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ORIGINAL RESEARCH

Association Between Pregnancy Outcomes and the Time of Progesterone Exposure of D6 Single-Blastocyst Transfer in Frozen-Thawed Cycles: A Retrospective Cohort Study

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Purpose: The objective of this study was to assess reproductive outcomes of D6 blastocysts transferred on day 6 in comparison to those transferred on day 7 of progesterone exposure in frozen-thawed embryo transfer cycles.

Patients and Methods: This retrospective cohort study included 2029 D6 single blastocysts from the first frozen-thawed embryo transfer cycles of patients at the Hospital for Reproductive Medicine Affiliated to Shandong University from February 2017 to January 2020. Participants were divided into Group A (blastocyst transferred on the 6th day of progesterone exposure, n=1634) and Group B (blastocyst transferred on the 7th day of progesterone exposure, n=395).

Results: The live birth rate was comparable between Group A and Group B (38.7% versus 38.7%, P=0.999). Subgroup analysis revealed a significantly higher preterm birth rate in D6 single blastocysts transferred on the 7th day than in those transferred on the 6th day of progesterone exposure for natural cycle frozen-thawed embryo transfer (5.2% versus 11.3%, P=0.020). After adjustment for potential confounders, the differences in the preterm birth rate in natural cycles persisted (adjusted odds ratio 2.347, 95% confidence interval 1.129–4.877, P=0.022).

Conclusion: In frozen-thawed embryo transfer cycles, transferring on the 6th or 7th day of progesterone exposure of D6 blastocysts did not affect the live birth rate; however, when a natural cycle protocol is adopted, the possible preterm risk of transferring D6 blastocysts on the 7th day of progesterone exposure should be noted.

Keywords: frozen-thawed embryo transfer, blastocyst transfer, endometrium preparation, live birth rate

Introduction

Over the last 40 years, in vitro fertilization (IVF) has become increasingly common. In the past, frozen-thawed embryo transfer (FET) has been associated with lower pregnancy rates compared with fresh transfers, likely due to suboptimal embryo survival after slow freezing.[1] Refinement of vitrification techniques for vitrified-warmed embryos has made it easier to conserve embryos for further use.[2] A small randomized controlled trial showed a higher clinical pregnancy rate with frozen-thawed embryo transfer than with fresh embryo transfer.[3] A multicenter, randomized trial demonstrated that

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the live birth rate did not differ significantly between fresh and frozen embryo transfers among ovulating women with infertility, but frozen embryo transfer resulted in a lower risk of ovarian hyperstimulation syndrome.[4] Thus, FET has enabled the more widespread use of embryo transfer.

Embryos cultured in vitro usually develop to the blastocyst stage by the 5th day (D5 blastocyst) after fertilization, but slower embryos can reach the blastocyst stage by day 6 (D6 blastocyst) or later. Previous studies have demonstrated that the clinical pregnancy rate (CPR) and live birth rate (LBR) are better with D5 blastocyst transfer than with D6 blastocyst transfer.[5] The hypothesized reasons for D6 blastocysts resulting in decreased reproductive outcomes include the quality of D6 blastocysts, which is lower than that of D5 blastocysts, and embryo–endometrium asynchrony.[6]

It is well known that synchronization between the embryonic stage and the endometrial window of implantation (WOI) is crucial for the success of FET cycles.[7] The optimal window for embryo transfer has been shown to be narrow, with the highest rates occurring during a 2-day window.[8] Considering embryo–endometrium synchronization, D6 blastocysts are selected for transfer on the 6th or 7th day of progesterone exposure in FET. The reported pregnancy outcomes for D6 blastocysts transferred on the 6th day compared with those transferred on the 7th day remain controversial.

