RESEARCH PAPER

A novel personalized sequential embryo transfer strategy for recurrent implantation failure: clinical outcomes from dual-stage timing

Rajyalakshmi Adusumilli[1,2], Navatha Pendyala[1,3], Pasupuleti Visweswara Rao[1,4]*

[1]Department of Biotechnology, School of Applied Sciences, REVA University, Rukhmini Knowledge Park, Bangalore- 560064, India
[2]Chief Embryologist, Ferticare IVF Asist, Banjara Hills, Hyderabad - 500034, Telangana, India
Mail id: raji.ivf@gmail.com
[3]Senior Embryologist, Dr Padmaja Fertility Center, Habsiguda, Hyderabad - 500007, Telangana, India
Mail id: navatha.pentyala@gmail.com
[4]Department of Biotechnology, School of Applied, REVA Research Centre, REVA University, Rukhmini Knowledge Park, Bangalore- 560064, India

***Corresponding author: Prof Dr Pasupuleti Visweswara Rao**
Department of Biotechnology, School of Applied Sciences & REVA Research Centre, REVA University, Rukhmini Knowledge Park, Bangalore- 560064, India
Mail id: visweswararao@reva.edu.in

Abstract
Background: Recurrent implantation failure (RIF) remains a major barrier to success in frozen embryo transfer (FET) programs, even when embryo morphology and chromosomal status appear favourable. Personalized sequential embryo transfer (SET), combining cleavage-stage and blastocyst transfer within the same programmed cycle, may improve outcomes by addressing subtle embryo–endometrial timing mismatch.
Methods: This prospective randomized trial enrolled 160 women with RIF and allocated them 1:1 to SET (n=80) or standard single blastocyst transfer (SBT; n=80). Analyses followed the intention-to-treat principle, with complete follow-up and all participants receiving the assigned intervention. Baseline characteristics were comparable between groups, including age (35.6±3.8 vs 35.9±4.1 years), BMI (24.8±3.2 vs 25.1±3.5 kg/m²), infertility duration (6.2±2.9 vs 6.5±3.1 years), and PGT-A use (40.0% vs 42.5%). Endometrial preparation was similar, with thickness ≥8 mm in 75.0% vs 72.5% and trilaminar pattern in 95.0% vs 92.5% (SET vs SBT).
Results: SET improved pregnancy outcomes compared with SBT. Biochemical pregnancy occurred in 55.0% vs 40.0% (RR 1.38, 95% CI 1.01–1.90; p=0.044), clinical pregnancy in 50.0% vs 35.0% (RR 1.43, 95% CI 1.01–2.02; p=0.041), ongoing pregnancy in 45.0% vs 30.0% (RR 1.50, 95% CI 1.01–2.23; p=0.039), and live birth in 42.5% vs 27.5% (RR 1.55, 95% CI 1.02–2.35; p=0.036). Absolute improvement was ~15% (NNT=7). Safety outcomes were comparable, with no serious adverse events and similar miscarriage and ectopic pregnancy rates.
Conclusion: In women with RIF, personalized SET increased live birth and pregnancy rates by ~15% versus SBT without added safety concerns, supporting SET as a pragmatic option in difficult RIF cases.

Keywords: Recurrent implantation failure; Sequential embryo transfer; Single blastocyst transfer; Frozen embryo transfer; Live birth; PGT-A
How to cite this article: Adusumilli R, Pendyala N, Rao PV. A Novel Personalized Sequential Embryo Transfer Strategy for Recurrent Implantation Failure: Clinical Outcomes from Dual-Stage Timing. Int J Drug Deliv Technol. 2026;16(22s): 973-984. DOI: 10.25258/ijddt.16.22s.116

Introduction

Recurrent implantation failure (RIF) remains one of the most frustrating challenges in assisted reproductive technology (ART). Despite advances in ovarian stimulation, embryo culture, and endometrial preparation, a significant subset of patients experience repeated negative outcomes even after transfer of morphologically good-quality embryos. Clinically, RIF has been variably defined, but in most contemporary studies it refers to failure to achieve a clinical pregnancy after multiple embryo transfers commonly three or more despite transferring good-quality embryos in each cycle (Lu, 2025 Such cases account for roughly 8–15% of ART cycles, highlighting both the clinical importance and biological complexity of implantation failure (Gao et al., 2023). Implantation is a coordinated event requiring competent embryos, receptive endometrium, and precise temporal alignment between the two. Even when chromosomally normal embryos are transferred, endometrial-embryo asynchrony can substantially reduce the probability of successful implantation. Similarly, meta-analyses of RIF pregnancies show that overall clinical pregnancy and live birth rates remain significantly lower compared with general ART populations, underscoring that repeated failures are not random events but reflect underlying pathophysiological mechanisms that are poorly understood and often multifactorial (Cimadomo et al., 2023). A pivotal variable in implantation success is embryo developmental stage at transfer. Traditionally, cleavage-

*Author for Correspondence: visweswararao@reva.edu.in

stage (Day-3) transfer places embryos into the uterine environment earlier, potentially allowing them to encounter a receptive endometrium under natural timing. Blastocyst (Day-5) transfer, by contrast, allows embryos to demonstrate developmental viability before transfer, often enriching for more competent embryos and aligning closer with the presumed in vivo implantation window (Franasiak & Scott, 2021). Indeed, aggregated data indicate that blastocyst transfer is associated with higher implantation and clinical pregnancy rates in RIF populations compared to cleavage-stage transfer alone, although the magnitude of benefit varies by patient characteristics and cycle type (He et al., 2025). Despite potential advantages associated with single-stage transfers, they may be insufficient for patients whose window of implantation is shifted or unpredictable, as is increasingly recognized in personalized reproductive medicine. To address this challenge, sequential embryo transfer has been proposed as a strategy to expand the opportunity for embryo–endometrium synchrony. This approach involves transferring a cleavage-stage embryo on Day 3 followed by a blastocyst on Day 5 in the same cycle, theoretically increasing the likelihood that at least one embryo encounters a receptive endometrium within the dynamic implantation window (Arefi et al., 2022). Several clinical studies support the potential benefit of sequential strategies for RIF patients. Randomized and prospective comparative research suggests that sequential transfer can improve implantation, clinical pregnancy, and ongoing pregnancy rates compared with double blastocyst transfer or conventional single-stage protocols (Salehpour et al., 2023), and retrospective cohorts report significantly higher clinical pregnancy and live birth outcomes with sequential protocols (Shalma et al., 2025). These effects are attributed to enhanced temporal coverage of the implantation window, which may better accommodate individual variations in endometrial receptivity that are not easily detected by static tests. However, not all studies have demonstrated consistent superiority of sequential transfer, and differences in patient selection, embryo quality, and preparation protocols contribute to heterogeneous findings (Torky et al., 2021). This variation underscores an important consideration: sequential transfer should be understood not as a universal solution but as a personalized approach aimed at optimizing timing and synchrony for patients with documented RIF. Moreover, personalized strategies that consider individual reproductive histories, endometrial markers, and ovarian response may improve the clinical utility and cost-effectiveness of sequential protocols in practice. Taken together, these insights suggest that a tailored sequential embryo transfer paradigm specifically integrating Day-3 cleavage and Day-5 blastocyst transfer may enhance implantation opportunities in RIF patients by increasing the overlap between embryo presence and endometrial receptivity. The present investigation therefore evaluates this novel timing strategy in a clinical cohort, with an aim to refine ART transfer practices and contribute evidence toward individualized reproductive care.

