seq-ET group (OR 0.67, 95%CI: 0.36–0.97, $P = 0.034$). Female BMI was negatively correlated with the live birth rate (OR 0.96, 95% CI: 0.92-1.00, $P = 0.040$). Endometrial thickness was positively correlated with the live birth rate (OR 1.08, 95% CI: 1.00-1.16, $P = 0.042$) and healthy birth rate (OR 1.10, 95% CI: 1.02–1.19, $P = 0.018$). As for endometrial preparation methods, stimulation cycles had a lower live birth rate than natural cycles (OR 0.47, 95% CI: 0.26–0.84, $P = 0.010$) (Table 1).

We compared the baseline characteristics of couples with live-born infants among the three groups. Patients in the D3-dET group had fewer previous failed cycles than patients in the Seq-ET and D5/6-dET groups (3.01 ± 1.47 vs. 4.64 ± 2.36 and 4.18 ± 2.13, $P < 0.001$). The cycle of artificial endometrial preparation method in the Seq-ET group was higher than in the D3-dET and D5/6-dET groups (66.4% vs. 52.8% and 48.3%, $P = 0.036$). No statistically significant differences were found in parental age, female BMI, infertility duration, primary infertility, fertilization method, or endometrial thickness (Table 2).

The number of gestational weeks at delivery for singleton infants was lower in the D5/6-dET group than in the Seq-ET group and the D3-dET group (38.05 ± 1.88 vs. 38.43 ± 2.09 and 38.45 ± 1.80, $P = 0.013$), and the number

