



Comparison of live birth rates following the transfer of day-6 blastocysts on the 6th versus 7th day of progesterone exposure in hormone replacement treatment–frozen embryo transfer cycles

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Objective: The duration of progesterone exposure is critical for establishing and maintaining a pregnancy in hormone replacement treatment (HRT)–frozen embryo transfer (FET) cycles. This study compared the live birth rates (LBRs) of patients undergoing day-6 warmed blastocyst transfer on either the 6th or 7th day of progesterone administration in HRT-FET cycles.

Methods: A retrospective cohort study was conducted using data from the central registry of Indira IVF Hospital Private Limited. In total, 2,633 patients who underwent day-6 blastocyst transfer cycles with HRT-FET were identified. The cycles were categorized based on the timing of the day-6 blastocysts transfer: P+6 vs. P+7 (initiation date: P+1). Propensity scores were calculated. The primary outcome measure was the LBR.

Results: Following propensity score matching, a total of 1,401 patients were included in the final analysis (P+6: n=1,212; P+7: n=189). The number of previous attempts was significantly higher in the P+6 group, whereas the rate of preimplantation genetic testing for aneuploidy was significantly higher in the P+7 group. The LBRs were 59.2% and 54.5% in the P+6 and P+7 groups, respectively ($p=0.21$). Multivariate regression analysis revealed that the number of previous *in vitro* fertilization attempts (odds ratio [OR], 3.246; 95% confidence interval [CI], 2.429 to 4.337; $p<0.001$), the number of blastocysts transferred (OR, 2.011; 95% CI, 1.461 to 2.768; $p<0.01$), and endometrial thickness on the day of progesterone initiation (OR, 1.143; 95% CI, 1.022 to 1.28; $p=0.02$) were independent predictors of live birth.

Conclusions: The LBRs were comparable in patients who underwent day-6 warmed blastocyst transfer on day 6 or 7 post-progesterone initiation in HRT-FET cycles.

Keywords: Day-6 blastocyst; Hormone replacement treatment; Live birth; Progesterone; Vitrified warmed blastocyst transfer

Introduction

Over the past decade, advancements in vitrification techniques and a rise in "freeze-all" cycles have significantly increased the num-

ber of frozen embryo transfer (FET) cycles worldwide [1]. Despite emerging data indicating increased maternal and obstetric risks [2], the hormone replacement treatment (HRT) protocol remains the most commonly used [3]. The optimal timing and concentration of progesterone exposure are essential for establishing and maintaining a successful pregnancy [4]. In this context, the administration route, dosage, and initiation timing of progesterone must be carefully considered. In an HRT cycle, where no corpus luteum is present, all progesterone provided is exogenously sourced.

The common practice is to transfer warmed blastocysts on the 6th day of progesterone initiation in HRT-FET cycles [4]. In this scenario, both day-5 and day-6 vitrified blastocysts are typically transferred on

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the 6th day after starting progesterone [4]. Since day-6 vitrified blastocysts are biologically delayed, there is limited data on whether it is more beneficial to transfer day-6 vitrified and warmed blastocysts on the 6th or 7th day after progesterone initiation. The only available small-scale retrospective study indicated numerically higher live birth rates (LBRs) when day-6 vitrified and warmed blastocysts were transferred on the 7th day of progesterone initiation compared to the 6th day; the respective rates were 35.5% and 21.5% ($p=0.06$). The increase in LBR was primarily attributed to a lower miscarriage rate on the 7th day of progesterone (21.4% vs. 50.0%, $p=0.02$) [5].

The objective of this study is to compare the LBRs of patients who underwent day-6 warmed blastocyst transfer on either the 6th or 7th day of progesterone administration in HRT-FET cycles.

Methods

1. Patients

This retrospective cohort study utilized data from 85 centers of Indira IVF Hospital Udaipur across India. A total of 62,227 FET cycles conducted between April 2019 and July 2021 were examined. Among these, 2,633 day-6 vitrified and warmed blastocyst transfer cycles undergoing HRT-FET were identified (Figure 1A).