Commonly used protocols for FET in ovulatory women are natural cycles (NC), stimulated cycles (SC), and artificial cycles (AC). NC treatment has a higher chance of live birth and lower risks of pregnancy-induced hypertension (PIH), postpartum hemorrhage (PPH), and very preterm birth (VPTB) than AC for endometrial preparation in women receiving FET cycles.[9] The corpus luteum can produce not only estradiol (E2) and progesterone but also vasoactive products, such as relaxin and vascular endothelial growth factor, which are important for initial placentation. It is possible that the absence of a corpus luteum during the AC may contribute to these differences.[10] A previous retrospective study reported that D6 blastocysts transferred on the 6th day had higher CPRs and LBRs than those transferred on the 7th day of progesterone exposure.[11] However, another study suggested that the CPR and LBR of frozen-thawed D6 blastocysts transferred on the 6th day were not statistically different from those with blastocysts transferred on the 7th day of progesterone exposure in hormone replacement cycles (HRC).[12] A subgroup analysis in another study of D6 blastocysts showed that D6 blastocysts transferred on the 6th day were associated with higher miscarriage rates than those transferred on the 7th day of progesterone exposure in HRC with FET.[13] However, there is no consensus on the optimal transfer time for frozen-thawed D6 blastocysts. Therefore, we designed this retrospective cohort study to assess reproductive outcomes of D6 blastocysts transferred on day 6 in comparison to those transferred on day 7 of progesterone exposure in frozen-thawed embryo transfer cycles.

Materials and Methods

Study Population

This retrospective study included D6 single-blastocyst transfers in their first frozen-thawed embryo transfer cycle of IVF or intracytoplasmic sperm injection (ICSI) at the Reproductive Hospital Affiliated to Shandong University from February 2017 to January 2020. Although most of the D6 vitrified-warmed blastocysts were transferred on the 6th day of progesterone exposure, some D6 vitrified-warmed blastocysts were transferred on the 7th day of progesterone exposure. Supernumerary embryos after fresh-embryo transfer and embryos from whole-embryo freezing cycles were included in the analysis. Patients were excluded if they: (i) were ≥38 years old, (ii) were diagnosed with a double uterus with or without a double vagina and double cervix, (iii) had undergone preimplantation genetic testing, (iv) had transferred two blastocysts at a time, and (v) were oocyte recipients, defined as patients receiving oocytes from a donor. Finally, 2029 D6 single blastocysts in FET cycles were included in the study, with 1634 on the 6th day of progesterone exposure (Group A) and 395 on the 7th day of progesterone exposure (Group B).

Measures

Ovarian Stimulation and Embryo Scoring

Individualized protocols for controlled ovarian hyperstimulation were determined by experienced clinicians and were initiated using either recombinant follicle-stimulating hormone or human menopausal gonadotropin, as previously

described.[14] When at least two follicles were 18 mm or greater in mean diameter, human chorionic gonadotropin (hCG) at a dose of 4000–10,000 IU was administered to induce the final maturation of oocytes. Oocyte retrieval was performed 34–36 h after hCG injection by experienced physicians. Fertilization was performed using conventional IVF or ICSI according to sperm quality. All oocyte retrieval and fertilization procedures were implemented in accordance with our hospital standards, as previously described.[15] According to morphologic criteria, embryos with scores of 6–8C at cleavage stage ≥2 were defined as excellent embryos. The degree of expansion, quality of the internal cell mass, and quality of trophectoderm cells were considered in the assessment of the quality of blastocysts according to the Gardner scoring system.[16] Blastocysts were divided into three groups according to morphologic quality score: Excellent (3–6 AA/AB/BA), Good (2–6 BB), and Poor (3–6 BC/CB/CC). The choice of fresh embryo transfer or frozen embryo transfer was determined based on the patient risk for ovarian hyperstimulation syndrome. The procedure for supernumerary blastocyst vitrification in our center has been previously described.[17]

Endometrial Preparation Protocols for FET Cycles

FET was performed using NC, SC, or HRC. The choice of endometrial preparation protocol was based on patient characteristics and the physician's preference. In general, patients with regular ovulation were allocated to NC, whereas those with irregular ovulation were allocated to modified NC or HRC. For conventional NC, the development of ovarian follicles was monitored using transvaginal ultrasound. Tests for serum E2, luteinizing hormone (LH) and progesterone were carried out to ascertain the timing of ovulation and the start time of progesterone administration. For SC, if the dominant follicle was not monitored using transvaginal ultrasound when the patient chose NC, human menopausal gonadotropin was administered to promote follicle growth. The monitoring of ultrasound and serum hormones was the same as in NC. Oral dydrogesterone (20–40 mg daily) was administered for luteal-phase support after ovulation in NC and SC. In HRC, oral E2 valerate, at a dose of 4–8 mg daily, was initiated on day 1–3 of the menstrual cycle; vaginal progesterone gel (90 mg per day) and oral dydrogesterone (10 mg twice daily) were added when the endometrial thickness reached 7 mm or more.[18] Transfer was considered for planning when the endometrium had a tri-laminar appearance and a thickness of at least 8 mm. FET protocols are illustrated in Figure 1.

