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The Role of Vitrification, Endometrial Receptivity, and Blastocyst Stage in Determining Reproductive Outcomes in Donor IVF Programmes

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Abstract

The 'frozen advantage' in IVF results from two scientific mechanisms which exist because of enhanced endometrial receptivity used in FET cycles and the selection process which occurs during the prolonged development of embryos until they become blastocysts. Donor IVF research has not yet established how vitrification technology interacts with endometrial preparation methods and the embryonic development stage. The study aims to assess how vitrification and endometrial receptivity and embryo stage (Day 3 vs. Day 5) impact reproductive success in donor IVF through assessment of mechanistic as well as clinical outcomes while focusing on subgroup interaction analysis. Research analyzed data from 1,089 donor IVF cycles which occurred between 2019 and 2023 to examine endometrial thickness as a continuous variable and the Group × Stage interaction term in logistic regression and the subgroup analyses which divided patients according to their embryo developmental stage. The study derived its mechanistic conclusions through studying endometrial gene expression and implantation window physiology and vitrification's epigenetic effects which previous studies published about these topics.

The study found that frozen transfer benefits mainly occur with blastocyst-stage embryos and this result emerged from a Group × Stage interaction which reached statistical significance ($p = 0.038$). The highest live birth rate (63.2%) occurred when endometrial thickness reached 9 mm or more but fresh cycles with thickness below 8 mm had the lowest live birth rate (38.7%). The study showed that vitrification did not increase miscarriage risk because it maintained epigenetic safety at the embryo level.

The frozen blastocyst transfer advantage results from a confluence of superior endometrial receptivity in programmed FET cycles, the self-selecting nature of blastocyst culture, and the biological safety of vitrification. The clinicians must establish optimal endometrial thickness for transfer procedures while they should use blastocyst culture as their primary method to increase success rates in donor programs.

Keywords: *endometrial receptivity, frozen embryo transfer, implantation window, cryopreservation, endometrial thickness, mechanistic analysis, vitrification, blastocyst, donor IVF, Day 5 transfer*

1. Introduction

The dramatic improvement in IVF success rates over the past two decades cannot be attributed to a single innovation. Rather, it reflects the convergence of multiple technical advances: improved ovarian stimulation protocols, refined oocyte handling, extended embryo culture, and — perhaps most transformatively — the advent of vitrification as the dominant cryopreservation paradigm. Vitrification, through ultra-rapid cooling that prevents ice crystal formation, has elevated embryo post-thaw survival rates from a variable 60–80% with slow cooling to a consistent 95% or above (Kuwayama et al., 2005). This technological watershed has enabled the widespread adoption of a 'freeze-all' strategy in IVF programmes globally.

Yet the clinical superiority of frozen embryo transfer (FET) over fresh ET in certain populations is not merely a consequence of vitrification's technical prowess. It also reflects a fundamental physiological insight: that the endometrium in a natural or programmed FET cycle is biologically better prepared for implantation than the endometrium during an ovarian stimulation cycle. In fresh IVF cycles, supraphysiological levels of oestradiol during stimulation alter endometrial gene expression, advance the implantation window, and may impair receptivity even in the absence of stimulation in the recipient (as in donor cycles). FET cycles allow a 'clean slate' — the endometrium is prepared under controlled hormonal conditions, independently of any follicular stimulation (Shapiro et al., 2011).

The research framework becomes more complicated because of the relationship between embryo developmental stage and transfer modality. Day 5 blastocysts are not merely older embryos because they have reached a vital developmental stage which separates abnormal embryos from normal ones. Day 3 embryos develop differently because their inner cell mass and trophectoderm have started to form their distinct identities (Gardner & Schoolcraft, 1999). FET and blastocyst culture interaction results in clinical effects which depend on whether their relationship functions in an additive or synergistic manner.

This paper investigates mechanistic relationships through a five-year retrospective analysis of 1,089 donor IVF cycles which includes analytical rigour. The study aims to explain why frozen blastocyst transfers achieve better results than other combinations while demonstrating its implications for clinical practice and research development in reproductive medicine.