The inclusion criteria were: (1) female, aged 21 to 45 years; (2) body mass index (BMI) ≤ 35 kg/m²; and (3) endometrial thickness ≥ 7 mm on the day progesterone administration began. Exclusion criteria included azoospermia, egg donation, surrogacy, cycles involving preimplantation genetic testing for monogenic disorders and structural rearrangement, uncontrolled diabetes mellitus, hyperprolactinemia, abnormal thyroid function tests, congenital uterine anomalies, a history of Asherman syndrome, submucous fibroids, and previous uterine surgery. Cycles were also excluded if the serum progesterone level was ≤ 1.5 ng/mL on the day of progesterone administration. These 2,633 cycles were divided into two groups based on the transfer day of day-6 blastocysts: P+6 versus P+7, considering the start date of progesterone as P+1 (Figure 1B).

We utilized propensity score matching (PSM) to select patients who tested negative for β -human chorionic gonadotropin (β -hCG) and paired them with those who tested positive and were followed until live birth (LB). This approach was adopted to maintain a consistent β -hCG test positive rate between the final study population and the initial cohort. Specifically, from the initial cohort, 332 cases were selected from the 627 with negative β -hCG tests in the P+6 arm, and 64 cases were chosen from the 117 in the P+7 arm (Figure 1B). For the PSM, we initially calculated the propensity scores for each patient using binary logistic regression. The variables included in this calculation were female age, male age, BMI, anti-Müllerian hormone (AMH) levels, and endometrial thickness. These variables were cho-

sen because they are pertinent to the treatment groups and represent potential confounders that could affect the outcomes. The subsequent step involved matching patients from the P+6 and P+7 groups based on their propensity scores using the nearest neighborhood method. For each patient in the P+6 group, a counterpart from the P+7 group with a similar propensity score was identified. This matching process was systematically applied to all patients in the P+6 group, ensuring that each was paired with a counterpart in the P+7 group who shared similar characteristics.

2. Ovarian stimulation and laboratory procedures

A gonadotropin-releasing hormone antagonist protocol was used for all patients. Final oocyte maturation was induced using either recombinant human chorionic gonadotropin (250 μ g) or triptorelin (0.2 mg), with oocyte retrieval occurring 36 hours later. Triptorelin was used for triggering when the number of follicles >11 mm exceeded 15, and a freeze-all strategy was implemented in these cases. If a fresh embryo transfer was conducted, any surplus eligible blastocysts were vitrified. The current study included day-6 blastocysts that were either vitrified during a freeze-all cycle or as surplus embryos in a fresh transfer cycle.

The collected oocytes were denuded to assess their maturation status. As our standard protocol, we conducted intracytoplasmic sperm injection (ICSI) and maintained an extended culture until the blastocyst stage for all patients.

A 35 $\times$ 10 mm Petri dish was prepared, and 30 μ L droplets of pre-equilibrated single-step culture media were dispensed into it, each droplet then being overlaid with pre-equilibrated paraffin oil. The dishes were placed in a benchtop incubator, which was maintained at 37 °C until ICSI was performed. Following ICSI, the oocytes were distributed among four primary culture droplets, with no more than five oocytes per droplet. The cultures were then left undisturbed for the next 5 days, at which point the embryos were first assessed for staging and grading [4]. The slow-growing embryos were reassessed on the 6th day of culture. The culture conditions were carefully controlled to ensure optimal temperature, pH, and osmolality for embryo development. The benchtop incubators were tri-gas models, maintaining CO₂ levels between 6.2% and 6.4% and O₂ levels between 6.0% and 6.2%.

Vitrification was performed on day 5 or 6 based on embryonic development. Blastocyst grading was performed using Gardner's criteria [6]. Briefly, blastocysts were categorized into four groups based on their morphological grading prior to cryopreservation: excellent (≥ 3 AA), good (3–6AB, 3–6BA, 1–2AA), average (3–6BB, 3–6AC, 3–6CA, 1–2AB, 1–2BA), and poor (1–6BC, 1–6CB, 1–6CC, 1–2BB).