Methodology

Study design

This study was conducted as a prospective, randomized, controlled, assessor-blinded clinical trial designed to evaluate whether sequential embryo transfer (SET) improves reproductive outcomes in women with recurrent implantation failure (RIF). Participants were randomly assigned in a 1:1 ratio to either the sequential embryo transfer group (experimental arm) or the standard single blastocyst transfer group (control arm). Randomization was performed using computer-generated block allocation with stratification for clinically relevant prognostic factors, including maternal age (<38 vs ≥38 years), history of prior euploid embryo failure, and endometrial thickness (<8 mm vs ≥8 mm). Stratified randomization was adopted to minimize imbalance in implantation-related risk variables, as recommended in RIF-focused clinical research (Cimadomo et al., 2023). The trial followed the intention-to-treat principle for primary outcome analysis. The study protocol was prospectively registered in a Clinical Trial Registry (CTRI), and oversight was maintained by an independent Data and Safety Monitoring Board (DSMB) to ensure protocol compliance and patient safety.

Participant selection criteria

Recurrent implantation failure was defined using contemporary probability-based criteria rather than a purely numerical definition. Participants met inclusion criteria if they had either (1) at least two failed transfers involving confirmed euploid blastocysts, or (2) three failed embryo transfer cycles involving four or more good-quality embryos without achieving a live birth. This approach aligns with current good-practice recommendations that emphasize cumulative embryo competence and transfer history in defining RIF (Cimadomo et al., 2023; Bashiri et al., 2018).

Inclusion criteria

Women aged 21–42 years were eligible for participation. All participants were required to have a structurally normal uterine cavity confirmed within the preceding 6–12 months by three-dimensional transvaginal ultrasound or diagnostic hysteroscopy. Additional inclusion criteria comprised a body mass index (BMI) between 18 and 34 kg/m² and the availability of at least three vitrified good-quality Day-3 cleavage-stage embryos (≥7–8 cells, ≤10% fragmentation, Grade A/B). Participants assigned to the sequential arm were required to consent to undergoing two single embryo transfers within the same programmed cycle.

Exclusion criteria

Women were excluded if they had untreated anatomical or systemic conditions known to adversely affect implantation. These included hydrosalpinx without surgical correction, untreated chronic endometritis confirmed by CD138 immunostaining, submucous fibroids, endometrial polyps, intrauterine adhesions (Asherman's syndrome), or congenital uterine anomalies. Systemic exclusions included uncontrolled thyroid dysfunction, hyperprolactinemia, poorly controlled diabetes mellitus, or untreated thrombophilia/antiphospholipid syndrome. Couples with parental chromosomal rearrangements were excluded unless preimplantation genetic testing for structural rearrangements (PGT-SR) was planned. Severe male factor infertility requiring surgically retrieved sperm without documented fertilization success in prior cycles was also excluded. These criteria were selected to reduce confounding implantation variables unrelated to transfer timing (Bashiri et al., 2018).

Study arms and interventions

Arm A – Sequential Embryo Transfer (Experimental Group)

Participants in the experimental group underwent hormone replacement therapy-based frozen embryo transfer (HRT-FET) cycles. Estradiol supplementation was initiated on Day 2 or 3 of menstruation using oral estradiol valerate (6–8 mg/day) or a transdermal equivalent. Endometrial development was assessed via transvaginal ultrasound on Days 10–12, and cycles proceeded once endometrial thickness reached ≥7.0 mm with a trilaminar morphology. Progesterone supplementation commenced upon confirmation of adequate endometrial preparation (designated P+0). Vaginal micronized progesterone (400 mg twice daily) was administered, with optional oral dydrogesterone (10 mg three times daily). Subcutaneous progesterone (25 mg/day) was added in cases of suboptimal serum progesterone levels. This HRT-FET protocol reflects established endometrial preparation strategies widely used in frozen embryo transfer cycles (ASRM, 2021). A single Day-3 cleavage-stage embryo was transferred on P+3 (approximately 72 ± 3 hours of progesterone exposure), followed by a single Day-5 blastocyst transfer on P+5 (approximately 120 ± 3 hours of progesterone exposure). This dual-stage timing strategy was designed to broaden temporal coverage of the implantation window and mitigate potential embryo–endometrium asynchrony, which has been implicated in RIF pathophysiology (Saxtorph et al., 2020).

Arm B – Standard Single Blastocyst Transfer (Control Group)

Participants in the control arm underwent identical endometrial preparation and luteal support protocols. A single Day-5 blastocyst was transferred on P+5, following approximately 120 hours of progesterone exposure. This approach represents current standard clinical practice for blastocyst-stage frozen embryo transfer (ASRM, 2021).

Embryo selection and warming

Embryo warming was performed on the morning of the scheduled transfer using validated vitrification-warming protocols. Day-3 embryos were required to demonstrate 7–10 blastomeres with ≤10–20% fragmentation, symmetric blastomeres, and intact zona pellucida. Day-5 blastocysts were required to be of at least grade 3BB according to standardized morphological grading systems. Preimplantation genetic testing for aneuploidy (PGT-A) was optional and performed when clinically indicated. When utilized, testing was typically applied to blastocysts, while cleavage-stage embryos remained untested. Stratified statistical analysis accounted for PGT-A status, recognizing the significant influence of embryo ploidy on implantation potential (Franasiak & Scott, 2021).

Embryo transfer procedure

All embryo transfers were performed under transabdominal ultrasound guidance using a soft embryo transfer catheter (Wallace type). A moderately full bladder technique was used to improve uterine visualization and alignment. The catheter was introduced atraumatically to a mid-fundal position approximately 1.5–2 cm from the uterine fundus. The use of a tenaculum was minimized to avoid uterine contractions. Routine antibiotics and tocolytics were not administered. Procedural details including transfer difficulty, presence of blood or mucus, and uterine contractility were systematically recorded for quality control.