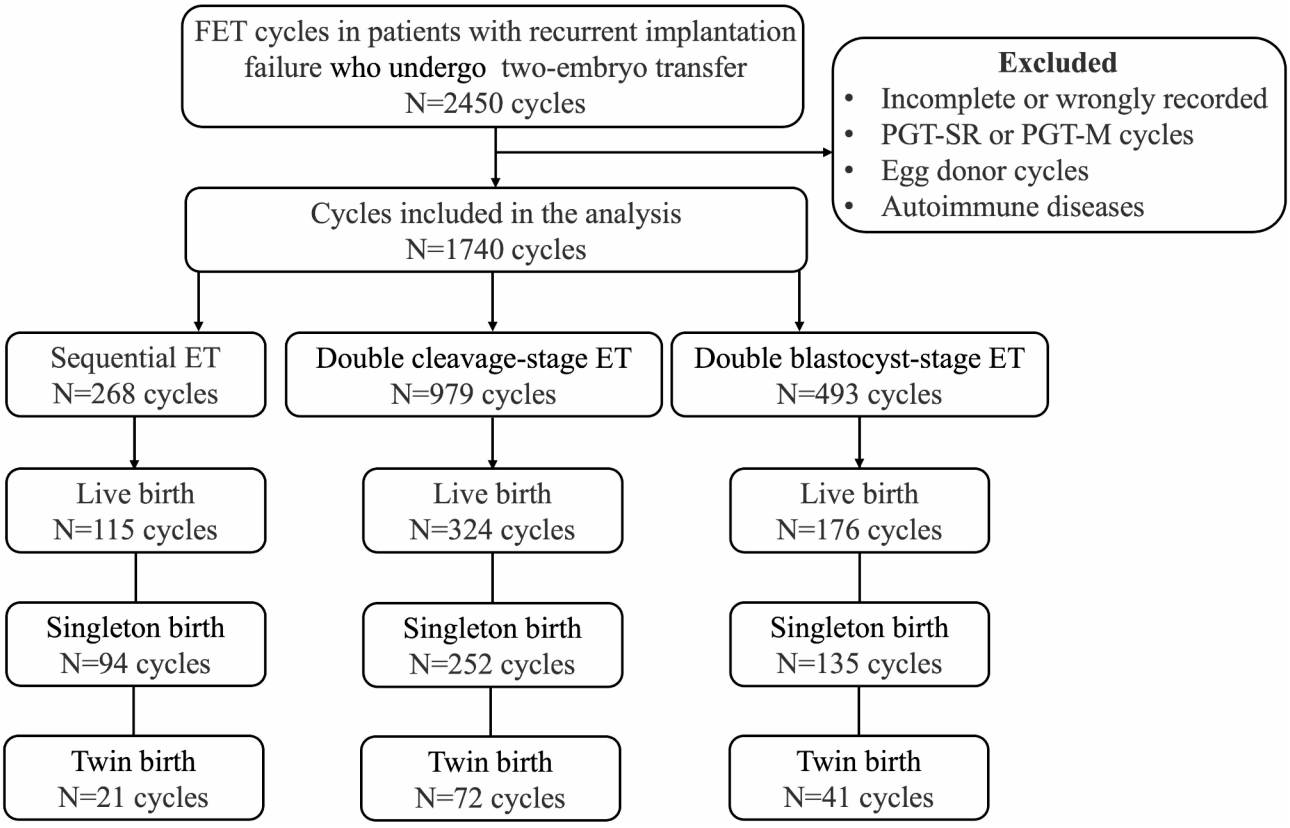

Fig. 1 Flow chart of the study. FET, frozen-thawed embryo transfer; ET, embryo transfer