For the vitrification and warming procedures, the Cryotop device and solutions (Kitazato Biopharma) were used [7]. After warming,

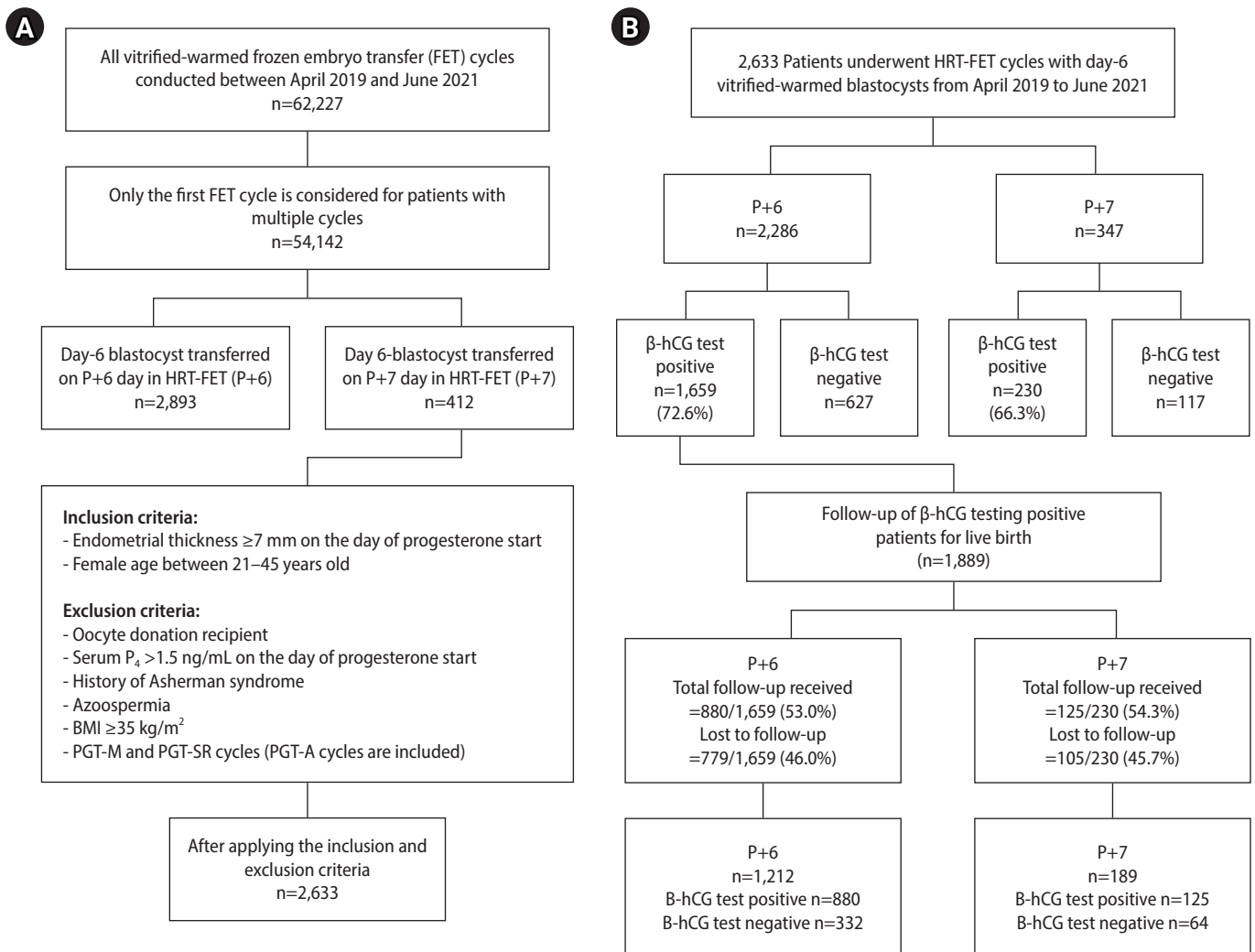


Figure 1. (A) Study flow diagram illustrating the selection process of the targeted population from the initial cohort. (B) Study flow diagram depicting the final study population after propensity score matching. HRT-FET, hormone replacement treatment–frozen embryo transfer; P+6, 6th day after progesterone initiation; P+7, 7th day after progesterone initiation; BMI, body mass index; PGT-M, pre-implantation genetic testing for monogenic disorders; PGT-SR, preimplantation Genetic Testing for Structural Rearrangements; PGT-A, preimplantation genetic testing for aneuploidy; β -hCG, β -human chorionic gonadotropin.

the blastocysts were transferred to pre-made culture dishes containing one-step medium, and placed into the incubator. Before embryo transfer, the post-warming survival status was checked.

One or two blastocysts were transferred under ultrasonographic guidance using a soft catheter. When multiple embryos were transferred, the one with the best morphological grading was included in the analysis.