Luteal Phase Support

Luteal support started from the day of ovulation and continued until the day of serum hCG testing. Biochemical pregnancy was defined as a β-hCG level >10 U/L. For women with a positive result, progesterone was continued until 10 weeks of gestation. If conception occurred and an intrauterine gestational sac was detected using transvaginal ultrasound at 7 weeks of gestational age, clinical pregnancy was confirmed. Information on pregnancy, obstetric, and perinatal outcomes was obtained through a review of obstetric and neonatal medical records.

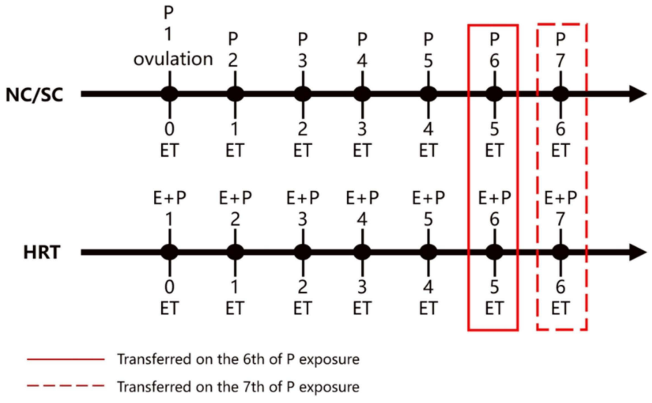

Figure 1 FET protocols.

Outcome Measures

The primary outcome was live birth rate. Live birth rate was defined as the delivery of any surviving newborn at ≥24 weeks of gestation. Secondary efficacy outcomes included the biochemical pregnancy rate (biochemical pregnancy was defined as hCG >10 mIU/mL, measured 14 days after embryo transfer), the clinical pregnancy rate (the presence of a gestational sac in uterine cavity 35 days after embryo transfer), miscarriage rate (spontaneous loss of clinical pregnancy before 24 completed weeks of gestational age), and preterm birth rate (preterm birth was defined as births occurring after 22 weeks and before 37 completed weeks of gestational age). These definitions were in accordance with the latest revision of The International Glossary on Infertility and Fertility Care (2017).[19]

Analysis

All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA), and two-sided P-values <0.05 were considered statistically significant. The chi-squared test was used to compare categorical data. The normal distribution of continuous data was evaluated. Continuous variables are presented as mean and standard deviation. The data were compared using a *t*-test when they were normally distributed and the Mann–Whitney *U*-test when they were not normally distributed. Multivariate logistic regression was performed to identify the factors influencing preterm birth in NC; adjusted odds ratios (aOR) and 95% confidence intervals (CI) are presented. The candidate variables for multivariate logistic regression were those with $P < 0.05$ in a univariate analysis as well as those known to affect preterm birth.

Results

In total, 2029 FET cycles of D6 single blastocysts were performed; the number of D6 single-blastocysts transferred on the 6th and 7th days of progesterone exposure were 1634 (Group A) and 395 (Group B), respectively.

Characteristics of the 2029 patients are shown in Table 1. The results showed a significant difference in antral follicle count (AFC) between the two groups (15.14±9.03 vs 14.15±8.28, P=0.048), but there were no statistically significant differences in age (31.33±3.88 vs 31.06±3.70, P=0.205), BMI (23.87±3.79 vs 23.88±3.65, P=0.972), AMH (3.76±3.10 vs 3.90±3.10, P=0.439), type of infertility (P=0.094), etiology of infertility (P=0.274), FSH (6.82±2.32 vs 6.62±2.18,

