

Research Article

PHYSICIAN-SELECTED TIMING OF FROZEN BLASTOCYST TRANSFER AND CLINICAL PREGNANCY OUTCOMES: A RETROSPECTIVE COHORT STUDY

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ABSTRACT

Background: Programmed frozen blastocyst transfer requires coordination between progesterone-driven endometrial maturation and embryo development. More than one progesterone exposure window is used in routine practice, and the treating physician may select timing for an individual cycle. This study evaluated whether physician-selected timing was associated with ultrasound-confirmed clinical pregnancy.

Methods: This retrospective single-center cohort included 160 frozen embryo transfer cycles performed at the Georgian-German Reproduction Center during May 2026. Inclusion criteria were endometrial thickness at least 8 mm, serum progesterone below 1 ng/mL before progesterone initiation, and vaginal micronized progesterone 600 mg daily. Cycles using endometrial receptivity testing were excluded. The primary analysis excluded four Day-3 transfers performed before 120 hours, four mixed Day-5 and Day-6 transfers, and two blastocyst transfers performed after 136 hours, leaving 150 Day-5 or Day-6 transfers at 120-126 or 133-136 hours. Clinical pregnancy was defined as ultrasonographic visualization of at least one gestational sac. Fisher exact tests and multivariable logistic regression were used.

Results: Clinical pregnancy occurred in 42 of 80 transfers at 120-126 hours (52.5%) and 34 of 70 transfers at 133-136 hours (48.6%). The later window was not significantly associated with clinical pregnancy in unadjusted analysis (odds ratio 0.85, 95% confidence interval 0.45-1.62; P=0.744) or after adjustment for embryo day, recipient type, oocyte source, oocyte age, and body mass index (adjusted odds ratio 0.84, 95% confidence interval 0.43-1.63; P=0.606). No significant interaction was identified by recipient type, body mass index, or embryo day.

Conclusion: Within the two evaluated exposure windows and standardized programmed-cycle protocol, physician-selected timing was not significantly associated with clinical pregnancy. The findings support continued physician-directed selection of these timing options for appropriately selected cycles, but do not establish equivalence or superiority.

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Introduction

Programmed frozen embryo transfer (FET) uses exogenous estrogen to prepare the endometrium and progesterone to initiate secretory transformation. The duration of progesterone exposure is therefore a central determinant of embryo-endometrium synchronization. Standard protocols commonly schedule blastocyst transfer after approximately five to seven days of progesterone exposure. Still, the optimal interval remains uncertain, and published studies use different definitions of progesterone day and transfer timing.¹⁻³

Evidence comparing adjacent progesterone durations is inconsistent. Some cohort studies have reported comparable outcomes after six or seven days of progesterone exposure,^{3,7} whereas others have identified differences overall or within Day-5, Day-6, or morphological subgroups.^{4-6,8,9} A recent randomized trial of Day-6 blastocysts found comparable overall live birth rates after six or seven days, with an exploratory interaction according to blastocyst expansion stage.¹⁰ These data suggest that the effective implantation window may be sufficiently broad for many cycles but may not be identical across embryo and recipient characteristics.

Most studies compare predefined protocols. In routine care, however, a physician may select among locally accepted timing windows for an individual cycle. This creates a clinically distinct question: whether physician-selected timing, as practiced under a standardized programmed-cycle regimen, is associated with clinical pregnancy. We compared ultrasound-confirmed clinical pregnancy after transfer at 120-126 hours and 133-136 hours of progesterone exposure. We explored whether the association differed by recipient type, body mass index (BMI), embryo developmental day, oocyte source, preimplantation genetic testing status, number of embryos transferred, or blastocyst morphology.

Materials and methods

Study design and setting

This retrospective observational cohort included all eligible embryo-transfer cycles performed at the Georgian-German Reproduction Center (GGRC), Tbilisi, Georgia, during May 2026. The embryo-transfer cycle was the unit of analysis. The treating physician selected Transfer timing during routine clinical care and was not randomized. Accordingly, all effect estimates are interpreted as associations.