3. Endometrium preparation

For HRT-FET cycles, oral estradiol valerate was administered at a dosage of 6 to 8 mg/day starting on the 2nd day of the cycle. An assessment of the endometrium was conducted after 10 to 14 days of estradiol treatment. If the endometrial thickness was 7 mm or great-

er, the serum progesterone level was measured. Should the progesterone level be below 1.5 ng/mL, exogenous progesterone administration commenced. The transfer of the warmed blastocyst occurred either on the 6th day (P+6) or the 7th day (P+7) following the initiation of progesterone, with the 1st day of progesterone administration designated as P+1. Progesterone was administered intramuscularly at 100 mg once daily.

4. Outcome measures

The primary outcome measure was the LBR. The secondary outcome measures included the rates of positive pregnancy tests, biochemical loss, clinical pregnancies, and miscarriages.

The Institutional Ethics Committee approved the study under the

reference ECR/1627/inst/WB/2021/IIHPL-UDR/RCT/009_2022. Additionally, the Institutional Review Board sanctioned the study in accordance with the Helsinki Declaration. Written informed consent by the patients was waived due to a retrospective nature of our study.

5. Statistical analysis

After PSM, we conducted statistical analyses to compare the outcomes between the two matched groups. We assessed categorical variables, such as the positive β -hCG rate, using either the chi-square test or Fisher exact test, depending on the data's appropriateness. For continuous variables, such as endometrial thickness, we employed the Student *t*-test or the Mann-Whitney *U* test, based on whether the data distribution was normal. We also performed univariate and multivariate logistic regression analyses to identify independent predictors of LBs. In our analysis, we considered *p*-values <0.05 to be statistically significant, indicating a meaningful difference or association between variables.

The statistical analyses, including PSM and forest plot calculations, were conducted using statistical software such as IBM SPSS ver. 28.0 (IBM Co.) and MedCalc software (MedCalc Software Ltd.). PSM is an effective method for controlling confounding factors, thereby enhancing the reliability of comparisons between different treatment groups. By ensuring that the groups compared have similar baseline characteristics, this approach strengthens the robustness of the study's findings and reduces susceptibility to bias.

Results

The baseline demographic features and embryological data for the P+6 and P+7 groups are presented in Tables 1 and 2, respectively. Most of the demographic features and embryological data were similar between the two groups. However, the P+6 group had a significantly higher number of previous attempts, while the rate of preimplantation genetic testing for aneuploidy (PGT-A) was significantly higher in the P+7 group. When two blastocysts were transferred, the P+7 group received a significantly greater number of excellent-quality blastocysts (Table 2).

The reproductive outcomes for the P+6 and P+7 groups are presented in Table 2. The LBRs for the P+6 and P+7 groups were similar, at 59.2% and 54.5%, respectively ($p=0.21$). The rates of positive pregnancy tests were 72.6% for the P+6 group and 66.1% for the P+7 group ($p=0.07$). The biochemical loss rates were 3.0% for the P+6 group and 4.8% for the P+7 group ($p=0.27$), while the clinical pregnancy rates were 70.5% and 63.0%, respectively ($p=0.04$). The miscarriage rates for both groups were comparable, at 15.9% for the P+6 group and 13.4% for the P+7 group.

The demographic and embryological data for cycles with or with-

out LB are presented in Table 3. Females were significantly younger in cycles that resulted in LB. The maturity rate, the number of blastocysts transferred, and the rate of double blastocyst transfer were all significantly higher in the LB group. Additionally, the number of previous failed cycles was significantly lower in the LB group.

A multivariate regression analysis was conducted with LB as the dependent variable. Independent variables included female age, BMI, number of previous attempts, the AMH level, number of oocytes retrieved, metaphase-II rate, PGT-A, number of blastocysts transferred, blastocyst morphology, duration of progesterone exposure (P+6 or P+7), and endometrial thickness on the day progesterone treatment began (Figure 2). The number of previous *in vitro* fertilization attempts (odds ratio [OR], 3.246; 95% confidence interval [CI], 2.429 to 4.337; $p<0.001$), the number of blastocysts transferred (double embryo transfer; reference: single embryo transfer) (OR, 2.011; 95% CI, 1.461 to 2.768; $p<0.01$), and endometrial thickness on the day progesterone treatment started (OR, 1.143; 95% CI, 1.022 to 1.28; $p=0.02$) were significant independent predictors of LB.