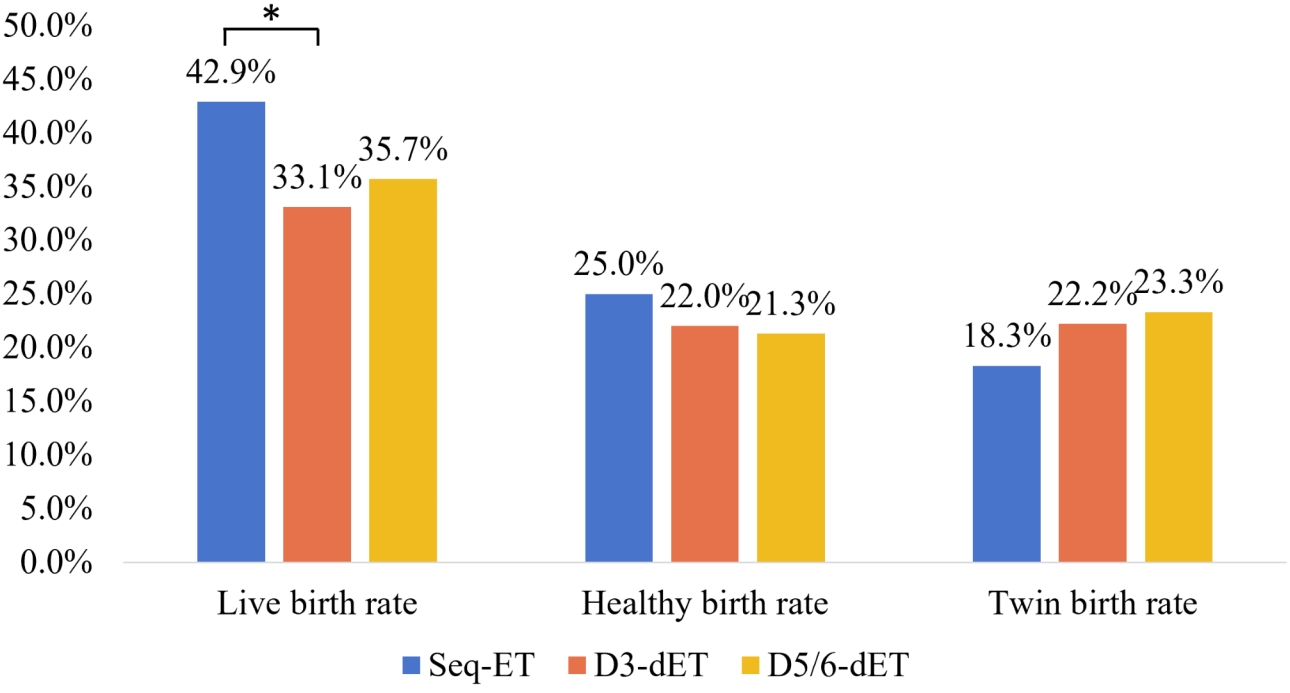

Fig. 2 Birth outcomes in each group. *$P = 0.003$

Table 1 Logistic regression analyses on the birth outcomes

Variable	Live birth		Healthy birth		Twin birth	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Protocol of embryo transfer		0.092		0.707		0.707
Seq-ET	Ref		Ref		Ref	
D3-dET	0.67 (0.36, 0.97)	**0.034**	0.81 (0.54, 1.24)	0.333	1.23 (0.57, 2.64)	0.595
D5/6-dET	0.80 (0.54, 1.18)	0.260	0.80 (0.52, 1.24)	0.327	1.39 (0.64, 3.02)	0.407
Female age (y)	0.96 (0.92, 1.00)	0.073	1.00 (0.95, 1.05)	0.975	0.95 (0.85, 1.06)	0.368
Male age (y)	0.99 (0.95, 1.02)	0.491	0.97 (0.93, 1.01)	0.104	0.96 (0.87, 1.06)	0.404
Female BMI (kg/m^2)	0.96 (0.92, 1.00)	**0.040**	0.96 (0.92, 1.01)	0.099	0.96 (0.88, 1.05)	0.358
Infertility duration (y)	0.94 (0.93, 1.02)	0.240	0.98 (0.94, 1.03)	0.471	1.02 (0.92, 1.13)	0.692
Infertility type						
Primary infertility	Ref		Ref		Ref	
Secondary infertility	0.03 (0.78, 1.35)	0.857	1.06 (0.78, 1.44)	0.720	0.69 (0.39, 1.24)	0.217
Previous failed cycles (n)	1.02 (0.95, 1.10)	0.551	1.00 (0.92, 1.08)	1.000	1.06 (0.92, 1.22)	0.438
Fertilization method						
IVF	Ref		Ref		Ref	
ICSI	1.00 (0.76, 1.33)	0.981	0.90 (0.66, 1.24)	0.525	0.90 (0.51, 1.59)	0.712
Endometrial preparation method		**0.016**		0.286		0.327
Natural cycle	Ref		Ref		Ref	
Artificial cycle	0.77 (0.59, 1.00)	0.053	0.84 (0.62, (1.12)	0.235	0.84 (0.50, 1.42)	0.508
Stimulation cycle	0.47 (0.26, 0.84)	**0.010**	0.65 (0.35, 1.22)	0.183	0.22 (0.03, 1.76)	0.155
Endometrial thickness (mm)	1.08 (1.00, 1.16)	**0.042**	1.10 (1.02, 1.19)	**0.018**	0.89 (0.76, 1.05)	0.169

The values in bold are statistically significant

Table 2 Comparison of the baseline characteristics of women with live-born infants among the three groups

	Seq-ET(*n* = 115)	D3-dET (*n* = 324)	D5/6-dET (*n* = 176)	*P* value
Female age (y)	32.87 ± 3.94	32.62 ± 3.59	33.14 ± 3.81	0.358
Male age (y)	34.34 ± 4.86	34.03 ± 4.58	34.16 ± 4.59	0.898
Female BMI (kg/m^2)	22.21 ± 3.35	22.12 ± 3.15	21.74 ± 2.95	0.416
Infertility duration (y)	5.17 ± 2.93	4.83 ± 3.03	5.17 ± 3.00	0.181
Primary infertility	76 (66.1%)	227 (70.1%)	110 (62.5%)	0.220
Previous failed cycles (n)	4.64 ± 2.36	3.01 ± 1.47[§]	4.18 ± 2.13	**< 0.001**
Fertilization method				0.078
IVF	82 (71.3%)	206 (63.6%)	107 (60.8%)	
ICSI	29 (25.2%)	107 (33.0%)	55 (31.3%)	
Others	4 (3.5%)	11 (3.4%)	14 (8.0%)	
Endometrial preparation method				**0.036**
Natural cycle	31 (27.4%) [£]	131 (40.4%)	80 (45.5%)	
Artificial cycle	75 (66.4%)	171 (52.8%)	85 (48.3%)	
Stimulation cycle	7 (6.2%)	22 (6.8%)	11 (6.3%)	
Endometrial thickness (mm)	10.12 ± 1.69	10.24 ± 1.83	10.09 ± 1.42	0.959

[§]Cleavage-stage embryo transfer vs. sequential embryo transfer and blastocyst embryo transfer

[£] Sequential embryo transfer vs. Cleavage-stage embryo transfer and Blastocyst embryo transfer

of singleton infants with macrosomia was higher in the Seq-ET group than in the D3-dET group and the D5/6-d ET group (11.7% vs. 3.2% and 3.7%, $P = 0.005$). There was no statistically significant difference in the preterm delivery, birth weight or length, low birthweight, SGA, LGA, GDM, GHD, or female sex in singleton deliveries among the three groups (Table 3). The gestational weeks, preterm delivery, birth weight or length, low birthweight, macrosomia, SGA, LGA, GDM, GHD, or female sex were also not significantly different in twin deliveries, among the three groups (Supplemental Table 1).