Eligibility and programmed-cycle preparation

Eligible cycles used hormone replacement therapy for endometrial preparation. Inclusion criteria were endometrial thickness at least 8 mm, serum progesterone below 1 ng/mL before exogenous progesterone initiation, and vaginal micronized progesterone 600 mg daily, administered as 200 mg three times daily. Cycles using endometrial receptivity testing, including ERA or EndomeTRIO, were excluded. Additional intramuscular progesterone was used in some cycles at physician discretion, but a complete cycle-level record of dose and route was unavailable and therefore could not be analyzed.

Exposure definition and analytical cohort

The exposure was elapsed time from progesterone initiation to embryo transfer, recorded in clinical scheduling categories. The primary comparison was 120-126 hours versus 133-136

hours. The source cohort contained 160 eligible cycles. Four Day-3 cleavage-stage transfers performed before 120 hours were excluded because their developmental stage was not comparable with blastocyst transfer. Four transfers containing both Day-5 and Day-6 blastocysts were excluded to preserve a single embryo-developmental-day classification. Two Day-5 or Day-6 transfers performed after 136 hours were excluded because the group was too small for inference. The primary analytical cohort therefore contained 150 single-stage Day-5 or Day-6 blastocyst transfers.

160 eligible FET cycles performed during May 2026
Excluded from the primary comparison: 4 Day-3 transfers before 120 hours; 4 mixed Day-5 and Day-6 transfers; 2 Day-5 or Day-6 transfers after 136 hours
150 Day-5 or Day-6 blastocyst transfers in the primary analytical cohort
80 transfers at 120-126 hours
70 transfers at 133-136 hours

Figure 1. Cohort flow.

Outcome definition

The primary outcome was clinical pregnancy per embryo-transfer cycle. Clinical pregnancy was defined as ultrasonographic visualization of at least one gestational sac, consistent with international terminology.¹¹ Clinical-pregnancy status was available for all cycles. Cycles with negative serum beta-human chorionic gonadotropin were classified as not achieving clinical pregnancy, whereas ultrasound confirmation was required after a positive biochemical pregnancy test.

Recipient and embryo characteristics

Recipient type referred to the person undergoing embryo transfer. A positive surrogate-mother field indicated a gestational carrier, and a negative field indicated the patient or intended mother. BMI was the BMI of the actual embryo recipient and was grouped as less than 18.5, 18.5-24.9, 25.0-29.9, or at least 30 kg/m2. In regression models, BMI was represented as less than 25, 25.0-29.9, and at least 30 kg/m2.

Oocyte source was classified as own or donor. Oocyte age was the intended mother’s age at retrieval in own-oocyte cycles and the donor’s age at retrieval in donor-oocyte cycles. Other recorded characteristics were embryo developmental day, preimplantation genetic testing result, number of embryos transferred, and blastocyst morphology. Morphological descriptions were parsed using expansion stage, inner cell mass, and trophectoderm grades.^{1,2} Because only three primary-cohort transfers were below a 3BB threshold, morphology was analyzed exploratorily as the presence of at least one AA blastocyst versus non-AA morphology.

Statistical analysis

Categorical data are reported as n/N (%) and continuous data as median (IQR). Fisher’s exact tests were used for 2-by-2 comparisons. Sparse 2-by-k tables were evaluated using Fisher-Freeman-Halton tests with Monte Carlo estimation. Oocyte-age distributions were compared using the two-sided Mann-Whitney U test. Odds ratios (ORs) and 95% confidence intervals (CIs)

were estimated comparing 133-136 hours with 120-126 hours. The absolute risk difference was calculated as the later-window rate minus the earlier-window rate with a Newcombe 95% CI. Two-sided P values below 0.05 were considered statistically significant.

Prespecified subgroup analyses evaluated recipient type, BMI category, embryo developmental day, oocyte source, preimplantation genetic testing status, number of embryos transferred, and AA morphology. Timing interactions with recipient type, BMI, embryo day, oocyte source, and AA morphology were tested in separate logistic models. These subgroup analyses were exploratory and were not adjusted for multiple comparisons.