Discussion

In this study, we observed that the LBRs are comparable when slowly growing day-6 vitrified blastocysts are transferred on either the 6th or 7th day after progesterone initiation in an HRT-FET protocol. The interaction between the blastocyst and a receptive endometrium is a complex molecular process essential for successful implantation [8]. Synchronization between the embryonic stage and the endometrial window of implantation (WOI) is crucial for the success of a FET cycle [9]. Progesterone is a crucial determinant of the WOI and initiates its opening [9]. There is a paucity of data regarding the impact of the duration of progesterone exposure on reproductive outcomes in HRT-FET cycles [4]. To our knowledge, there are four randomized controlled trials (RCTs), three published in peer-reviewed journals [8,10,11] and one abstract [12], evaluating the impact of the duration of progesterone exposure on reproductive outcomes in HRT-FET cycles. Of those four RCTs, embryo transfer was carried out at the cleavage-stage in two trials [8,10], whereas the remaining two conducted embryo transfer at the blastocyst stage [11,12]. The earliest RCT [8], which was conducted in an oocyte donation model and transferred day-3 embryos, compared progesterone initiation on the 2nd (egg retrieval+1 day; group C; $n=91$), 3rd (egg retrieval day, group B; $n=94$), or 4th (egg retrieval+1 day; group A; $n=97$) day of progesterone administration in the recipients. The ongoing pregnancy rates per embryo transfer were similar in all groups except for a higher biochemical pregnancy rate in group A (12.9%) than in groups B (6.6%) and C (2.3%) [8]. In another RCT, when cleavage-stage day-3 embryos were warmed and cultured

Table 1. Comparison of baseline demographic features among patients undergoing day-6 blastocyst transfers on the 6th versus 7th day of progesterone exposure in HRT-FET cycles

Characteristic	Sixth day of progesterone administration (n = 1,212)	Seventh day of progesterone administration (n = 189)	p-value
Female age at FET (yr)	31.10 ± 4.41	31.61 ± 4.47	0.137 ^{a)}
Male age (yr)	35.12 ± 4.89	35.71 ± 4.45	0.105 ^{a)}
Female body mass index (kg/m ²)	25.05 ± 4.01	24.93 ± 4.08	0.704 ^{a)}
Anti-Müllerian hormone (ng/mL)	3.34 ± 2.59	2.92 ± 2.48	0.041 ^{a)}
M-II rate (%)	70.17 ± 17.18	71.27 ± 18.77	0.461 ^{a)}
Retrieval rate (%)	97.44 ± 26.86	102.74 ± 29.81	0.057 ^{a)}
Oocytes retrieved (count)	13.76 ± 7.72	14.03 ± 8.81	0.691 ^{a)}
Total gonadotropin used (IU)	3,072.95 ± 872.52	3,073.42 ± 859.29	0.996 ^{a)}
Stimulation protocol			0.665
Agonist	920/1,018 (90.4)	133/145 (91.7)	
Antagonist	87/1,018 (8.5)	12/145 (8.3)	
Minimal	11/1,018 (1.1)	0/145 (0)	
Sperm washing technique			0.135
Density gradient	152/1,118 (13.6)	24/177 (13.6)	
Qualis	65/1,118 (5.8)	18/177 (10.2)	
Simple wash	9/1,118 (0.8)	0/177 (0.0)	
Swim-up	892/1,118 (79.8)	135/177 (76.3)	
Sperm remarks			0.502
Asthenoteratozoospermia	263/1,080 (24.4)	38/165 (23)	
Asthenozoospermia	20/1,080 (1.9)	1/165 (0.6)	
Cryptozoospermia	5/1,080 (0.5)	0/165 (0)	
Normozoospermia	342/1,080 (31.7)	52/165 (31.5)	
Oligoasthenoteratozoospermia	126/1,080 (11.7)	18/165 (10.9)	
Oligoasthenozoospermia	2/1,080 (0.2)	0/165 (0)	
Oligoteratozoospermia	4/1,080 (0.4)	3/165 (1.8)	
Oligozoospermia	1/1,080 (0.1)	0/165 (0)	
Teratozoospermia	317/1,080 (29.4)	53/165 (32.1)	
No. of previous IVF attempts			0.003
0	650 (53.6)	126 (66.7)	
1	366 (30.2)	43 (22.8)	
2	196 (16.2)	20 (10.6)	
No. of patients with PGT-A	38 (3.1)	12 (6.3)	0.027

Values are presented as mean ± standard deviation or number (%).