Table 1 Patients Characteristics Between Group A and Group B

Parameters	Group A (n=1634)	Group B (n=395)	P
Age	31.33±3.88	31.06±3.70	0.205
BMI	23.87±3.79	23.88±3.65	0.972
AMH	3.76±3.10	3.90±3.10	0.439
Type of infertility			
Primary	46.2% (755/1634)	50.9% (201/395)	0.094
Secondary	53.8% (879/1634)	49.1% (194/395)	
Etiology of infertility			
Female factors	74.6% (1219/1634)	72.7% (287/395)	0.274
Male factors	18.3% (299/1634)	17.5% (69/395)	
Mixed	2.0% (33/1634)	3.3% (13/395)	
Unexplained	5.1% (83/1634)	6.6% (26/395)	
FSH	6.82±2.32	6.62±2.18	0.125
LH	5.60±3.87	5.52±3.71	0.702
E_2	45.12±49.93	44.64±51.34	0.865
AFC	15.14±9.03	14.15±8.28	0.048*
No. of retrieved oocytes	10.35±6.10	10.51±6.25	0.649
No. of Excellent embryos	4.38±3.16	4.46±3.279	0.692
Fresh embryo transfer rate	42.8% (700/1634)	40.5% (160/395)	0.400

Note: *P<0.05.

Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; AFC, number of antral follicle count.

P=0.125), LH (5.60±3.87 vs 5.52±3.71, P=0.702), E2 (45.12±49.93 vs 44.64±51.34, P=0.865), the number of retrieved oocytes (10.35±6.10 vs 10.51±6.25, P=0.649), number of excellent embryos (4.38±3.16 vs 4.46±3.279, P=0.692), and fresh embryo transfer rate (42.8% vs 40.5%, P=0.400) between Group A and Group B.

The FET cycle parameters are presented in Table 2. Endometrial thickness (0.95±0.17 vs 0.95±0.17, P=0.654) and blastocyst morphological grade (P=0.777) were not significantly different between the groups. However, the proportion of patients receiving different protocols for endometrium preparation, including NC (58.0% vs 55.2%, respectively), SC (5.8% vs 10.1%, respectively), and HRC (36.2% vs 34.7%, respectively) differed statistically (P=0.009) between Group A and Group B.

Based on the actual treatment received by the patients, we carried out intragroup comparisons. The biochemical pregnancy rate was higher in Group A than in Group B (60.2% vs 53.2%, P=0.011). There was no significant difference in the CPR (49.8% vs 47.1%, P=0.330), miscarriage rate (21.6% vs 17.7%, P=0.240), preterm birth rate (6.8% vs 8.6%, P=0.377), or LBR (38.7% vs 38.7%, P=0.999) between the groups (Table 2).

A subgroup analysis was performed to compare pregnancy outcomes of D6 single blastocysts transferred on the 6th day with those transferred on the 7th day of progesterone exposure in different types of endometrium preparation. The preterm birth rate was higher for blastocysts transferred on the 7th day than those transferred on the 6th day of progesterone exposure in NC (Table 3, 11.3% vs 5.2%, P=0.020). The LBR did not differ significantly between groups for NC, SC, and HRC (Table 3).

Multivariate regression of the preterm birth rate showed the same result (Table 4; aOR 2.347, 95% CI [1.129–4.877], P=0.022). Factors with significant differences in the univariate analysis (Table 5) and those known to affect preterm birth were included in the multivariate logistic regression.

In total, 1528 patients with excellent and average blastocyst morphological grades were included in the multivariate analysis. The number of cycles of D6 single blastocysts transferred on the 6th and 7th day of progesterone exposure was 1228 (Group C) and 300 (Group D), respectively. The demographics of this group were similar to those of the overall study sample. Characteristics of the 1528 patients are shown in Table 6. There were no statistically significant differences in age (31.22±3.91 vs 30.97±3.67, P=0.306), BMI (23.85±3.81 vs 23.94±3.76, P=0.708), AMH (3.79±3.04 vs 3.94±3.11, P=0.453), type of infertility (P=0.169), etiology of infertility (P=0.115), FSH (6.85±2.66 vs 6.76±3.51, P=0.607), LH (5.66±3.92 vs 5.57±3.84, P=0.713), E2 (45.52±45.25 vs 46.20±57.95, P=0.589), antral follicle count (15.09±8.80 vs 14.26±8.46, P=0.140), number of retrieved oocytes (10.56±6.09 vs 10.55±6.33, P=0.973), number of