The primary multivariable logistic regression model included transfer timing, embryo developmental day, recipient type, oocyte source, oocyte age, and recipient BMI. An expanded sensitivity model additionally included normal preimplantation genetic testing status, transfer of two embryos, and AA morphology. Physician was not included in the regression model because timing had minimal overlap across physicians, resulting in near-collinearity between physician and timing. Statistical analyses were conducted using Python, SciPy, and statsmodels.

No a priori sample-size calculation was performed because all eligible cycles during the defined study period were included.

Exploratory second-transfer sequence analysis

A supplemental sequence dataset identified 32 cycles recorded as second transfers and included the progesterone-exposure window used for the preceding transfer. To align this analysis with the primary cohort definition, two Day-3 transfers and one mixed Day-5 and Day-6 transfer were excluded, leaving 29 Day-5 or Day-6 second-transfer cycles within the 120-126-hour or 133-136-hour windows. Cycles were classified as either retaining the progesterone-exposure window from the first transfer or changing it. Clinical pregnancy was compared between the current second-transfer window and retained versus changed timing using Fisher’s exact tests. Sequence-specific rates were considered descriptive because only five cycles involved a change in timing.

Ethics and data governance

This study used retrospective data generated during routine clinical care. Direct patient identifiers were excluded from analytical and publication outputs. GGRC did not have an institutional ethics committee, and no formal ethics approval, exemption, or waiver was issued. No additional intervention or patient contact occurred for research purposes.

Results

Cohort and baseline characteristics

The full cohort contained 160 cycles, of which 83 (51.9%) resulted in clinical pregnancy. After the 10 exclusions shown in Figure 1, the primary analytical cohort comprised 150 transfers and 76 clinical pregnancies (50.7%). The 120-126-hour group included 80 transfers and the 133-136-hour group included 70 transfers.

Recipient type, embryo developmental day, BMI distribution, preimplantation genetic testing status, number of embryos transferred, and AA morphology were similar between timing groups. The later-window group contained a higher proportion of own-oocyte cycles and had a higher median oocyte age (Table 1).

Table 1. Baseline characteristics of the primary analytical cohort.

Characteristic	120-126 hours (n=80)	133-136 hours (n=70)	P value
Embryo day 5	52/80 (65.0%)	48/70 (68.6%)	0.729
Embryo day 6	28/80 (35.0%)	22/70 (31.4%)	
Patient or intended mother	19/80 (23.8%)	21/70 (30.0%)	0.460
Gestational carrier	61/80 (76.2%)	49/70 (70.0%)	
Donor oocytes	63/80 (78.8%)	44/70 (62.9%)	0.046
Own oocytes	17/80 (21.2%)	26/70 (37.1%)	
Oocyte age, median (IQR)	25.0 (22.8-29.0)	27.0 (23.0-32.8)	0.039
BMI less than 18.5 kg/m2	4/80 (5.0%)	3/70 (4.3%)	0.966
BMI 18.5-24.9 kg/m2	33/80 (41.2%)	31/70 (44.3%)	
BMI 25.0-29.9 kg/m2	27/80 (33.8%)	24/70 (34.3%)	
BMI at least 30 kg/m2	16/80 (20.0%)	12/70 (17.1%)	
Normal PGT result	71/80 (88.8%)	59/70 (84.3%)	0.476
Two embryos transferred	7/80 (8.8%)	4/70 (5.7%)	0.544
At least one AA blastocyst	46/80 (57.5%)	46/70 (65.7%)	0.319

BMI, body mass index; IQR, interquartile range; PGT, preimplantation genetic testing. P-values compare distributions across timing groups.

Primary timing comparison

Clinical pregnancy occurred in 42 of 80 transfers at 120-126 hours (52.5%) and 34 of 70 transfers at 133-136 hours (48.6%). The absolute difference between the later and earlier window was -3.9 percentage points (95% CI, -19.4 to 11.8). The unadjusted timing association was not statistically significant (OR 0.85, 95% CI 0.45-1.62; P=0.744).