HRT-FET, hormone replacement treatment–frozen embryo transfer; M-II, metaphase-II; IVF, *in vitro* fertilization; PGT-A, preimplantation genetic testing for aneuploidy.

^{a)}Mann-Whitney.

overnight to day 4 and transferred on the 5th (n=150) or 3rd (n=150) day of progesterone administration, similar clinical pregnancy rates were noted (37/137 [27.0%] vs. 26/138 [18.8%], respectively; OR, 1.6; 95% CI, 0.9 to 2.82; *p*=0.11) [10]. However, the early pregnancy loss rate was significantly higher following embryo transfer on the 3rd day of progesterone administration (32/58 [55.2%] vs. 21/58 [36.2%]; OR, 0.46; 95% CI, 0.22 to 0.97; *p*=0.04) [10]. For the blastocyst stage transfer, as mentioned, there are two RCTs; one was presented as an abstract [12] and one was published in a peer-reviewed journal [11].

In the study by Ding et al. [12], frozen/thawed blastocysts were transferred on the 6th (n=23) or 7th day (n=26) of progesterone administration, and higher clinical pregnancy, ongoing pregnancy, and implantation rates were reported in the day-6 group (60.9% vs. 53.8%, 56.5% vs. 50.0%, and 40.7% vs. 30.0%, respectively); however, the differences did not reach statistical significance (*p*>0.05). Moreover, a recent RCT compared the outcomes of blastocyst transfer on the 5th (n=151) or 7th (n=152) day of progesterone administration. In that study, LBRs tended to be higher for the 5th day of progesterone ad-

Table 2. Comparison of embryological data and reproductive outcomes among patients undergoing day-6 blastocyst transfers on the 6th versus 7th day of progesterone exposure in HRT-FET cycles

Variable	Sixth day of progesterone administration (n = 1,212)	Seventh day of progesterone administration (n = 189)	p-value
Endometrial thickness at the day of progesterone initiation (mm)	8.95 ± 1.27	8.88 ± 1.18	0.492 ^{a)}
No. of blastocysts transferred			0.761
1	327 (27)	49 (25.9)	
2	885 (73)	140 (74.1)	
Blastocyst morphology of SET			0.716
Excellent	150 (45.9)	23 (46.9)	
Good	144 (44)	24 (49)	
Average	24 (7.3)	2 (4.1)	
Poor	9 (2.8)	0	
Blastocyst morphology of DET (best-quality embryos out of both)			0.004
Excellent	546 (61.7)	107 (76.4)	
Good	308 (34.8)	29 (20.7)	
Average	26 (2.9)	3 (2.1)	
Poor	5 (0.6)	1 (0.7)	
Blastocyst morphology of DET			> 0.999
Excellent and good	266 (30.1)	48 (34.3)	
Excellent and average	28 (3.2)	5 (3.6)	
Excellent and poor	2 (0.2)	0	
Good and average	99 (11.2)	7 (5)	
Good and poor	5 (0.6)	1 (0.7)	
Average and poor	3 (0.3)	1 (0.7)	
Both excellent	250 (28.2)	54 (38.6)	
Both good	204 (23.1)	21 (15)	
Both average	23 (2.6)	2 (1.4)	
Both poor	5 (0.6)	1 (0.7)	
β-hCG rate	880/1,212 (72.6)	125/189 (66.1)	0.066
Biochemical loss rate	26/880 (3.0)	6/125 (4.8)	0.271
Clinical pregnancy rate	854/1,212 (70.5)	119/189 (63)	0.037
Clinical miscarriage rate	136/854 (15.9)	16/119 (13.4)	0.485
Live birth rate	718/1,212 (59.2)	103/189 (54.5)	0.218
Multiple live birth rate	200/1,212 (16.5)	28/189 (14.8)	0.559

Values are presented as mean±standard deviation or number (%).

HRT-FET, hormone replacement treatment–frozen embryo transfer; SET, single embryo transfer; DET, double embryo transfer; β-hCG, β-human chorionic gonadotropin.

^{a)}Mann-Whitney, Morphology Grading: excellent (3AA,4AA,5AA), good (3,4,5,6 AB or BA), average (3,4,5,6 BB or AC or CA), poor (3,4,5,6 BC or CC).

ministration, although without statistical significance (31.1% vs. 25.7%; OR, 0.76; 95% CI, 0.46 to 1.26) [11].