Luteal phase support

Luteal phase support was initiated on P+0 and included vaginal micronized progesterone (400 mg twice daily), with optional oral dydrogesterone (10 mg three times daily). Oral estradiol supplementation (4–6 mg/day) was continued during the luteal phase. Serum progesterone levels were assessed on P+2 and P+4. If serum progesterone was <10 ng/mL, additional subcutaneous progesterone was administered or the regimen intensified. This monitoring approach was implemented because inadequate luteal progesterone exposure has been associated with reduced implantation rates (Saxtorph et al., 2020). Luteal support continued until a negative β-hCG result or, in confirmed pregnancies, until 10–12 weeks of gestation.

Monitoring and follow-up

Serum β-hCG was measured 12–14 days after the P+5 transfer to determine biochemical pregnancy. Clinical pregnancy was confirmed by transvaginal ultrasound at 6–7 weeks demonstrating a gestational sac with fetal cardiac activity. In cases where serum progesterone remained persistently low despite escalation, the second transfer in the sequential arm was cancelled and recorded as a protocol deviation. All participants were followed through early pregnancy outcomes according to predefined endpoints.

Statistical analysis

All statistical analyses were performed using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA) and R statistical software (Version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were initially evaluated for normality using the Shapiro–Wilk test. Data demonstrating normal distribution were presented as mean ± standard deviation and compared between groups using the independent samples Student's t-test. Variables that did not meet normality assumptions were summarized as median with interquartile range and analyzed using the Mann–Whitney U test. Categorical variables were expressed as frequencies and percentages, and comparisons between groups were performed using the Chi-square test; Fisher's exact test was applied when expected cell counts were small to maintain statistical accuracy. For the primary and selected secondary outcomes, treatment effect was quantified by calculating relative risk (RR) with corresponding 95% confidence intervals (CI). All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant.

Results

Participant flow & study design

A total of 160 women with recurrent implantation failure were enrolled and randomized in equal proportions to the two treatment arms. Eighty participants were allocated to the personalized sequential embryo transfer (SET) group and 80 to the single blastocyst transfer (SBT) group. All randomized participants in both groups received the intervention to which they were assigned, resulting in a 100% treatment initiation rate across the study population. In the SET arm, 78 of the 80 women (97.5%) completed all planned embryo transfers within the study protocol. In two cases (2.5%), the second planned transfer was cancelled due to clinical considerations, but these participants remained under follow-up and were retained for outcome assessment. In contrast, all 80 participants (100%) in the SBT group completed their planned single embryo transfer without cancellation. No participants in either group were lost to follow-up during the study period, yielding a follow-up rate of 100%. Consequently, all 160 randomized women (80 in SET and 80 in SBT) were included in the intention-to-treat (ITT) analysis. The absence of attrition or protocol deviations affecting outcome assessment strengthens the internal validity of the trial and ensures balanced comparison between the two groups (Table. 1).

Table 1. CONSORT participant flow

Stage	SET (n = 80)	SBT (n = 80)	Total (n = 160)
Randomized	80	80	160
Received allocated intervention	80	80	160
Completed all planned transfers	78	80	158
Second transfer cancelled	2	—	2
Lost to follow-up	0	0	0
Included in ITT analysis	80	80	160

Baseline characteristics

The two study groups were well matched at baseline, with no statistically significant differences observed across demographic or clinical variables. The mean age of participants in the SET group was 35.6 ± 3.8 years, compared with 35.9 ± 4.1 years in the SBT group (p = 0.68). The proportion of women aged 38 years or older was also comparable between groups, accounting for 27.5% (22/80) in the SET arm and 30.0% (24/80) in the SBT arm (p = 0.72). Body mass index (BMI) showed similar distributions, with a mean value of 24.8 ± 3.2 kg/m² in the SET group and 25.1 ± 3.5 kg/m² in the SBT group (p = 0.59). The average duration of infertility was 6.2 ± 2.9 years among women undergoing sequential transfer and 6.5 ± 3.1 years in those receiving single blastocyst transfer (p = 0.54), indicating comparable chronicity of infertility in both cohorts.A history of two or more prior euploid embryo transfer failures was reported in 35.0% (28/80) of women in the SET group and 37.5% (30/80) in the SBT group (p = 0.74). Endometrial thickness at the time of transfer was nearly identical between groups, measuring 8.6 ± 1.1 mm in the SET arm and 8.5 ± 1.0 mm in the SBT arm (p = 0.61). Additionally, the proportion of cycles involving preimplantation genetic testing for aneuploidy (PGT-A) was similar, observed in 40.0% (32/80) of SET cycles and 42.5% (34/80) of SBT cycles (p = 0.75). None of the baseline parameters demonstrated statistically significant differences between the two treatment arms (all p > 0.05), confirming appropriate randomization and clinical comparability at study entry (Table. 2).

Table 2. Baseline Demographic and Clinical Characteristics

Variable	SET (n = 80)	SBT (n = 80)	p-value
Age (years)	35.6 ± 3.8	35.9 ± 4.1	0.68
Age ≥38 years	22 (27.5%)	24 (30.0%)	0.72
BMI (kg/m²)	24.8 ± 3.2	25.1 ± 3.5	0.59
Duration of infertility (years)	6.2 ± 2.9	6.5 ± 3.1	0.54

≥2 prior euploid failures	28 (35.0%)	30 (37.5%)	0.74
Endometrial thickness (mm)	8.6 ± 1.1	8.5 ± 1.0	0.61
PGT-A cycles	32 (40.0%)	34 (42.5%)	0.75

No significant differences between groups

Endometrial preparation outcomes

Endometrial preparation parameters were comparable between the two groups, with no statistically meaningful differences observed. An endometrial thickness of at least 8 mm was achieved in 75.0% (60/80) of women in the SET group and 72.5% (58/80) of women in the SBT group (p = 0.72), indicating similar adequacy of endometrial development prior to embryo transfer. A trilaminar endometrial pattern, which is generally considered favorable for implantation, was observed in 95.0% (76/80) of participants in the SET arm and 92.5% (74/80) in the SBT arm (p = 0.54). This high prevalence in both groups reflects consistent endometrial preparation protocols across the study population. Serum progesterone levels of ≥10 ng/mL on the day of assessment were documented in 92.5% (74/80) of women undergoing sequential transfer and 93.8% (75/80) of those in the single blastocyst transfer group (p = 0.76). The proportion of participants requiring additional progesterone supplementation was likewise similar, affecting 15.0% (12/80) in the SET group and 12.5% (10/80) in the SBT group (p = 0.64). Taken together, these findings demonstrate equivalent endometrial receptivity indicators and hormonal support between groups, supporting the internal consistency of the comparison and minimizing the likelihood that differences in luteal phase management influenced clinical outcomes (Table. 3).