Table 2 Frozen-Embryo Transfer Cycles Parameters and Pregnancy Outcomes Between Group A and Group B

Parameters/Outcomes	Group A (n=1634)	Group B (n=395)	P
Endometrium preparation			
NC	58.0% (947/1634)	55.2% (218/395)	0.009*
SC	5.8% (95/1634)	10.1% (40/395)	
HRC	36.2% (592/1634)	34.7% (137/395)	
Endometrial thickness	0.95±0.17	0.95±0.17	0.654
Blastocysts morphological grade			
Excellent	16.3% (266/1634)	17.7% (70/395)	0.777
Average	58.9% (962/1634)	58.2% (230/395)	
Poor	24.8% (406/1634)	24.1% (95/395)	
Biochemical pregnancy rate	60.2% (984/1634)	53.2% (210/395)	0.011*
CPR	49.8% (814/1634)	47.1% (186/395)	0.330
Miscarriage rate	21.6% (176/814)	17.7% (33/186)	0.240
Preterm birth rate	6.8% (55/814)	8.6% (16/186)	0.377
LBR	38.7% (633/1634)	38.7% (153/395)	0.999

Note: *P<0.05.

Abbreviations: FET, Frozen-thawed embryo transfer; NC, natural cycle; SC, stimulated cycle; HRC, hormone replacement cycle; LBR, live birth rate; CPR, clinical pregnancy rate.

Table 3 Pregnancy Outcomes of the Subgroup Analysis Comparing Group A with Group B in NC, SC and HRC Respectively

Outcomes	Group A	Group B	P
Natural cycles	n=947	n=218	
Biochemical pregnancy rate	59.2% (561/947)	56.4% (123/218)	0.446
CPR	50.5% (478/947)	48.6% (106/218)	0.622
Miscarriage rate	19.2% (92/478)	16.0% (17/106)	0.443
Preterm birth rate	5.2% (25/478)	11.3% (12/106)	0.020*
LBR	40.3% (382/947)	40.8% (89/218)	0.895
Stimulated cycles	n=95	n=40	
Biochemical pregnancy rate	60.0% (57/95)	47.5% (19/40)	0.181
CPR	48.4% (46/95)	40.0% (16/40)	0.370
Miscarriage rate	26.1% (12/46)	18.8% (3/16)	0.555
Preterm birth rate	13.0% (6/46)	0.0% (0/16)	0.128
LBR	35.8% (34/95)	32.5% (13/218)	0.714
Hormone replacement cycles	n=592	n=137	
Biochemical pregnancy rate	61.8% (366/592)	49.6% (68/137)	0.009*
CPR	49.0% (290/592)	46.7% (64/137)	0.632
Miscarriage rate	24.8% (72/290)	20.3% (13/64)	0.444
Preterm birth rate	8.3% (24/290)	6.3% (4/64)	0.587
LBR	36.7% (217/592)	37.2% (51/137)	0.901

Note: *P<0.05.
Abbreviations: NC, natural cycle; SC, stimulated cycle; HRC, hormone replacement cycle; LBR, live birth rate; CPR, clinical pregnancy rate.

Table 4 Multivariable Regression Analysis of Preterm Birth Rate

Preterm Birth Rate	cOR (95% CI)	P	aOR (95% CI)	P
The 7th day of progesterone exposure	2.313 (1.122–4.768)	0.023*	2.347 (1.129–4.877)	0.022*
Secondary infertility	2.375 (1.128–5.002)	0.023*	2.360 (1.115–4.996)	0.025*
BMI	1.087 (0.991–1.192)	0.076	1.084 (0.987–1.190)	0.091

Notes: *P<0.05. Potential confounders included time of transfer, type of infertility, BMI.
Abbreviations: BMI, body mass index, cOR, crude odds ratio, aOR, adjust odds ratio.