Through logistic regression analysis adjusted for female age, female BMI, infertility duration, primary infertility, number of previous failed cycles, fertilization method, endometrial preparation method, and endometrial thickness, we found that the ET method was not associated with the risk of perinatal outcomes, except that D5/6-dET had a higher risk of LGA than D3-dET (OR2.15, 95% CI: 1.00-4.62, $P = 0.049$) (Table 4).

Table 3 Comparison of perinatal outcomes in Singleton deliveries among the three groups

	Seq-ET(*n* = 115)	D3-dET (*n* = 324)	D5/6-dET (*n* = 176)	*P* value
Singleton deliveries	94 (81.7%)	252 (77.8%)	135 (76.7%)	0.574
Gestational week	38.43 ± 2.09	38.45 ± 1.80	38.05 ± 1.88[§]	**0.013**
Preterm delivery	13 (13.8%)	22 (8.7%)	20 (14.8%)	0.169
Birth weight (g)	3267.6 ± 666.5	3201.7 ± 501.3	3254.5 ± 522.5	0.373
Birth length (cm)	49.64 ± 2.84	49.97 ± 1.90	49.88 ± 1.97	0.941
Low birthweight	10 (9.9%)	13 (5.2%)	10 (7.5%)	0.353
Macrosomia	11 (11.7%) [£]	8 (3.2%)	5 (3.7%)	**0.005**
SGA	5 (5.3%)	18 (7.1%)	10 (7.4%)	0.801
LGA	14 (14.9%)	24 (9.5%)	23 (17.0%)	0.083
GDM	10 (10.6%%)	29 (11.5%)	14 (10.4%)	0.906
GHD	5 (5.3%)	10 (4.0%)	3 (2.2%)	0.461
Female sex	44 (46.8%)	123 (48.8%)	70 (51.9%)	0.737

SGA, small for gestational age; LGA, large for gestational age; GDM, gestational diabetes mellitus; GHD, gestational hypertensive disease

[§]Blastocyst embryo transfer vs. sequential embryo transfer and cleavage-stage embryo transfer

[£]Sequential embryo transfer vs. cleavage-stage embryo transfer and blastocyst embryo transfer

Table 4 Logistic regression analyses of perinatal outcomes in Singleton infants and women with different ET methods

Variable	D3-dET vs. Seq-ET		D5/6-dET vs. Seq-ET		D5/6-dET vs. D3-dET	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Singleton deliveries	0.80 (0.37, 1.70)	0.558	0.70 (0.32, 1.52)	0.367	0.83 (0.46, 1.49)	0.529
Preterm delivery	0.39 (0.14, 1.12)	0.081	1.06 0.40, 2.82)	0.913	2.42 (0.98, 5.99)	0.056
Low birthweight	0.65 (0.19, 2.24)	0.494	0.61 (0.17, 2.20)	0.452	0.97 (0.31, 2.97)	0.952
Macrosomia	0.32 (0.92, 1.11)	0.073	0.32 (0.08, 1.32)	0.115	0.97 (0.25, 3.77)	0.961
SGA	1.71 (0.44, 6.65)	0.440	1.70 (0.42, 6.85)	0.456	1.06 (0.39, 2.90)	0.904
LGA	0.81 (0.30, 2.18)	0.682	1.80 (0.68, 4.78)	0.236	2.15 (1.00, 4.62)	**0.049**
GDM	0.77 (0.31, 1.90)	0.570	0.73 (0.27, 1.96)	0.526	0.91 (0.38, 2.18)	0.837
GHD	1.14 (0.22, 5.83)	0.872	0.77 (0.13, 4.61)	0.774	0.71 (0.14, 3.59)	0.679
Female sex	1.11 (0.59, 2.09)	0.744	1.40 (0.71, 2.74)	0.329	1.27 (0.74, 2.17)	0.382