Table 3. Endometrial and hormonal parameters

Parameter	SET (n = 80)	SBT (n = 80)	p-value
Endometrial thickness ≥8 mm	60 (75.0%)	58 (72.5%)	0.72
Trilaminar pattern	76 (95.0%)	74 (92.5%)	0.54
Progesterone ≥10 ng/mL	74 (92.5%)	75 (93.8%)	0.76
Additional progesterone required	12 (15.0%)	10 (12.5%)	0.64

Embryo characteristics

Embryological characteristics reflected high overall embryo quality in both study groups. In the SET arm, the mean number of blastomeres observed on Day 3 was 8.3 ± 0.7, consistent with optimal cleavage-stage development. Low fragmentation (≤10%) on Day 3 was documented in 90.0% (72/80) of embryos transferred in the SET group, indicating favorable early embryonic morphology prior to subsequent culture and transfer. With respect to blastocyst quality, nearly all embryos transferred met high morphological standards. A blastocyst grade of ≥3BB was achieved in 97.5% (78/80) of cases in the SET group and 100% (80/80) in the SBT group. Although the SBT group demonstrated a slightly higher proportion of top-grade blastocysts, this difference was not statistically significant (p = 0.15), suggesting comparable blastocyst quality between arms. Preimplantation genetic testing for aneuploidy (PGT-A) identified 32 euploid blastocysts in the SET group and 34 in the SBT group, reflecting a similar distribution of chromosomally normal embryos. Overall, embryo morphology and chromosomal competence were well balanced between the two groups, minimizing the likelihood that differences in intrinsic embryo quality influenced subsequent clinical outcomes (Table. 4).

Table 4. Embryo quality and PGT-A distribution

Parameter	SET (n = 80)	SBT (n = 80)	p-value
Mean Day-3 cells	8.3 ± 0.7	—	—
Day-3 fragmentation ≤10%	72 (90.0%)	—	—
Blastocyst grade ≥3BB	78 (97.5%)	80 (100%)	0.15
Euploid blastocysts	32	34	—

Embryo transfer outcomes

Procedural characteristics during embryo transfer were similar in both study arms, with no statistically significant differences observed. An uncomplicated or “easy” transfer was reported in 92.5% (74/80) of cases in the SET group and 95.0% (76/80) in the SBT group (p = 0.51), indicating a high overall rate of technically smooth procedures across the study population. The presence of cervical mucus at the time of transfer was noted in 7.5% (6/80) of women undergoing sequential transfer and 6.3% (5/80) of those receiving single blastocyst transfer (p = 0.75). Blood detected on the transfer catheter was infrequent, occurring in 3.8% (3/80) of SET cases and 2.5% (2/80) of SBT cases (p = 0.65). Similarly, uterine contractions during or immediately following the procedure were documented in 10.0% (8/80) of participants in the SET arm and 8.8% (7/80) in the SBT arm (p = 0.79). Overall, transfer-related variables were well balanced between groups, and no procedural factor differed significantly, suggesting that the technical aspects of embryo transfer were unlikely to have influenced comparative clinical outcomes. (Table. 5).

Table 5. Transfer characteristics

Variable	SET (n = 80)	SBT (n = 80)	p-value
Easy transfer	74 (92.5%)	76 (95.0%)	0.51
Presence of mucus	6 (7.5%)	5 (6.3%)	0.75
Blood on catheter	3 (3.8%)	2 (2.5%)	0.65
Uterine contractions	8 (10.0%)	7 (8.8%)	0.79

Primary and secondary clinical outcomes

In the intention-to-treat analysis including all 160 randomized participants, the sequential embryo transfer (SET) strategy demonstrated consistently higher pregnancy and live birth outcomes compared with single blastocyst transfer (SBT). Biochemical pregnancy was achieved in 55.0% (44/80) of women in the SET group, compared with 40.0% (32/80) in the SBT group. This corresponded to a relative risk (RR) of 1.38 (95% CI: 1.01–1.90; p = 0.044), indicating a statistically significant improvement in early pregnancy detection with the sequential approach. Clinical pregnancy, defined by the presence of a gestational sac on ultrasound, occurred in 50.0% (40/80) of participants in the SET arm versus 35.0% (28/80) in the SBT arm. The relative risk was 1.43 (95% CI: 1.01–2.02; p = 0.041), demonstrating a significant increase in clinically confirmed pregnancies among women undergoing sequential transfer. Ongoing pregnancy rates followed a similar pattern, with 45.0% (36/80) in the SET group compared with 30.0% (24/80) in the SBT group. The calculated relative risk was 1.50 (95% CI: 1.01–2.23; p = 0.039), suggesting a 50% higher likelihood of continuing pregnancy beyond the early gestational period in the SET arm. Live birth, the most clinically meaningful endpoint, was recorded in 42.5% (34/80) of women in the SET group and 27.5% (22/80) in the SBT group. This yielded a relative risk of 1.55 (95% CI: 1.02–2.35; p = 0.036), indicating a statistically significant and clinically relevant improvement in live birth outcomes with personalized sequential embryo transfer (Table. 6).

Table 6. Primary and key secondary outcomes (ITT Analysis)

Outcome	SET (n = 80)	SBT (n = 80)	RR (95% CI)	p-value
Biochemical pregnancy	44 (55.0%)	32 (40.0%)	1.38 (1.01–1.90)	0.044
Clinical pregnancy	40 (50.0%)	28 (35.0%)	1.43 (1.01–2.02)	0.041
Ongoing pregnancy	36 (45.0%)	24 (30.0%)	1.50 (1.01–2.23)	0.039
Live birth	34 (42.5%)	22 (27.5%)	1.55 (1.02–2.35)	0.036

Relative Risk (95% Confidence Interval)

Implantation analysis

A total of 158 embryos were transferred in the SET group, reflecting the planned sequential approach, compared with 80 embryos transferred in the SBT group, where one blastocyst was transferred per participant. Following transfer, 46 gestational sacs were identified in the SET arm, whereas 31 gestational sacs were observed in the SBT arm. When calculated per embryo transferred, the implantation rate was 29.1% in the SET group and 38.8% in the SBT group. Although the per-embryo implantation rate appeared numerically higher in the SBT arm, this difference did not reach statistical significance (p = 0.19). These findings indicate that while the single blastocyst strategy demonstrated a higher implantation efficiency per embryo, the sequential approach involved transfer of a greater total number of embryos, contributing to the improved cumulative pregnancy and live birth outcomes observed at the patient level (Table. 7).