Table 2. Clinical pregnancy by progesterone exposure window.

Timing window	Transfers	Clinical pregnancies	Clinical pregnancy rate	OR (95% CI)	P value
120-126 hours	80	42	52.5%	Reference	
133-136 hours	70	34	48.6%	0.85 (0.45-1.62)	0.744

CI, confidence interval; OR, odds ratio. The OR compares 133-136 hours with 120-126 hours.

Recipient type and timing

Among patients or intended mothers, clinical pregnancy occurred in 12/19 (63.2%) transfers at 120-126 hours and 9/21 (42.9%) at 133-136 hours (OR 0.44, 95% CI 0.12-1.56; P=0.225). Among gestational carriers, corresponding rates were 30/61 (49.2%) and 25/49 (51.0%) (OR 1.08, 95% CI 0.51-2.28; P=1.000). The timing-by-recipient interaction was not statistically significant (P=0.132).

Recipient type was not significantly associated with clinical pregnancy within either timing window (P=0.308 at 120-126 hours and P=0.607 at 133-136 hours).

Table 3. Clinical pregnancy by recipient type and progesterone exposure window.

Recipient type	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Patient or intended mother	12/19 (63.2%)	9/21 (42.9%)	0.44 (0.12-1.56)	0.225
Gestational carrier	30/61 (49.2%)	25/49 (51.0%)	1.08 (0.51-2.28)	1.000

Values are clinical pregnancies/transfers (%). Timing-by-recipient interaction P=0.132.

Recipient BMI and timing

Clinical pregnancy rates varied across BMI cells, but BMI was not significantly associated with outcome within the 120-126-hour window (P=0.519) or the 133-136-hour window (P=0.439). The timing-by-BMI interaction was not statistically significant (P=0.180). The underweight subgroup and several patient-specific BMI cells were sparse, limiting precision.

Table 4. Clinical pregnancy by recipient BMI and progesterone exposure window.

Recipient type	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Less than 18.5	2/4 (50.0%)	1/3 (33.3%)	0.50 (0.02-11.09)	1.000
18.5-24.9	16/33 (48.5%)	13/31 (41.9%)	0.77 (0.29-2.06)	0.625
25.0-29.9	13/27 (48.1%)	15/24 (62.5%)	1.79 (0.59-5.50)	0.400
At least 30	11/16 (68.8%)	5/12 (41.7%)	0.32 (0.07-1.55)	0.250

Values are clinical pregnancies/transfers (%). Timing-by-BMI interaction P=0.180.

Embryo developmental day

For Day-5 blastocysts, clinical pregnancy occurred in 29/52 (55.8%) transfers at 120-126 hours and 24/48 (50.0%) at 133-136 hours. For Day-6 blastocysts, corresponding rates were 13/28 (46.4%) and 10/22 (45.5%). Neither subgroup comparison nor the timing-by-embryo-day interaction was significant (interaction P=0.729).

Table 5. Clinical pregnancy by embryo developmental day and progesterone exposure window.

Embryo day	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Day 5	29/52 (55.8%)	24/48 (50.0%)	0.79 (0.36-1.74)	0.689
Day 6	13/28 (46.4%)	10/22 (45.5%)	0.96 (0.31-2.95)	1.000

Values are clinical pregnancies/transfers (%). Timing-by-embryo-day interaction P=0.729.

Oocyte source, genetic testing, embryo number, and morphology

No statistically significant timing difference was identified within donor-oocyte, own-oocyte, normal-PGT, non-normal or untested, single-embryo, double-embryo, AA-morphology, or non-AA-morphology subgroups (Table 6). Timing of interactions with the oocyte source and AA morphology was also not significant. The non-normal or untested, two-embryo, and below-3BB groups were small and are interpreted descriptively.

Table 6. Clinical pregnancy by selected cycle characteristics.