The common practice is to treat day-6 vitrified blastocysts as day-5 and transfer on the 6th day after progesterone initiation (P+6) in HRT-FET cycles [4]. In theory, day-6 vitrified “delayed” blastocysts may seem to encounter a different and possibly narrower WOI than day-5 embryos [5]. To our knowledge, only one retrospective study has compared the reproductive outcomes between the 6th and 7th days of progesterone administration for day-6 vitrified warmed blastocyst transfer cycles employing the HRT-FET protocol [5]. This study report-

ed a slightly higher, though not statistically significant, LBR on the 7th day after progesterone initiation compared to the 6th day (35.5% vs. 21.5%, $p=0.06$); this trend toward a higher LBR was primarily due to a lower miscarriage rate when transferred on the 7th day of progesterone (21.4% vs. 50.0%, $p=0.02$). The limitations of this study include its retrospective design and the subgroup analysis. Our results show comparable LBRs in the P+6 and P+7 groups, in contrast with those reported by Roelens et al. [5]. The reasons for these divergent results may include differences in sample size and the route of progesterone administration (intramuscular in the current study versus

Table 3. Univariate comparison of baseline demographic features, embryological data, and reproductive outcomes between patients with and without live birth

Variable	Live birth		p-value
	No (n = 580)	Yes (n = 821)	
Female age at FET (yr)			0.002
≤ 30	241/654 (36.9)	413/654 (63.1)	
31–35	220/504 (43.7)	284/504 (56.3)	
> 35	119/243 (49.0)	124/243 (51.0)	
Female body mass index (kg/m ²)			0.444
< 19	37/85 (43.5)	48/85 (56.5)	
19.1–25	245/623 (39.3)	378/623 (60.7)	
25.1–30	205/465 (44.1)	260/465 (55.9)	
≥ 30	77/182 (42.3)	105/182 (57.7)	
Anti-Müllerian hormone (ng/mL)			0.130
≤ 1.5	162/353 (45.9)	191/353 (54.1)	
1.5–4.5	285/702 (40.6)	417/702 (59.4)	
≥ 4.5	125/323 (38.7)	198/323 (61.3)	
M-II rate (%)			< 0.001
≤ 50	98/190 (51.6)	92/190 (48.4)	
> 50	390/1,023 (38.1)	633/1,023 (61.9)	
Retrieval rate (%)			0.721
≤ 50	24/55 (43.6)	31/55 (56.4)	
> 50	386/937 (41.2)	551/937 (58.8)	
No. of patients with PGT-A	17/50 (34)	33/50 (66)	0.279
No. of blastocysts transferred			< 0.001
1	193/376 (51.3)	183/376 (48.7)	
2	387/1,025 (37.8)	638/1,025 (62.2)	
No. of previous IVF attempts			< 0.001
0	409/776 (52.7)	367/776 (47.3)	
1	72/409 (17.6)	337/409 (82.4)	
2	99/216 (45.8)	117/216 (54.2)	
Blastocyst morphology of SET			0.054
Excellent	77/173 (44.5)	96/173 (55.5)	
Good	95/168 (56.5)	73/168 (43.5)	
Average	14/26 (53.8)	12/26 (46.2)	
Poor	7/9 (77.8)	2/9 (22.2)	
Blastocyst morphology of DET (best-quality embryo out of both)			0.001
Excellent	236/653 (36.1)	417/653 (63.9)	
Good	127/337 (37.7)	210/337 (62.3)	
Average	19/29 (65.5)	10/29 (34.5)	
Poor	5/6 (83.3)	1/6 (16.7)	
Duration of progesterone administration (6th or 7th days)			0.218
Day 6	494/1,212 (40.8)	718/1,212 (59.2)	
Day 7	86/189 (45.5)	103/189 (54.5)	

Values are presented as number (%).

FET, frozen embryo transfer; M-II, metaphase-II; PGT-A, preimplantation genetic testing for aneuploidy; IVF, *in vitro* fertilization; SET, single embryo transfer; DET, double embryo transfer.