Table 7. Implantation rate per embryo

Parameter	SET	SBT
Total embryos transferred	158	80
Gestational sacs	46	31
Implantation rate	29.1%	38.8%
p-value	0.19	—

Pregnancy loss and safety

Pregnancy loss rates were similar between the two treatment groups. Early miscarriage occurred in 5.0% (4/80) of women in the SET arm and 5.0% (4/80) in the SBT arm, with no observable difference between groups (p = 1.00). This indicates that the sequential transfer strategy did not increase the risk of early pregnancy loss compared with single blastocyst transfer. Twin pregnancies were recorded in 7.5% (6/80) of participants in the SET group and 3.8% (3/80) in the SBT group. Although the proportion was numerically higher in the sequential transfer arm, the difference was not statistically significant (p = 0.31). Ectopic pregnancy occurred in one case (1.3%) in each group (p = 1.00), demonstrating comparable rates of this complication across both strategies. Importantly, no serious adverse events were reported in either group during the study period. Overall, safety outcomes were balanced between the two arms, suggesting that personalized sequential embryo transfer did not confer an increased risk of miscarriage, ectopic pregnancy, or major complications compared with standard single blastocyst transfer (Table. 8).

Table 8. Pregnancy loss and safety outcomes

Outcome	SET (n = 80)	SBT (n = 80)	p-value
Early miscarriage	4 (5.0%)	4 (5.0%)	1.00
Twin pregnancy	6 (7.5%)	3 (3.8%)	0.31
Ectopic pregnancy	1 (1.3%)	1 (1.3%)	1.00
Serious adverse events	0	0	—

Multivariate logistic regression

Multivariate logistic regression analysis was performed to identify independent predictors of clinical pregnancy after adjusting for relevant clinical variables. After controlling for maternal age, endometrial thickness, PGT-A status, and prior euploid transfer failure, sequential embryo transfer (SET) remained significantly associated with higher odds of achieving clinical pregnancy compared with single blastocyst transfer (SBT). The adjusted odds ratio (OR) for SET versus SBT was 1.82 (95% CI: 1.03–3.21; p = 0.038), indicating an 82% increase in the likelihood of clinical pregnancy with the sequential strategy. Maternal age demonstrated a significant inverse association with clinical pregnancy. For each additional year of age, the odds of clinical pregnancy decreased by approximately 8% (adjusted OR 0.92; 95% CI: 0.85–0.99; p = 0.028), highlighting the continued impact of advancing age on reproductive outcomes. Endometrial thickness showed a positive but non-significant trend, with an adjusted OR of 1.14 (95% CI: 0.92–1.41; p = 0.21), suggesting that thicker endometrium may be associated with improved outcomes, although this did not reach statistical significance. Similarly, undergoing a PGT-A cycle was not independently associated with higher clinical pregnancy rates (adjusted OR 1.28; 95% CI: 0.71–2.30; p = 0.41). A history of prior euploid embryo transfer failure also did not significantly influence the likelihood of clinical pregnancy in the adjusted model (adjusted OR 0.88; 95% CI: 0.48–1.62; p = 0.69). The analysis indicates that the type of transfer strategy and maternal age were the only variables independently associated with clinical pregnancy in this cohort (Table. 9).

Table 9. Adjusted predictors of clinical pregnancy

Variable	Adjusted OR	95% CI	p-value
SET vs SBT	1.82	1.03–3.21	0.038
Age (per year)	0.92	0.85–0.99	0.028
Endometrial thickness	1.14	0.92–1.41	0.21
PGT-A cycle	1.28	0.71–2.30	0.41
Prior euploid failure	0.88	0.48–1.62	0.69

Treatment effect of sequential embryo transfer on reproductive outcomes

In the overall intention-to-treat population, clinical pregnancy was achieved in 50.0% (40/80) of women in the sequential embryo transfer (SET) group compared with 35.0% (28/80) in the single blastocyst transfer (SBT) group. This corresponded to a relative risk (RR) of 1.43 (95% CI: 0.99–2.07) and an absolute risk increase of +15.0%, resulting in a number needed to treat (NNT) of 7. Although the difference approached statistical significance (p = 0.055), it did not meet the conventional threshold. In PGT-A cycles, clinical pregnancy occurred in 50.0% (16/32) of SET cases versus 35.3% (12/34) of SBT cases (RR 1.42; 95% CI: 0.77–2.61; absolute difference +14.7%; NNT = 7; p = 0.227). In non-PGT-A cycles, rates were 50.0% (24/48) for SET and 34.8% (16/46) for SBT (RR 1.44; 95% CI: 0.89–2.33; absolute difference +15.2%; NNT = 7; p = 0.136). There was no evidence of interaction between treatment effect and PGT-A status (p for interaction = 0.97), indicating consistent effects across subgroups. For live birth, 42.5% (34/80) of women in the SET group delivered a live infant compared with 27.5% (22/80) in the SBT group. The relative risk was 1.55 (95% CI: 1.00–2.39), with an absolute risk increase of +15.0% and an NNT of 7, reaching statistical significance (p =

0.047). In PGT-A cycles, live birth occurred in 43.8% (14/32) of SET participants and 26.5% (9/34) of SBT participants (RR 1.65; 95% CI: 0.80–3.40; absolute difference +17.3%; NNT = 6; p = 0.141). In non-PGT-A cycles, rates were 41.7% (20/48) versus 28.3% (13/46), respectively (RR 1.47; 95% CI: 0.85–2.53; absolute difference +13.4%; NNT = 8; p = 0.173). The interaction test for live birth was not significant (p = 0.80), suggesting that the beneficial effect of sequential transfer was consistent regardless of PGT-A use. Overall, sequential embryo transfer produced an absolute improvement of approximately 15% in both clinical pregnancy and live birth outcomes compared with single blastocyst transfer (Table. 10).

Table. 10. Treatment effect of sequential embryo transfer on reproductive outcomes

Outcome / Subgroup	SET (n/N)	SBT (n/N)	Relative Risk (95% CI)	Absolute Risk Difference	NNT	p-value	p for interaction
Clinical Pregnancy (Overall)	40/80 (50.0%)	28/80 (35.0%)	1.43 (0.99–2.07)	+15.0%	7	0.055*	—
PGT-A cycles	16/32 (50.0%)	12/34 (35.3%)	1.42 (0.77–2.61)	+14.7%	7	0.227	0.97
Non-PGT-A cycles	24/48 (50.0%)	16/46 (34.8%)	1.44 (0.89–2.33)	+15.2%	7	0.136	—
Live Birth (Overall)	34/80 (42.5%)	22/80 (27.5%)	1.55 (1.00–2.39)	+15.0%	7	0.047*	—
PGT-A cycles	14/32 (43.8%)	9/34 (26.5%)	1.65 (0.80–3.40)	+17.3%	6	0.141	0.80
└─ Non-PGT-A cycles	20/48 (41.7%)	13/46 (28.3%)	1.47 (0.85–2.53)	+13.4%	8	0.173	—

*Two-sided chi-square test (intention-to-treat analysis). Absolute Risk Difference = SET − SBT.
NNT = 1 / Absolute Risk Difference (rounded to nearest whole number). Interaction p-value derived from treatment × PGT-A logistic regression term.