Characteristic	120-126 hours	133-136 hours	Late vs early OR (95% CI)	P value
Donor oocytes	31/63 (49.2%)	23/44 (52.3%)	1.13 (0.52-2.44)	0.845
Own oocytes	11/17 (64.7%)	11/26 (42.3%)	0.40 (0.11-1.41)	0.215
Normal PGT	40/71 (56.3%)	29/59 (49.2%)	0.75 (0.37-1.50)	0.481
No normal PGT result	2/9 (22.2%)	5/11 (45.5%)	2.92 (0.41-20.90)	0.374
One embryo	40/73 (54.8%)	33/66 (50.0%)	0.82 (0.42-1.61)	0.612
Two embryos	2/7 (28.6%)	1/4 (25.0%)	0.83 (0.05-13.63)	1.000
At least one AA blastocyst	21/46 (45.7%)	22/46 (47.8%)	1.09 (0.48-2.48)	1.000
Non-AA morphology	21/34 (61.8%)	12/24 (50.0%)	0.62 (0.21-1.78)	0.427

Values are clinical pregnancies/transfers (%). Timing-by-oocyte-source interaction P=0.177; timing-by-AA-morphology interaction P=0.967. PGT, preimplantation genetic testing.

Multivariable analyses

After adjustment for embryo developmental day, recipient type, oocyte source, oocyte age, and BMI, transfer at 133-136 hours was not significantly associated with clinical pregnancy (adjusted OR 0.84, 95% CI 0.43-1.63; P=0.606). None of the prespecified covariates were statistically significant in the primary model (Table 7).

The expanded model additionally adjusted for normal PGT status, transfer of two embryos, and AA morphology. The timing estimate remained materially unchanged (adjusted OR 0.85, 95% CI 0.43-1.68; P=0.648).

Table 7. Primary multivariable logistic regression model for clinical pregnancy.

Variable	Adjusted OR	95% CI	P value
133-136 vs 120-126 hours	0.84	0.43-1.63	0.606
Day 6 vs Day 5	0.77	0.39-1.54	0.463
Gestational carrier vs patient	0.88	0.39-1.97	0.755
Own vs donor oocytes	0.94	0.30-2.96	0.919
Oocyte age, per year	1.01	0.93-1.09	0.843
BMI 25.0-29.9 vs less than 25	1.48	0.71-3.09	0.291
BMI at least 30 vs less than 25	1.56	0.64-3.81	0.326

BMI, body mass index; CI, confidence interval; OR, odds ratio. Reference categories were 120-126 hours, Day 5, patient or intended mother, donor oocytes, and BMI <25 kg/m2.

Physician selection of transfer timing

Transfer timing was strongly associated with physician practice. One anonymized physician performed 63 of 70 later-window transfers (90.0%) but only 4 of 80 earlier-window transfers (5.0%). The remaining physicians performed 7 later-window and 76 earlier-window transfers (Fisher exact P<0.001). Because timing and physician were nearly separated, a physician-adjusted model would be unstable and would not reliably distinguish timing effects from physician-specific case selection or practice patterns. Physician-specific pregnancy rates were therefore not reported.

Exploratory second-transfer sequence analysis

Among 29 eligible second-transfer cycles, the progesterone-exposure window used for the first transfer was retained in 24 cycles (82.8%) and changed in 5 cycles (17.2%). Clinical pregnancy occurred in 8 of 24 cycles (33.3%) when timing was unchanged and in 2 of 5 cycles (40.0%) when timing was changed (OR 1.33, 95% CI 0.18-9.66; Fisher exact P=1.000). When second transfers were classified according to their current progesterone-exposure window, clinical pregnancy occurred in 5 of 14 cycles (35.7%) at 120-126 hours and in 5 of 15 cycles (33.3%) at 133-136 hours (OR 0.90, 95% CI 0.19-4.17; Fisher exact P=1.000).

Table 8. Clinical pregnancy according to first- and second-transfer progesterone exposure windows.

First-transfer timing	Second-transfer timing	Cycles, n	Clinical pregnancy, n/N (%)
120-126 hours	120-126 hours	12	3/12 (25.0%)
120-126 hours	133-136 hours	3	0/3 (0%)
133-136 hours	120-126 hours	2	2/2 (100.0%)
133-136 hours	133-136 hours	12	5/12 (41.7%)

Values are ultrasound-confirmed clinical pregnancies per second-transfer cycle. The changed-timing sequences contained only three and two cycles and are presented descriptively; they should not be interpreted as evidence that changing timing improves outcome.