Adjusted for female age and BMI, infertility duration, primary infertility, number of previous failed cycles, fertilization method, endometrial preparation method, and endometrial thickness. The values in bold are statistically significant

SGA, small for gestational age; LGA, large for gestational age; GDM, gestational diabetes mellitus; GHD, gestational hypertensive disease; OR, odds ratio; CI, confidence interval

Discussion

To our knowledge, this is the first dataset published thus far on perinatal outcomes following Seq-ET, which was compared to both D3-dET and D5/6-dET. Herein, we used this analysis of 615 deliveries to demonstrate that Seq-ET does not increase the risk of adverse perinatal outcomes. In this study, the live birth rate of the Seq-ET group was significantly greater than that of the D3-dET group and was comparable to that of the D5/6-dET group. To date, only two studies on the live birth rate after sequential ET [11, 16] have been published, and the results of our study are consistent with these studies. The healthy live birth rate was greater in the Seq-ET group than in the D3-dET and D5/6-dET groups, although the differences were not significant. Compared to the D3-dET group and the D5/6-dET group, no differences were found in the rates of preterm delivery, birth weight or length, low birthweight, macrosomia, SGA, LGA, GDM, GHD, or the sex ratio in the Seq-ET group.

The incidence of multiple pregnancies in IVF patients is much greater than that in patients who become pregnant naturally [20]. With an increase in the multiple pregnancy rate, the rates of perinatal and neonatal complications also increase [21]. In recent years, single ET has been recommended for use in ART treatment to reduce the multiple pregnancy rate. However, in most patients with RIF, it is important to improve the IVF-ET success rate to reduce financial and psychological burdens. In our study, patients in the Seq-ET group had a greater number of previous failed cycles but had a greater live birth rate than patients in the D3-dET group. In addition, no more than two newborns were born during the cycles in this study, and the twin birth rate was 18.3% in patients who underwent Seq-ET, which was lower than that in patients who underwent D3-dET and D5/6-dET, although no significant difference was found. Our study revealed that, compared with conventional IVF-ET, sequential ET can benefit RIF patients by allowing them to become pregnant without increasing the risk of multiple pregnancy.

This finding is consistent with the results of previous studies [15].

The comparison of perinatal outcomes following blastocyst and cleavage-stage ET is still controversial. Earlier meta-analyses suggested that blastocyst ET is associated with a greater risk of preterm birth, very preterm birth, and congenital malformations [22, 23]. Wang et al. claimed that cleavage-stage ET was associated with a high risk of small for gestational age and a low risk of large for gestational age [24]. However, Zhu et al. showed that there was no difference in the risk of early preterm delivery, low birth weight, very low birth weight, high birth weight or very high birth weight between frozen-thawed cleavage-stage ET and frozen-thawed blastocyst ET [25] Recently, some studies demonstrated that infants born after blastocyst transfer had a higher risk of preterm birth and LGA compared to infants born after cleavage-stage embryo transfer [26, 27]. In our study, the perinatal outcomes of double frozen-thawed blastocyst ET were comparable to those of double frozen-thawed cleavage-stage ET, except for the number of gestational weeks at birth for singleton infants. After adjustment, D5/6-dET was associated with a higher risk of LGA compared to D3-dET. However, Seq-ET in this study did not affect the obstetric or perinatal outcomes compared to D3-dET and D5/6-dET in patients with RIF undergoing FET cycles.

In our study, the gestational age at birth of singleton infants was lower in the D5/6-dET group than in the Seq-ET group and D3-dET group. This is probably due to the greater rate of preterm delivery in the D5/6-dET group than in the other two groups, although no significant difference was found. ET at the blastocyst stage is associated with a greater risk of preterm delivery [22, 25]. In addition, the proportion of macrosomia among singleton infants was greater in the Seq-ET group than in the D3-dET group and D5/6-dET group before adjustment in logistic regression analysis. This could be because the proportion of patients undergoing artificial cycles in the sequential ET group was relatively high, and artificial-cycle FET had a greater risk of large for gestational age and macrosomia than natural-cycle FET [28, 29]. After adjustment, there was no significant difference in macrosomia among the three ET groups. However, more work needs to be done to verify and confirm the results, since another possible reason for these uncertain conclusions is that the sample size in this study was limited.