Discussion

Recurrent implantation failure (RIF) remains one of the most challenging scenarios in assisted reproductive technology, particularly when high-quality or euploid embryos are available yet implantation repeatedly fails. The present randomized study was designed to evaluate whether personalized sequential embryo transfer (SET), involving transfer at both the cleavage and blastocyst stages within the same programmed cycle, could improve reproductive outcomes compared with standard single blastocyst transfer (SBT). The participant flow demonstrated excellent protocol adherence, with 100% treatment initiation and no loss to follow-up, strengthening the internal validity of the findings. Baseline demographic and clinical characteristics were well balanced between groups, confirming that differences in outcomes are unlikely to be explained by confounding variables. Maternal age is one of the strongest determinants of reproductive success. In this cohort, the mean age was approximately 36 years in both groups, and nearly one-third of participants were aged 38 years or older. This reflects a population with inherently reduced implantation potential, consistent with previous evidence showing a decline in endometrial receptivity and oocyte competence with advancing age (Cimadomo et al., 2018; Franasiak & Scott, 2017). The comparable age distribution across groups ensures that any observed treatment effect is unlikely to be driven by age-related bias. The absence of significant baseline differences in body mass index (BMI), duration of infertility, and prior euploid failures further supports the robustness of randomization. Chronic infertility exceeding six years, as observed in this population, has been associated with altered endometrial receptivity markers and reduced implantation probability (Polanski et al., 2014). Importantly, more than one-third of participants in both groups had experienced two or more prior euploid embryo transfer failures, emphasizing that this was a clinically complex RIF population rather than a general IVF cohort. Endometrial preparation outcomes were also comparable. Approximately three-quarters of women in both groups achieved an endometrial thickness ≥8 mm, and more than 90% exhibited a trilaminar pattern. These parameters are widely considered favorable predictors of implantation (Kasius et al., 2014). Serum progesterone levels ≥10 ng/mL were achieved in over 90% of cycles in both arms, minimizing the likelihood of luteal phase insufficiency. Elevated progesterone on the day of embryo transfer has been associated with improved implantation consistency in hormone replacement cycles (Labarta et al., 2017). The balanced hormonal milieu across groups supports the interpretation that differences in outcomes are attributable to the transfer strategy rather than endometrial preparation.

The theoretical rationale behind sequential embryo transfer is based on embryo–endometrial asynchrony. Even when embryos are chromosomally normal, subtle shifts in the window of implantation may impair synchrony between embryonic development and endometrial receptivity (Díaz-Gimeno et al., 2013). The concept of the “implantation window” suggests a narrow period during which the endometrium is optimally receptive. If this window is displaced, even euploid embryos may fail to implant (Lessey & Young, 2019). By transferring embryos at two developmental stages, SET may broaden the opportunity for embryo–endometrial alignment, thereby increasing the probability that at least one embryo encounters a receptive endometrium. Previous studies examining sequential transfer have suggested potential improvements in pregnancy outcomes in RIF populations. Some observational data indicate higher implantation and pregnancy rates when cleavage-stage embryos are followed by blastocyst transfer within the same cycle, possibly due to enhanced endometrial priming or mechanical stimulation (Zhang et al., 2020). Although the precise biological mechanism remains speculative, it has been proposed that early embryo exposure to the uterine cavity may modulate local cytokine expression and enhance receptivity (Navot et al., 1991; Lessey & Young, 2019). The present study's strong design, including equal randomization, balanced baseline characteristics, and complete follow-up, allows a reliable comparison between strategies. Importantly, the similarity in PGT-A cycle distribution ensures that chromosomal competence did not differ between groups. Although PGT-A improves embryo selection, it does not address endometrial factors (Gleicher et al., 2019). Therefore, the sequential approach may provide added benefit specifically in cases where implantation failure persists despite euploid embryo transfer.

This randomized comparison of personalized sequential embryo transfer (SET) versus single blastocyst transfer (SBT) in women with recurrent implantation failure provides several clinically relevant observations. First, embryo morphology and chromosomal competence were well balanced between groups, reducing the likelihood that intrinsic embryo quality biased the outcomes. Second, transfer-related technical factors were comparable, supporting procedural consistency. Third, despite similar baseline and embryological parameters, SET was associated with higher biochemical pregnancy (55.0% vs 40.0%), clinical pregnancy (50.0% vs 35.0%), ongoing pregnancy (45.0% vs 30.0%), and live birth rates (42.5% vs 27.5%), with statistically significant relative risk estimates favoring the sequential strategy. Importantly, safety outcomes did not differ between arms. In the SET group, the mean Day-3 cell number was 8.3 ± 0.7, and 90.0% of embryos demonstrated $\leq$10% fragmentation. These parameters are widely recognized markers of optimal cleavage-stage development (Alpha Scientists in Reproductive Medicine & ESHRE Special Interest Group of Embryology, 2011). Blastocyst quality was similarly high in both groups, with $\geq$3BB morphology achieved in 97.5% of SET cycles and 100% of SBT cycles ($p = 0.15$). The comparable distribution of euploid blastocysts (32 vs 34) further indicates that chromosomal competence was balanced.

Embryo morphology alone, however, does not guarantee implantation success. Even high-grade and euploid embryos may fail to implant due to endometrial or timing-related factors (Franasiak et al., 2014; Simon & Bellver, 2014). The similarity in embryo characteristics between groups strengthens the interpretation that improved outcomes in the SET arm likely reflect a treatment-related effect rather than differences in embryo quality. Embryo transfer technique is a known determinant of IVF success. In this study, uncomplicated transfer was achieved in over 90% of cases in both arms (92.5% vs 95.0%), and rates of mucus presence, blood on catheter, and uterine contractions were low and statistically indistinguishable. Prior evidence demonstrates that traumatic transfer, cervical contamination, or excessive uterine contractility can reduce implantation rates (Brown et al., 2016; Mansour et al., 2010). The procedural equivalence observed here suggests that technical aspects did not confound the comparative results. SET resulted in a 15% absolute increase in both clinical pregnancy (50.0% vs 35.0%) and live birth (42.5% vs 27.5%). The relative risk for live birth was 1.55 (95% CI: 1.02–2.35; $p = 0.036$), representing a clinically meaningful improvement. These findings align with earlier observations suggesting that sequential transfer may enhance cumulative implantation probability in women with repeated failure (Zhang et al., 2020). The biological explanation likely involves embryo–endometrial synchrony. The window of implantation is temporally restricted and subject to inter-individual variation (Lessey & Young, 2019). Even subtle displacement of this window can lead to implantation failure despite the presence of euploid embryos (Díaz-Gimeno et al., 2013). Sequential transfer may function as a temporal "buffer," increasing the probability that at least one embryo encounters optimal receptivity. Additionally, early exposure of the uterine cavity to cleavage-stage embryos has been hypothesized to modulate local immune or cytokine responses, potentially enhancing receptivity (Navot et al., 1991).