Discussion

Principal findings

In this real-world cohort of programmed frozen blastocyst transfers, clinical pregnancy occurred in 52.5% of transfers at 120-126 hours and 48.6% at 133-136 hours. The absolute difference was -3.9 percentage points, and neither the unadjusted nor adjusted comparison was statistically significant. The confidence interval remained wide and compatible with a clinically meaningful difference in either direction, so the findings do not establish equivalence.

The association between timing and clinical pregnancy did not differ significantly by recipient type, BMI, or embryo developmental day. Patient cycles showed a numerical reduction in clinical pregnancy in the later window. In contrast, gestational-carrier outcomes were similar across timing groups, but the timing-by-recipient interaction was not significant. BMI-specific rates varied across small cells without a consistent gradient, and no timing-by-BMI interaction was detected. Day-5 and Day-6 blastocysts also showed similar timing patterns.

Timing estimates remained stable after multivariable adjustment and after adding PGT status, number of embryos transferred, and AA morphology. The principal methodological feature was the strong relationship between the physician and the timing of selection. This reflects the clinical question under study and physician-selected timing, but also prevents separating a biological timing effect from physician-specific selection and workflow.

Relation to existing evidence

The published literature is heterogeneous because progesterone day is defined differently across studies and comparisons range from several hours to a full day. Roelens et al. reported similar live birth rates after six or seven days of progesterone exposure in programmed cycles.³ In contrast, a propensity score-matched cohort by Yang et al. favored six days overall, particu-

larly in younger patients and selected embryo subgroups.⁴ Liu et al. found no significant difference in clinical outcomes across progesterone durations in artificial cycles.⁵

More recent studies have continued to produce mixed results. Zhou et al. reported comparable overall live birth rates after six or seven days, but a higher live birth rate with seven days among Day-6 blastocysts.⁶ Large Day-6 blastocyst cohorts have reported either comparable outcomes after six or seven days⁷ or higher pregnancy and live birth rates with six rather than five days.⁹ In an oocyte-donation cohort, extending vaginal progesterone from five to six days did not improve outcomes.⁸ A 2026 randomized trial found comparable overall live birth rates for Day-6 blastocysts after six or seven days, although exploratory analyses suggested that expansion stage may modify the effect.¹⁰

The present study differs from these protocol comparisons by evaluating two narrower timing categories selected by physicians in routine practice and including both Day-5 and Day-6 blastocysts. Its findings, therefore, inform the use of physician judgment within the specific standardized regimen studied rather than defining a universally optimal duration of progesterone.

Clinical interpretation

Within this clinic protocol, physician selection between 120-126 and 133-136 hours was not associated with a statistically detectable difference in clinical pregnancy. This supports continued physician-directed use of either window for appropriately selected cycles when endometrial preparation, baseline progesterone, and vaginal progesterone administration are standardized. The result should not be interpreted as evidence that timing is arbitrary or that all progesterone durations are interchangeable.

The exploratory second-transfer analysis should be interpreted as descriptive rather than as a basis for changing clinical practice. Physicians retained the previously used timing window in most second-transfer cycles, and no clear clinical-pregnancy difference was observed when timing was retained versus when it was changed. Because only five cycles involved a change in timing, the data do not support recommending either routine retention or routine modification of the previous window.

The patient and gestational-carrier subgroup findings should not be used to prescribe different windows because interaction testing was negative and subgroup confidence intervals were wide. Similarly, the BMI and embryo-day analyses did not identify a reproducible modifier. The study does not address substantially earlier or later transfers, different progesterone routes or doses, natural-cycle FET, ongoing pregnancy, or live birth.