Conclusions

In conclusion, our statistical results suggest that the use of sequential ET in patients with RIF undergoing FET cycles can improve the live birth rate without increasing the risk of adverse perinatal outcomes for mothers and newborns. Our study is the first to evaluate the impact of sequential ET on the perinatal outcomes of mothers and newborns, and it included patients undergoing two types of ET (double cleavage-stage ET and double blastocyst ET) as controls.

However, this study has several limitations that warrant consideration. First, its retrospective design and relatively small sample size may introduce inherent confounding factors and bias, thereby limiting the generalizability of the findings. Specifically, the insufficient sample size, particularly in the subgroup of twin live births, rendered certain data unanalyzable and may have led to potential deviations in the results. Additionally, the lack of stratified analysis based on embryo quality, a critical factor influencing ART outcomes, represents a significant limitation. Finally, the mechanisms by which sequential embryo transfer (ET) improves clinical outcomes, including the underlying molecular pathways, remain unclear and require further investigation through well-designed prospective studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07557-5.

Supplementary Material 1

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Author contributions

XL and JG played a key role in the study design, execution, analysis, interpretation of data, and discussion. JG wrote the main manuscript text, designed the figures and tables. RL, PL and HW coordinated the study and revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

In compliance with the Declaration of Helsinki, this study received ethical approval from the Institutional Review Board of Peking University Third Hospital. All patients provided written informed consent for the embryo transfer procedure and participation in the follow-up protocol. Data used in this retrospective analysis were de-identified upon extraction from the electronic health records database to ensure confidentiality.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Coughlan C, Ledger W, Wang Q, Liu FH, Demirol A, Gurgan T, Cutting R, Ong K, Sallam H, Li TC. Recurrent implantation failure: definition and management. Reprod Biomed Online. 2014;28(1):14–38.
2. Polanski LT, Baumgarten MN, Quenby S, Brosens J, Campbell BK. Raine-Fenning, what exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion. Reprod Biomed Online. 2014;28(4):409–23.
3. Mak JSM, Chung CHS, Chung JPW, Kong GWS, Saravelos SH, Cheung LP, Li TC. The effect of endometrial scratch on natural-cycle cryopreserved embryo transfer outcomes: a randomized controlled study. Reprod Biomed Online. 2017;35(1):28–36.
4. Pirtea P, de Ziegler D, Ayoubi JM. Recurrent implantation Failure-Is it the egg or the chicken?? Life-Basel 12(1) (2022).
5. Pirtea P, Scott RT, de Ziegler D, Ayoubi JM. Recurrent implantation failure: how common is it? Curr Opin Obstet Gyn. 2021;33(3):207–12.
6. Nejat EJ, Ruiz-Alonso M, Simón C, Meier UT. Timing the window of implantation by nucleolar channel system prevalence matches the accuracy of the endometrial receptivity array. Fertil Steril 102(5) (2014).
7. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. Fertil Steril. 2003;79(6):1317–22.
8. Li MZ, Kang Y, Wang QF, Yan L. Efficacy of autologous intrauterine infusion of Platelet-Rich plasma in patients with unexplained repeated implantation failures in embryo transfer: A systematic review and Meta-Analysis. J Clin Med 11(22) (2022).
9. Makrigiannakis A, Makrygiannakis F, Vrekoussis T. Approaches to improve endometrial receptivity in case of repeated implantation failures. Front Cell Dev Biol. 2021;9:613277.
10. Zhang J, Wang C, Zhang H, Zhou Y. Sequential cleavage and blastocyst embryo transfer and IVF outcomes: a systematic review. Reprod Biol Endocrinol. 2021;19(1):142.
11. Torky H, Ahmad A, Hussein A, El-Desouky ES, Aly R, Ragab M, Abo-Louz A. Comparing sequential vs day 3 vs day 5 embryo transfers in cases with recurrent implantation failure: randomized controlled trial. JBRA Assist Reprod. 2021;25(2):185–92.