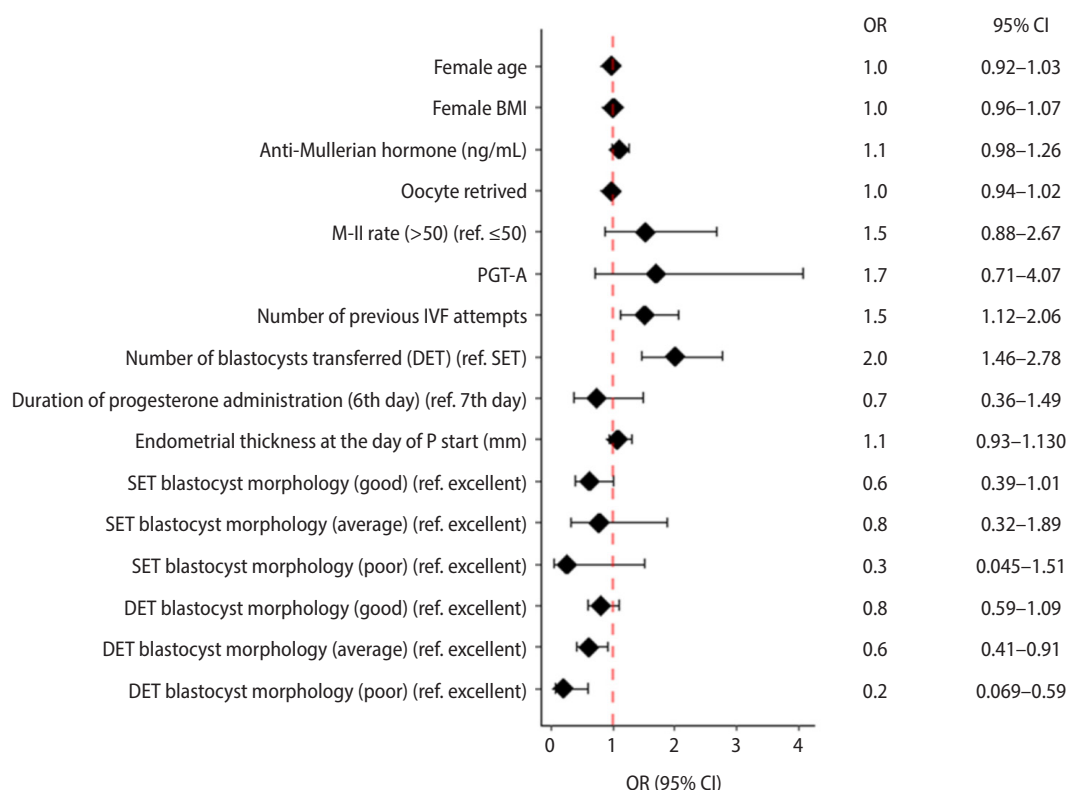


Figure 2. Multivariate logistic regression analysis to evaluate the independent effect of the duration of progesterone exposure (P+6 vs. P+7) following the transfer of day-6 blastocysts on live birth. The model included female age, body mass index (BMI), number of previous attempts, anti-Müllerian hormone levels, number of oocytes retrieved, metaphase-II (M-II) rate, preimplantation genetic testing for aneuploidy (PGT-A), number of blastocysts transferred, blastocyst morphology, duration of progesterone administration (P+6 or P+7), and endometrial thickness on the day of progesterone initiation as potential independent predictors and covariates. OR, odds ratio; CI, confidence interval; IVF, *in vitro* fertilization; DET, double embryo transfer; SET, single embryo transfer.

vaginal in the study by Roelens et al. [5]).

A recent European Society for Human Reproduction and Embryology (ESHRE) 2023 abstract compared the reproductive outcomes of 718 warmed blastocyst transfer cycles employing the HRT protocol according to whether FET was performed on the 5th or 6th day of progesterone administration [13]. Micronized vaginal progesterone was administered to all patients. The rates of overall biochemical pregnancy, implantation, clinical pregnancy, and early pregnancy loss were comparable between the two groups.

Despite using PSM, the limitations of the current study include its retrospective design and the small sample size of the P+7 group. The practice of performing double-warmed blastocyst transfer in the majority of the cycles could restrict the generalizability of the findings. Additionally, the high rate of loss to follow-up among patients before LB data became available from the initial cohort may have introduced selection bias.

In conclusion, we conclude that the LBRs are comparable for day-6 vitrified blastocysts when transferred on the 6th or 7th day after pro-

gesterone initiation. However, it is important to acknowledge that this study relies on retrospective data from a specific period and location. Further research, including prospective RCTs in various clinical settings, is necessary to validate and generalize these findings.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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