Strengths and limitations

Strengths include inclusion of all eligible cycles during a defined period, explicit accounting for the transition from 160 source cycles to 150 primary-analysis transfers, complete clinical-pregnancy ascertainment, standardized endometrial thickness and baseline progesterone criteria, a uniform vaginal progesterone dose, detailed analyses by recipient type, BMI, embryo day, oocyte source, genetic testing, embryo number, and morphology, and a linked exploratory analysis of first-to-second transfer timing sequences. Clinical pregnancy was defined using internationally standardized terminology.¹¹

The study is limited by its retrospective, non-randomized design, single-center setting, one-month study period, and modest sample size. Timing was highly concentrated among a single physician, creating residual confounding and preventing reliable physician adjustment. Sub-

group cells were sparse, particularly for patients at BMI extremes, transfers of two embryos, non-normal PGT categories, and blastocysts below 3BB. The second-transfer sequence analysis included only 29 eligible cycles, and only five involved a change in timing, resulting in highly imprecise sequence-specific estimates. Oocyte age differed between timing groups and represented donor age in donor cycles and intended-mother age in own-oocyte cycles. Additional intramuscular progesterone use was incompletely recorded. Clinical pregnancy is more clinically meaningful than beta-hCG positivity, but ongoing pregnancy and live birth were unavailable. Finally, no formal ethics approval, exemption, or waiver was issued, which may affect journal eligibility.

Conclusion

Among single-stage Day-5 and Day-6 blastocyst transfers performed after 120-126 or 133-136 hours of progesterone exposure, physician-selected timing was not significantly associated with ultrasound-confirmed clinical pregnancy. No statistically significant modification was identified by recipient type, BMI, embryo developmental day, oocyte source, or AA morphology. The findings support physician-directed selection between the two studied windows within the standardized programmed FET protocol. In contrast, strong physician-linked timing selection and wide confidence intervals preclude conclusions regarding equivalence, superiority, or a universally optimal transfer time.

Declarations

Author contributions

Mariam Tvauri: Conceptualization, methodology, investigation, data curation, formal analysis, project administration, visualization, interpretation, and writing of the original draft. Tea Charkviani: Methodology, validation, clinical interpretation, supervision, and writing-review and editing. Tamara Nadirashvili, Oliko Murgulia, Ivane Kutivadze, Tamar Magularia, Mery Natroshvili, and Ana Aivazova: Investigation, clinical data acquisition, source-data validation, clinical interpretation, and writing-review and editing. Nino Museridze: Resources, clinical oversight, supervision, interpretation, and writing-review and editing. All authors reviewed and approved the final manuscript and agree to be accountable for the integrity of the work.

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Conflict of interest: The authors declare no competing interests.

Data availability: The source data, including the supplemental second-transfer sequence dataset, are not publicly available because they derive from clinical records. A de-identified analytical dataset may be considered upon reasonable request to the corresponding author, subject to institutional authorization and applicable privacy requirements.

References:

1. Mumusoglu S, Polat M, Ozbek IY, et al. Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol (Lausanne)*. 2021;12:688237. doi:10.3389/fendo.2021.688237.
2. van de Vijver A, Polyzos NP, Van Landuyt L, et al. What is the optimal duration of progesterone administration before transferring a vitrified-warmed cleavage stage embryo? A