Table 5 Univariable Analysis of Patients (with No Preterm Birth or Preterm Birth)' Basic Characteristics in Natural Cycles

Parameters	No Preterm Birth (n=547)	Preterm Birth (n=37)	P
The 7th day of progesterone exposure	17.2% (94/547)	32.4% (12/37)	0.020*
Age	31.35±3.74	31.95±3.61	0.338
BMI	23.42±3.30	24.44±4.19	0.155
AMH	3.19±2.33	3.33±2.30	0.721
Type of infertility			
Primary	46.8% (256/547)	27.0% (10/37)	0.019*
Secondary	53.2% (291/547)	73.0% (27/37)	
Etiology of infertility			
Female factors	74.4% (396/547)	64.9% (24/37)	0.522
Male factors	20.1% (110/547)	27.0% (10/37)	
Mixed	2.2% (12/547)	0.0% (0/37)	
Unexplained	5.3% (29/547)	8.1% (3/37)	

(*Continued*)

Table 5 (Continued).

Parameters	No Preterm Birth (n=547)	Preterm Birth (n=37)	P
FSH	7.00±2.33	6.58±2.48	0.323
LH	5.27±3.36	5.26±3.56	0.978
E2	44.46±45.68	54.87±91.70	0.498
AFC	15.10±8.56	14.97±7.07	0.927
No. of retrieved oocytes	10.06±5.63	10.92±6.60	0.375
No. of Excellent embryos	4.38±3.12	4.76±3.48	0.476
Fresh embryo transfer	45.2% (247/547)	45.9% (17/37)	0.925
Endometrial thickness	1.00±0.17	1.01±0.18	0.579
Blastocysts morphological grade			
Excellent	21.2% (116/547)	27.0% (10/37)	0.610
Average	60.5% (331/547)	59.5% (22/37)	
Poor	18.3% (100/547)	13.5% (5/37)	

Note: *P<0.05.
Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; AFC, number of antral follicle count.

excellent embryos (4.56±3.15 vs 4.61±3.40, P=0.800), and the fresh embryo transfer rate (45.8% vs 40.0%, P=0.072) between Group C and Group D.

The FET cycle parameters are presented in Table 7. Protocols of endometrium preparation (P=0.094), endometrial thickness (0.97±0.17 vs 0.96±0.17, P=0.549), and blastocyst morphological grade (P=0.110) were not significantly different between the groups.

Table 6 Characteristics of Patients with Excellent and Average Blastocyst Morphological Grades Between Group C and Group D

Parameters	Group C (n=1228)	Group D (n=300)	P
Age	31.22±3.91	30.97±3.67	0.306
BMI	23.85±3.81	23.94±3.76	0.708
AMH	3.79±3.04	3.94±3.11	0.453
Type of infertility			
Primary	46.6% (572/1228)	51.0% (153/300)	0.169
Secondary	53.4% (656/1228)	49.0% (147/300)	
Etiology of infertility			
Female factors	74.1% (910/1228)	72.7% (218/300)	0.115
Male factors	19.1% (235/1228)	16.7% (50/300)	
Mixed	1.9% (23/1228)	3.3% (10/300)	
Unexplained	4.9% (60/1228)	7.3% (22/300)	
FSH	6.85±2.66	6.76±3.51	0.607
LH	5.66±3.92	5.57±3.84	0.713
E_2	45.52±45.25	46.20±57.95	0.589
AFC	15.09±8.80	14.26±8.46	0.140
No. of retrieved oocytes	10.56±6.09	10.55±6.33	0.973
No. of Excellent embryos	4.56±3.15	4.61±3.40	0.800
Fresh embryo transfer rate	45.8% (562/1228)	40.0% (120/299)	0.072

Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; AFC, number of antral follicle count.

Table 7 Frozen-Embryo Transfer Cycles Parameters and Pregnancy Outcomes Between Group C and Group D in Patients with Excellent and Average Blastocyst Morphological Grades