2. Background and Mechanistic Framework

2.1 Endometrial Receptivity: Biology and Clinical Assessment

Endometrial receptivity describes the ability of the uterine lining to allow trophoblasts to penetrate and begin the process of implantation. The implantation window of receptivity begins on day 20 of the natural menstrual cycle and ends on day 24 which corresponds to 6 to 10 days after the luteinising hormone (LH) surge according to Paulson 2011. The endometrium undergoes multiple transformations during this period because it produces specific adhesion molecules which include integrins αvβ3 and LIF while it develops pinopodes which are uterodomes that form on its luminal epithelium and its immune system undergoes a change from rejection to acceptance.

The time frame of this process can shift from its established period during new IVF cycles which use ovarian stimulation. Research that used endometrial receptivity arrays (ERA) found that high progesterone levels during the follicular phase which results from strong gonadotropin stimulation causes implantation to begin three days earlier which creates a mismatch between the embryo's developmental stage and the endometrium's receptive phase (Simón et al., 2020). This specific mechanism does not function in donor recipients who do not receive stimulation. The fresh donor cycles face a synchronization issue because the timing of progesterone treatment needs to match the moment when the donor provides her eggs.

In FET cycles, endometrial preparation is entirely decoupled from follicular stimulation. Exogenous oestradiol is administered to build the endometrial lining, followed by progesterone initiation at a precisely controlled time point. This approach produces a more predictable and physiological endometrial environment. Clinical monitoring of endometrial thickness and pattern via transvaginal ultrasound provides an additional quality gate: transfer is only performed when the lining meets predefined criteria. In the present study, a threshold of ≥ 8 mm was applied for FET cycles, and mean endometrial thickness in the frozen group (9.1 ± 0.9 mm) was significantly greater than in the fresh group (8.4 ± 1.1 mm, $p < 0.001$), reflecting the operational advantage of the FET protocol.

2.2 Vitrification: Mechanism, Safety, and Epigenetic Implications

Vitrification is an ultra-rapid cryopreservation technique in which an embryo is exposed to high concentrations of cryoprotectants — typically a combination of ethylene glycol, DMSO, and sucrose — and then plunged directly into liquid nitrogen at a cooling rate exceeding 15,000°C per minute. At this rate, the cryoprotectant-

water mixture does not crystallise but instead forms a glass-like amorphous solid state, from which the embryo can be recovered by rapid warming without cellular damage (Edgar & Gook, 2012).

The main issue with all cryopreservation methods stems from their capacity to cause epigenetic changes. Epigenetic marks which include DNA methylation and histone modification and non-coding RNA expression patterns work as vital controllers for early embryonic development but they show sensitivity to environmental changes that include temperature fluctuations. Studies in both animal models and human embryos have examined whether vitrification alters these marks. The current scientific evidence shows that sensitive molecular analysis technologies can identify transcriptional differences between fresh and vitrified embryos yet these differences fail to produce noticeable clinical effects that involve miscarriage rates and congenital anomalies and perinatal outcomes (Chen et al., 2021; Pelkonen et al., 2015).

The present study shows equal miscarriage rates for fresh (16.5%) and frozen (14.9%) groups which supports the existing evidence. The literature and these data show a consistent absence of early pregnancy loss in FET cycles because vitrification should produce clinically important chromosomal and epigenetic damage.

2.3 Blastocyst Culture: Self-Selection and Implantation Advantage

Extended embryo culture to the blastocyst stage (Day 5 or Day 6) represents a laboratory-based form of natural selection. The human genome is activated at approximately the 4–8 cell stage (Day 3), and embryos with significant chromosomal abnormalities — particularly aneuploidies — frequently fail to progress beyond this point. By Day 5, the surviving blastocysts are enriched for chromosomal normality relative to a Day 3 cohort from the same oocyte cohort. This does not mean all blastocysts are euploid — the rate of aneuploidy at blastocyst is still approximately 40–60% depending on maternal age — but it does mean that the average developmental competence of blastocysts is higher than cleavage-stage embryos (Marek et al., 2019).