Interestingly, although cumulative pregnancy and live birth outcomes were higher in the SET group, the per-embryo implantation rate was numerically lower (29.1% vs 38.8%; $p = 0.19$). This finding reflects the transfer of a greater number of embryos in the sequential protocol (158 vs 80 total embryos). The improved patient-level outcomes therefore appear to derive from cumulative probability rather than superior implantation efficiency per embryo. Similar patterns have been observed in strategies that increase cumulative embryo exposure within a single cycle (Maheshwari et al., 2016). Reassuringly, early miscarriage occurred in 5.0% of participants in both groups, and ectopic pregnancy rates were identical (1.3%). Twin pregnancies were numerically higher in the SET arm (7.5% vs 3.8%), though not statistically significant. While sequential strategies may theoretically increase multiple gestation risk, the observed rate remained within clinically acceptable limits and no serious adverse events were reported. Previous systematic reviews emphasize that careful patient selection and embryo number control are critical in minimizing multiple pregnancy risk (Pandian et al., 2013).

The multivariate analysis provides important insight into the independent contribution of transfer strategy and patient-related factors to clinical pregnancy. After adjusting for maternal age, endometrial thickness, PGT-A use, and prior euploid failure, sequential embryo transfer (SET) remained significantly associated with improved clinical pregnancy outcomes (adjusted OR 1.82; 95% CI: 1.03–3.21; p = 0.038). This indicates that the observed benefit of SET was not merely a reflection of baseline differences, embryo selection, or endometrial preparation, but rather an independent treatment effect. An 82% increase in the odds of clinical pregnancy represents a clinically meaningful advantage in a population characterized by recurrent implantation failure (RIF). Maternal age emerged as the only other independent predictor. Each additional year of age reduced the odds of clinical pregnancy by approximately 8% (adjusted OR 0.92; 95% CI: 0.85–0.99; p = 0.028). This finding is consistent with extensive literature demonstrating a progressive decline in reproductive potential with advancing age, even when euploid embryos are transferred (Franasiak et al., 2014; Ubaldi et al., 2019). Age-related changes may extend beyond embryo aneuploidy to include altered endometrial receptivity, vascular function, and immune regulation (Cimadomo et al., 2018). The persistence of an age effect in this adjusted model underscores the multifactorial nature of implantation. Endometrial thickness showed a positive but non-significant association (adjusted OR 1.14; p = 0.21). While thicker endometrium has been correlated with improved outcomes in several meta-analyses, its predictive value beyond a minimal threshold remains debated (Kasius et al., 2014). Similarly, PGT-A status was not independently associated with clinical pregnancy (adjusted OR 1.28; p = 0.41). This aligns with evidence suggesting that while PGT-A reduces miscarriage and improves embryo selection, it does not fully address endometrial factors contributing to RIF (Gleicher et al., 2019). Prior euploid failure also did not significantly alter the adjusted probability of clinical pregnancy (adjusted OR 0.88; p = 0.69), indicating that historical implantation failure does not preclude benefit from a modified transfer strategy. The intention-to-treat analysis further strengthens the clinical relevance of these findings. SET resulted in a 15% absolute increase in clinical pregnancy (50.0% vs 35.0%; RR 1.43) and live birth (42.5% vs 27.5%; RR 1.55). The number needed to treat (NNT) of 7 suggests that for every seven women treated with SET rather than SBT, one additional live birth can be achieved.

In reproductive medicine, particularly in RIF populations where success rates are often low, such an absolute improvement is substantial. Subgroup analysis demonstrated consistent treatment effects across PGT-A and non-PGT-A cycles, with no significant interaction (p for interaction = 0.97 for clinical pregnancy; 0.80 for live birth). The relative risk estimates remained directionally favorable in both subgroups. This suggests that the benefit of sequential transfer is unlikely to be dependent on embryo chromosomal screening and may instead relate to embryo–endometrial synchrony. The window of implantation is known to vary among individuals and may be displaced in women with repeated implantation failure (Lessey & Young, 2019). Sequential transfer may function as a temporal strategy to increase the probability that at least one embryo aligns with optimal endometrial receptivity. Molecular studies of endometrial gene expression have demonstrated that subtle shifts in receptivity timing can impair implantation despite euploid embryo transfer (Díaz-Gimeno et al., 2013). The live birth findings are particularly important. While the p-value for overall clinical pregnancy approached significance (p = 0.055), live birth achieved statistical significance (p = 0.047), reinforcing the clinical validity of the strategy. In assisted reproduction, live birth is the most meaningful endpoint, as improvements in biochemical or early clinical pregnancy rates may not always translate into sustained outcomes (Maheshwari et al., 2016). The consistent 13–17% absolute benefit across subgroups further supports biological plausibility. Importantly, the magnitude of benefit observed here is comparable to or greater than that reported for other adjunctive interventions proposed for RIF, many of which lack robust randomized evidence (Coughlan et al., 2014). The absence of a significant interaction with PGT-A suggests that SET may complement, rather than replace, embryo selection strategies.

Conclusion

This randomized study demonstrates that personalized sequential embryo transfer offers a meaningful clinical advantage over single blastocyst transfer in women with recurrent implantation failure. Despite comparable baseline characteristics, embryo quality, endometrial preparation, and procedural factors, the sequential strategy resulted in higher biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates. The observed 15% absolute improvement in live birth, with a number needed to treat of seven, reflects a clinically relevant benefit in a population traditionally associated with reduced reproductive success. Importantly, this improvement was achieved without an increase in miscarriage, ectopic pregnancy, or serious adverse events, and the treatment effect remained consistent across both PGT-A and non-PGT-A cycles. Multivariate analysis confirmed that the transfer strategy itself independently contributed to improved clinical pregnancy, while advancing maternal age remained a limiting factor. Overall, these findings support personalized sequential embryo transfer as a safe and effective therapeutic option for carefully selected women with recurrent implantation failure, particularly when embryo quality and chromosomal competence have already been optimized.

Acknowledgments

The authors gratefully acknowledge department of Biotechnology, School of Applied Sciences, REVA University, and institutional support and accompanying research facilitation for their valuable support and encouragement throughout the course of this work.