Parameters/Outcomes	Group C (n=1228)	Group D (n=300)	P
Endometrium preparation			
NC	58.6% (719/1228)	55.3% (166/300)	0.094
SC	6.4% (79/1228)	10.0% (30/300)	
HRC	35.0% (430/1228)	34.7% (103/300)	
Endometrial thickness	0.97±0.17	0.96±0.17	0.549
Blastocysts morphological grade			
Excellent	7.8% (96/1228)	10.7% (32/300)	0.110
Average	92.2% (1132/1228)	89.3% (268/300)	
Biochemical pregnancy rate	63.4% (779/1228)	55.0% (165/300)	0.007*
CPR	54.2% (665/1228)	49.3% (148/300)	0.134
Miscarriage rate	20.0% (133/665)	16.2% (24/148)	0.292
Preterm birth rate	7.7% (51/665)	8.1% (12/148)	0.857
LBR	42.8% (526/1228)	41.0% (123/300)	0.565

Note: *P<0.05.
Abbreviations: FET, Frozen-thawed embryo transfer; NC, natural cycle; SC, stimulated cycle; HRC, hormone replacement cycle; LBR, live birth rate; CPR, clinical pregnancy rate.

The biochemical pregnancy rate was higher in Group C than in Group D (63.4% vs 55.0%, P=0.007). There was no significant difference in the CPR (54.2% vs 49.3%, P=0.134), miscarriage rate (20.0% vs 16.2%, P=0.292), preterm birth rate (7.7% vs 8.1%, P=0.857), or LBR (42.8% vs 41.0%, P=0.565) between the groups (Table 7).

Discussion

Several studies have explored the optimal transfer time for frozen-thawed D6 blastocysts, but controversy remains.[11–13,20] In this study, we found no difference in the LBR of D6 single-blastocyst embryos transferred on the 6th day compared with those transferred on the 7th day of progesterone exposure. Comparing subgroups of patients with excellent and average blastocyst morphological grades, there was also no difference in LBR. However, an additional detailed subgroup analysis of FET endometrium preparation protocols was performed, showing that the preterm birth rate was higher in blastocysts transferred on the 7th day than on the 6th day of progesterone exposure in NC. To our knowledge, this is currently the largest-sample study comparing D6 single blastocysts transferred on the 6th day compared with those transferred on the 7th day of progesterone exposure.

Blastocyst transfer may be advantageous in assisted reproduction techniques because the exposure of the embryo to the uterine environment is similar to the NC.[21] In addition, blastocyst culture improves synchronization of the uterus and embryo and the ability to self-select viable embryos, thereby increasing implantation rates.[22] Embryos that are cultured in vitro usually develop to D5 blastocysts after fertilization, but slower embryos can achieve blastulation on Day 6 (D6 blastocysts). The question of whether D6 blastocysts should be transferred on the 6th day of progesterone exposure poses a dilemma. There is often concern that the embryo–endometrium asynchrony when using D6 blastocysts may increase the risk of pregnancy failure.[23] The optimal duration of progesterone exposure in FET cycles thus assumes the utmost importance for ensuring the best FET outcomes. This information could be important to reassure couples who conceive following the transfer of a D6 blastocyst.

The outcomes of assisted reproductive technology pregnancies are influenced by endometrial receptivity and embryo–endometrium synchrony.[24] Endometrial receptivity is regulated by many factors, including uterine anatomical factors, immunity, and metabolism.[7,25] With the influence of inflammation or other factors, shortening of or missing the endometrial implantation window can lead to infertility or pregnancy failures.[26] Estrogen and progesterone are important regulatory factors in the implantation process. Estrogen and progesterone bind to specific high-affinity receptors, which, in turn, regulate the transcription of a large number of genes that drive the endometrium to enter a period of receptivity.[27]

The time of progesterone exposure is related to the endometrial implantation window. Whether the embryo is synchronized with the endometrium at the WOI determines whether a blastocyst can be successfully implanted.[28] A previous study found that D6 blastocysts transferred on the 6th day had a higher LBR compared with those transferred on the 7th day of progesterone exposure; it may be assumed that each embryo has to spend a certain amount of time in the uterus before implantation and that the WOI is more likely to have closed when the embryo is transferred on the 7th day of progesterone exposure.[11] However, the results of our study demonstrated similar LBRs between D6 blastocysts transferred on the 6th and 7th days of progesterone exposure.