The blastocyst's trophectoderm, which will form the placenta, also begins to secrete early implantation signals including early pregnancy factor (EPF) and human chorionic gonadotropin (hCG), which may facilitate endometrial dialogue and implantation. This biological communication between the blastocyst and the endometrium is not possible with Day 3 embryos, which have not yet reached the stage of trophectoderm differentiation.

3. Methods

The detailed methodology of this retrospective cohort is described in the companion paper (Research Paper I). The mechanistic analysis includes an analytical addition which uses formal interaction terms between Group and Stage as part of multivariate logistic regression analysis and it includes the analysis of live birth rates which researchers conducted by dividing endometrial thickness measurements into three groups of less than 8 millimeters, 8 to 9 millimeters, and 9 millimeters or more in each transfer category.

The interaction model was specified as: LBR ~ Group + Stage + Group × Stage + Recipient Age + BMI + Embryo Quality. A statistically significant interaction term ($p < 0.05$) would confirm that the effect of transfer modality on LBR differs by embryo stage — providing mechanistic evidence for a stage-dependent frozen advantage. The researchers conducted pre-specified subgroup analyses which they adjusted for multiple comparisons by using the Benjamini-Hochberg procedure to maintain control over the false discovery rate.

4. Results

4.1 Group × Stage Interaction Analysis

The interaction term (Group × Stage) showed statistical significance because it produced an odds ratio of 1.64 with a 95 percent confidence interval that ranged from 1.03 to 2.60 and a p value of 0.038. The frozen cycles at blastocyst transfers achieved better results than fresh cycles with a 9.7 percentage point lead in laboratory-based rate of birth (61.4% vs. 51.7%). The cleavage-stage transfer showed smaller differences between the two groups at 44.3% and 38.5% which failed to achieve statistical significance because $p = 0.09$. The data show that frozen

transfer and blastocyst stage interact through a synergistic relationship which produces results that exceed their individual effects.

Subgroup	Fresh ET LBR	Frozen ET LBR	Difference	p-value
Day 5 Blastocyst	51.7%	61.4%	+9.7%	0.001*
Day 3 Cleavage Stage	38.5%	44.3%	+5.8%	0.09 (NS)
Overall	46.2%	56.8%	+10.6%	0.001*

Table 1. Subgroup Analysis of Live Birth Rate by Transfer Modality and Embryo Stage. *Statistically significant; NS = Not Significant.

4.2 Endometrial Thickness and Outcome: Stratified Analysis

The LBR showed a clinically significant increase across different thickness categories and transfer groups. The LBR in FET cycles reached 63.2% when endometrial thickness reached 9 mm, which represented the highest value across all subgroups. The lowest LBR (38.7%) occurred in fresh cycles when endometrial thickness remained below 8 mm, demonstrating how both suboptimal endometrial preparation and synchronized fresh cycles restricted the results. The endometrial thickness dose response relationship with LBR showed stronger effects in FET cycles compared to fresh cycles because the FET endometrial preparation protocol boosts the advantages of sufficient endometrial thickness.

Endometrial Thickness	Fresh ET LBR	Frozen ET LBR
< 8 mm	38.7%	44.1%
8 – 9 mm	46.5%	55.8%
≥ 9 mm	51.2%	63.2%

Table 2. Live Birth Rate Stratified by Endometrial Thickness Category and Transfer Modality.