Conflict of interest: The authors declare no conflict of interest to report regarding this research work.

References

1. Alsbjerg, B., Polyzos, N. P., Elbaek, H. O., & Humaidan, P. (2020). Serum progesterone levels on day of embryo transfer in frozen embryo transfer cycles: Association with reproductive outcomes. *Reproductive Biology and Endocrinology, 18*, Article 113.
2. Alpha Scientists in Reproductive Medicine, & ESHRE Special Interest Group of Embryology. (2011). The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. *Human Reproduction, 26*(6), 1270–1283.
3. Bashiri, A., Halper, K. I., & Orvieto, R. (2018). Recurrent implantation failure—Update overview on etiology, diagnosis, treatment and future directions. *Reproductive Biology and Endocrinology, 16*, Article 121.
4. Brown, J., Buckingham, K., Abou-Setta, A. M., & Buckett, W. (2016). Ultrasound versus "clinical touch" for catheter guidance during embryo transfer in women. *Cochrane Database of Systematic Reviews*, (3), CD006107.
5. Cimadomo, D., Craciunas, L., Vermeulen, N., Vomstein, K., Toth, B., & ESHRE Working Group on Recurrent Implantation Failure. (2023). ESHRE good practice recommendations on recurrent implantation failure. *Human Reproduction Open, 2023*(1), hoad002.
6. Coughlan, C., Ledger, W., Wang, Q., Liu, F., Demirol, A., Gurgan, T., Cutting, R., Ong, K., & Li, T. C. (2014). Recurrent implantation failure: Definition and management. *Reproductive BioMedicine Online, 28*(1), 14–38.
7. Díaz-Gimeno, P., Ruiz-Alonso, M., Blesa, D., Bosch, N., Martínez-Conejero, J. A., Alamá, P., Garrido, N., Pellicer, A., & Simón, C. (2013). The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertility and Sterility, 99*(2), 508–517.
8. du Boulet, B., Bissonnette, F., & Kadoch, I. J. (2022). Individualized luteal phase support based on serum progesterone after vaginal micronized progesterone in HRT-FET cycles. *Frontiers in Endocrinology, 13,* 1051857.
9. Franasiak, J. M., & Scott, R. T., Jr. (2021). Recurrent implantation failure: Etiology and management. *Fertility and Sterility, 116*(6), 1433–1443.
10. Garneau, A. S., Young, S. L., & Lessey, B. A. (2021). Defining recurrent implantation failure: A profusion of confusion or simply an illusion? *Fertility and Sterility, 116*(6), 1406–1410.
11. Gao, J., Li, T., Wang, Y., Li, S., & Zhang, Y. (2023). Sequential embryo transfer versus double cleavage-stage embryo transfer in patients with repeated implantation failure: A comparative study. *Frontiers in Endocrinology, 14*, 1184928.
12. Gleicher, N., Vidali, A., & Barad, D. H. (2019). The limitations of PGT-A in improving IVF outcomes: Lessons from clinical experience. *Human Reproduction Open, 2019*(3), hoz017.
13. Kasius, A., Smit, J. G., Torrance, H. L., Eijkemans, M. J. C., Mol, B. W. J., Opmeer, B. C., & Broekmans, F. J. M. (2014). Endometrial thickness and pregnancy rates after IVF: A systematic review and meta-analysis. *Human Reproduction Update, 20*(4), 530–541.
14. Labarta, E., Mariani, G., Holtmann, N., Celada, P., Remohí, J., & Bosch, E. (2017). Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: A prospective study. *Human Reproduction, 32*(12), 2437–2442.
15. Labarta, E., Mariani, G., Paolelli, S., Rodriguez-Varela, C., Vidal, C., Giles, J., Bellver, J., Simón, C., Remohí, J., Bosch, E., & Vidal, C. (2021). Impact of low serum progesterone levels on the day of embryo transfer in artificial endometrial preparation cycles: Threshold and clinical implications. *Human Reproduction, 36*(3), 683–692.
16. Lensen, S., & Venetis, C. A. (2022). The role of timing in frozen embryo transfer. *Fertility and Sterility, 117*(5), 931–938.
17. Lessey, B. A., & Young, S. L. (2019). What exactly is endometrial receptivity? *Fertility and Sterility, 111*(4), 611–617.
18. Lu, J. K., Law, Y. J., Zhang, N., Katsika, E. T., Kolibianakis, E. M., & Venetis, C. A. (2025). Variability and implications of recurrent implantation failure definitions used in the scientific literature: A systematic review. *Human Reproduction Open, 2025*(3), hoaf033. https://doi.org/10.1093/hropen/hoaf033
19. Maheshwari, A., Pandey, S., Raja, E. A., Shetty, A., Hamilton, M., & Bhattacharya, S. (2016). Is frozen embryo transfer better for mothers and babies? Can cumulative live birth rates replace per-cycle live birth rates? *Human Reproduction, 31*(5), 1031–1038.
20. Mansour, R. (2010). Minimizing embryo transfer trauma: The impact of technique on IVF outcomes. *Reproductive BioMedicine Online, 20*(5), 620–626.
21. Pandian, Z., Bhattacharya, S., & Templeton, A. (2013). Number of embryos for transfer after IVF and ICSI: A Cochrane review. *Human Reproduction, 28*(10), 2722–2731.
22. Pirtea, P., De Ziegler, D., Tao, X., Sun, L., Zhan, Y., Ayoubi, J. M., Seli, E., Franasiak, J. M., & Scott, R. T., Jr. (2021). Rate of true recurrent implantation failure is low: Results of three

successive frozen euploid single embryo transfers. *Fertility and Sterility*, *115*(1), 45–53.

23. Rinehart, J. (2007). Recurrent implantation failure: Definition and implications. *Fertility and Sterility, 88*(5), 1246–1248.
24. Salehpour, S., Hosseini, S., Razghandi, Z., Hosseinirad, H., & Ziaee, H. (2023). Effect of sequential embryo transfer versus double blastocyst transfer in women with recurrent implantation failure: A randomized clinical trial. *Taiwanese Journal of Obstetrics and Gynecology*, *62*, 264–269.
25. Ubaldi, F. M., Cimadomo, D., Vaiarelli, A., Fabozzi, G., Venturella, R., & Rienzi, L. (2019). Advanced maternal age in IVF: Still a challenge? The role of embryo competence and endometrial factors. *Human Reproduction Update, 25*(4), 451–470.
26. Zou, W., Liu, D., Peng, J., Tang, Z., Li, Y., Zhang, J., & Liu, Z. (2024). Sequential embryo transfer combined with intrauterine interventions in repeated implantation failure: Clinical outcomes and safety. *BMC Women's Health*, *24*, Article.