Most previous studies have focused on the optimal duration of progesterone exposure before transferring frozen-thawed blastocysts in HRC.[12,13] However, this has remained elusive for different endometrium preparation protocols of FET, especially in NC, which is generally the preferred method for preparing the endometrium. Previous studies have indicated comparable pregnancy outcomes among NC, SC, and HRC, and there has been insufficient evidence to support the use of one protocol over another.[29,30] Reproductive clinicians should choose endometrium preparation protocols individually according to ovulation and other important factors. Thus, it is possible that the optimal duration of progesterone exposure has an effect on pregnancy outcomes in different endometrium preparation protocols. For this reason, we performed a subgroup analysis to compare pregnancy outcomes in NC, SC, and HRC regimens. We found that the LBR was not significantly different between D6 single blastocysts transferred on the 6th and 7th days of progesterone exposure in NC, SC, and HRC. However, a higher preterm birth rate was seen for blastocysts transferred on the 6th day than on the 7th day in NC. Thus, we speculated that the WOI of SC and HRC is more stable than that of NC due to the regulation of exogenous hormones. Franasiak[7] also proposed that, although implantation can occur in a broad window, the optimal time might be more restricted NC. Another consideration was that the histological dating may be inconsistent with the actual day after ovulation.[1] Further, delayed endometrial development in the luteal phase has been shown in around a quarter of women.[31] On the basis of this finding, when choosing NC as an endometrium preparation protocol for FET, one should note the preterm birth risk for patients transferring D6 blastocysts. Infection, cervical pathology, uterine overdistension, progesterone deficiency, stress on the mother and fetus, allograft reaction, and allergic phenomena, may lead to preterm birth. These several causes may improperly stimulate the usual pathway between the decidua and the fetal membranes, resulting in cervical ripening, membrane rupture, and uterine contractility, and finally, preterm birth occurs.[32]

Age and the aneuploidy rate may influence the embryo–endometrium synchrony.[3] One study found that, if embryos were selected on the basis of morphology alone, the euploidy rate was 86% in patients 22–34 years of age and 69% in those 35–44 years of age.[33] In our study, there was no difference in age between the two groups, and the effects of age on the euploidy rate and age itself on synchrony can be ignore assumed negligible. One previous study also suggested that D6 embryos have poor embryo quality and high aneuploidy rates.[34] However, another study compared D5 and D6 blastocysts that underwent a single biopsy in FET cycles and did not demonstrate differences in euploidy, aneuploidy, or mosaicism rates.[35] Even if patients underwent PGT, the false-negative rate of a low-level mosaic embryo and the health risk of the fetus cannot be avoided.[36] In our study, we excluded patients who underwent PGT, thus preventing us from determining whether the embryos had chromosomal abnormalities that might influence pregnancy outcomes. In the future, studies including D5 and D6 embryos that have undergone PGT may provide more convincing results.

This is the largest retrospective study of the effect of progesterone exposure time on pregnancy outcomes in D6 single blastocyst frozen-thawed embryo transfer. Subgroup analysis was performed to analyze the effects of different endometrial preparation protocols on the pregnancy outcomes of D6 single blastocyst embryo transfer. This study provides an important reference and basis for the time of D6 single blastocyst embryo transfer. This study had several limitations. A matched propensity score analysis may be necessary due to differences in sample size between groups. However, there were no significant differences in group characteristics, as a result, the findings are unlikely to have been skewed by this. Further, this study was retrospective, and we were not able to completely rule out all potential confounders. In addition, we did not measure hormone levels before luteal support, and more rigorous studies on this are needed. Meanwhile, further prospective research with sufficient sample sizes and more sophisticated stratified analyses is needed to confirm these findings. In addition, our study included only D6 blastocyst transfers; pregnancy outcomes of fresh blastocyst transfers and D5 blastocyst transfers during the same period should be compared.

Conclusion

In conclusion, in FET cycles, D6 blastocyst transfer on the 6th or 7th day of progesterone exposure did not affect the LBR; however, if the NC protocol was adopted, the higher possible preterm birth risk of blastocyst transferred on the 7th day should be noted.

Data Sharing Statement

The data used to support the findings of this study are included in the article. Also, if readers need detailed information, he/she can Email the corresponding author.

Ethics Approval and Informed Consent

This study complied with the Declaration of Helsinki. This study was approved by the independent ethics committee of the Center for Reproductive Medicine Shandong University. Informed consent was obtained from all the patients for this study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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