12. Arefi S, Ataei M, Maleki N, Yari N, Razi S, Amirajam S. Sequential (two-step) day 3/day 5 frozen-thawed embryo transfer: does it improve the pregnancy rate of patients suffering recurrent implantation failure? J Med Life. 2022;15(11):1365–70.
13. Tehraninejad ES, Raisi E, Ghaleh FB, Rashidi BH, Aziminekoo E, Kalantari V, Haghollahi F, Shariat M. The sequential embryo transfer compared to blastocyst embryo transfer in in vitro fertilization (IVF) cycle in patients with the three repeated consecutive IVF. A randomized controlled trial. Gynecol Endocrinol. 2019;35(11):955–9.
14. Stamenov GS, Parvanov DA, Chaushev TA. Mixed double-embryo transfer: A promising approach for patients with repeated implantation failure. Clin Exp Reprod Med. 2017;44(2):105–10.
15. Li J, Ji J, Guo H, Wang F, Fu Y, Hu R. Stratified analysis of clinical pregnancy outcomes of sequential embryo transfer in frozen embryo transfer cycles based on different factors: a retrospective study. BMC Pregnancy Childbirth. 2023;23(1):806.
16. Hu YL, Wang Y, Geng LH, Meng XQ, Xu HJ, Adu-Gyamfi EA, Zhong ZH, Wan Q, Ding YB. Effects of sequential cleavage and blastocyst embryo transfer on pregnancy outcomes in patients with poor ovarian response. J Reprod Immunol. 2023;155:103780.
17. Gao J, Yuan Y, Li J, Tian T, Lian Y, Liu P, Li R, Qiao J, Long X, Wang H. Sequential embryo transfer versus double cleavage-stage embryo or double blastocyst transfer in patients with recurrent implantation failure with frozen-thawed embryo transfer cycles: a cohort study. Front Endocrinol (Lausanne). 2023;14:1238251.
18. Dai L, Deng C, Li Y, Yi L, Li X, Mu Y, Li Q, Yao Q, Wang Y. Population-based birth weight reference percentiles for Chinese twins. Ann Med. 2017;49(6):470–8.
19. Dai L, Deng C, Li Y, Zhu J, Mu Y, Deng Y, Mao M, Wang Y, Li Q, Ma S, Ma X, Zhang Y. Birth weight reference percentiles for Chinese. PLoS ONE. 2014;9(8):e104779.
20. Chambers GM, Keller E, Choi S, Khalaf Y, Crawford S, Botha W, Ledger W. Funding and public reporting strategies for reducing multiple pregnancy from fertility treatments. Fertil Steril. 2020;114(4):715–21.
21. Martin C, Chang J, Boulet S, Jamieson DJ, Kissin D. Factors predicting double embryo implantation following double embryo transfer in assisted reproductive technology: implications for elective single embryo transfer. J Assist Reprod Genet. 2016;33(10):1343–53.
22. Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in Singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2013;100(6):1615–21.
23. Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among Singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis. Hum Reprod Update. 2014;20(3):439–48.
24. Wang XL, Du MZ, Guan YC, Wang BJ, Zhang JW, Liu ZH. Comparative neonatal outcomes in Singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. Reprod Biol Endocrin 15 (2017).
25. Zhu Q, Zhu J, Wang Y, Wang B, Wang N, Yin M, Zhang S, Lyu Q, Kuang Y. Live birth rate and neonatal outcome following cleavage-stage embryo transfer versus blastocyst transfer using the freeze-all strategy. Reprod Biomed Online. 2019;38(6):892–900.
26. Xie Q, Jiang W, Ji H, Li X, Zhou Y, Zhao C, Zhang J, Lu J, Ling X. Perinatal outcomes of singletons born after blastocyst or cleavage-stage embryo transfer in FET cycles. Eur J Obstet Gynecol Reprod Biol. 2022;271:265–70.
27. Raja EA, Bhattacharya S, Maheshwari A, McLernon DJ. A comparison of perinatal outcomes following fresh blastocyst or cleavage stage embryo transfer in Singletons and twins and between Singleton siblings. Hum Reprod Open. 2023;2023(2):hoad003.
28. Zaat TR, Kostova EB, Korsen P, Showell MG, Mol F, van Wely M. Obstetric and neonatal outcomes after natural versus artificial cycle frozen embryo transfer and the role of luteal phase support: a systematic review and meta-analysis. Hum Reprod Update. 2023;29(5):634–54.
29. Moreno-Sepulveda J, Espinos JJ, Checa MA. Lower risk of adverse perinatal outcomes in natural versus artificial frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. Reprod Biomed Online. 2021;42(6):1131–45.

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