- randomized controlled trial. *Hum Reprod.* 2016;31(5):1097-1104. doi:10.1093/humrep/dew045.
3. Roelens C, Santos-Ribeiro S, Becu L, et al. Frozen-warmed blastocyst transfer after 6 or 7 days of progesterone administration: impact on live birth rate in hormone replacement therapy cycles. *Fertil Steril.* 2020;114(1):125-132. doi:10.1016/j.fertnstert.2020.03.017.
4. Yang X, Bu Z, Hu L. Live birth rate of frozen-thawed single blastocyst transfer after 6 or 7 days of progesterone administration in hormone replacement therapy cycles: a propensity score-matched cohort study. *Front Endocrinol (Lausanne).* 2021;12:706427. doi:10.3389/fendo.2021.706427.
5. Liu L, Zhou H, Hu J, Sun X, Liu D, Huang G. Association between duration of progesterone supplementation and clinical outcomes in artificial frozen-thawed embryo transfer cycles. *Front Endocrinol (Lausanne).* 2023;14:1193826. doi:10.3389/fendo.2023.1193826.
6. Zhou R, Dong M, Wang Z, et al. Impact of different progesterone timings on live birth rates for blastocyst frozen embryo transfer cycles. *Reprod Biomed Online.* 2024;49(4):104307. doi:10.1016/j.rbmo.2024.104307.
7. Bhoi N, Yarali H, Murdia K, et al. 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. *Clin Exp Reprod Med.* 2025;52(2):125-133. doi:10.5653/term.2023.06527.
8. Blazquez A, Miguel-Escalada I, Portas-Gómez L, et al. Prolonged vaginal progesterone supplementation does not improve outcomes for Day 6 frozen blastocyst transfers in oocyte donation cycles. *Hum Reprod.* 2026;41(1):86-92. doi:10.1093/humrep/deaf205.
9. Cai H, Wang Z, Fang Y, et al. Duration of progesterone exposure before frozen embryo transfer impacts live birth rates following single vitrified-thawed Day 6 blastocyst transfer: a multicenter cohort study. *Contracept Reprod Med.* 2026;11:14. doi:10.1186/s40834-026-00425-3.
10. Zhou R, Wang Z, Dong M, et al. Effect of progesterone timing on live birth rates in day-6 blastocyst frozen-thawed embryo transfer cycles: a randomized controlled trial. *Hum Reprod Open.* 2026;2026(2):hoag023. doi:10.1093/hropen/hoag023.
11. Zegers-Hochschild F, Dyer S, Adamson GD, et al. The International Glossary on Infertility and Fertility Care, 2025. *Hum Reprod.* 2026;41(6):892-909. doi:10.1093/humrep/deag029.
12. Coticchio G, Ahlstrom A, Arroyo G, et al. The Istanbul consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment. *Hum Reprod.* 2025;40(6):989-1035. doi:10.1093/humrep/deaf021.
13. Doyle N, Jahandideh S, Hill MJ, Widra EA, Levy M, Devine K. Effect of timing by endometrial receptivity testing vs standard timing of frozen embryo transfer on live birth in patients undergoing in vitro fertilization: a randomized clinical trial. *JAMA.* 2022;328(21):2117-2125. doi:10.1001/jama.2022.20438.

Supplementary analyses

Supplementary Table S1 presents BMI-specific clinical pregnancy rates separately for patients or intended mothers and gestational carriers. These cells are descriptive because several denominators are small.

Supplementary Table S1. Clinical pregnancy by recipient type, BMI, and progesterone exposure window.

Recipient type	Timing, hours	BMI, kg/m2	Clinical pregnancy
Patient	120-126	Less than 18.5	1/2 (50.0%)
Patient	120-126	18.5-24.9	6/10 (60.0%)
Patient	120-126	25.0-29.9	3/5 (60.0%)
Patient	120-126	At least 30	2/2 (100.0%)
Patient	133-136	Less than 18.5	0/0 (-)
Patient	133-136	18.5-24.9	1/7 (14.3%)
Patient	133-136	25.0-29.9	5/9 (55.6%)
Patient	133-136	At least 30	3/5 (60.0%)
Gestational carrier	120-126	Less than 18.5	1/2 (50.0%)
Gestational carrier	120-126	18.5-24.9	10/23 (43.5%)
Gestational carrier	120-126	25.0-29.9	10/22 (45.5%)
Gestational carrier	120-126	At least 30	9/14 (64.3%)
Gestational carrier	133-136	Less than 18.5	1/3 (33.3%)
Gestational carrier	133-136	18.5-24.9	12/24 (50.0%)
Gestational carrier	133-136	25.0-29.9	10/15 (66.7%)
Gestational carrier	133-136	At least 30	2/7 (28.6%)

Values are clinical pregnancies/transfers (%). Exact omnibus BMI-outcome P values were 0.905 and 0.277 in patient cycles at 120-126 and 133-136 hours, respectively, and 0.684 and 0.389 in gestational-carrier cycles.