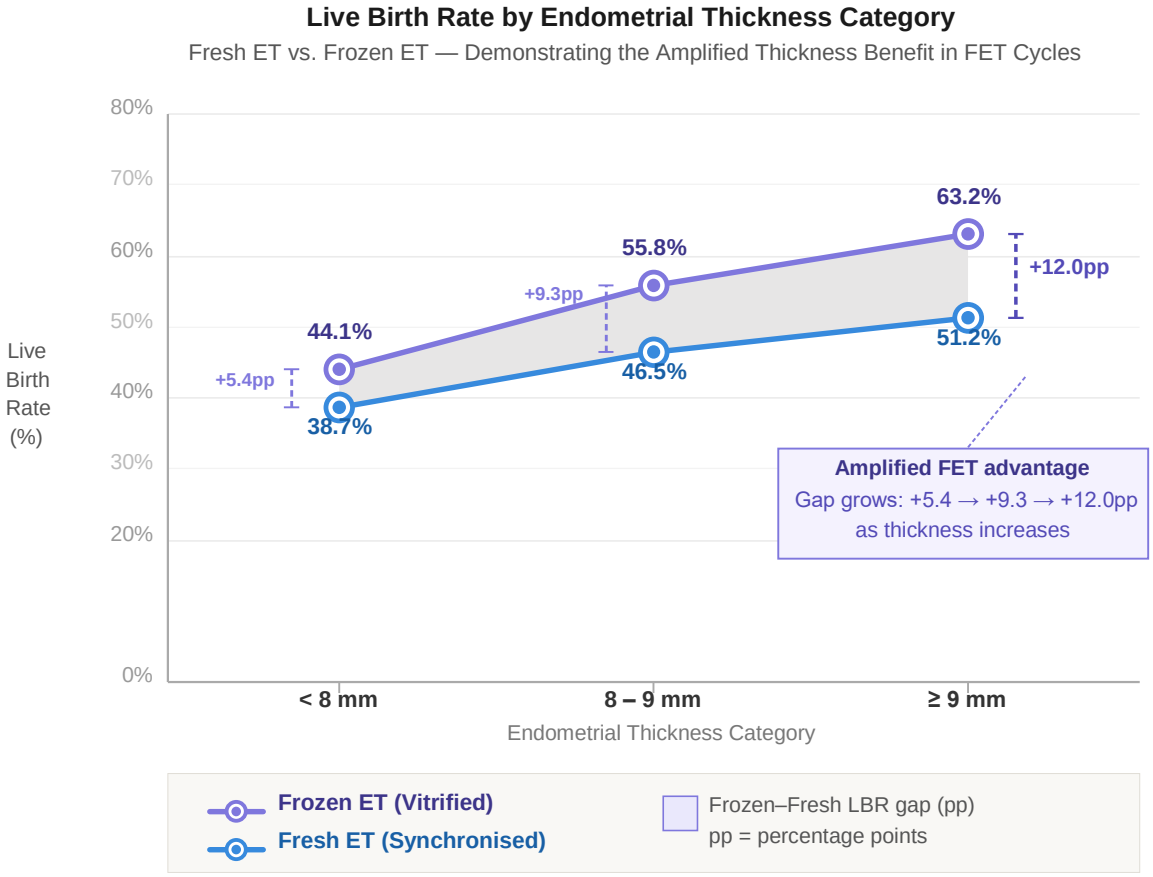

Figure 1: Line Graph: Live Birth Rate by Endometrial Thickness Category in Fresh vs. Frozen ET Groups — Demonstrating the Amplified Thickness Benefit in FET Cycles, Source: Author Generated

4.3 Vitrification Safety: Miscarriage Analysis

Across all subgroups analysed, miscarriage rates were statistically equivalent between fresh and frozen transfer cycles. Even in the blastocyst subgroup, where frozen transfer conferred the strongest LBR advantage, miscarriage rates remained comparable (Fresh: 15.8%; Frozen: 14.3%; p = 0.51). This consistency across subgroups reinforces that the vitrification process does not introduce genotoxic or epigenetic alterations that are clinically detectable through pregnancy loss rates.

Subgroup	Fresh ET Miscarriage Rate	Frozen ET Miscarriage Rate	p-value
Overall	16.5%	14.9%	0.52 (NS)
Day 5 Blastocyst	15.8%	14.3%	0.51 (NS)
Day 3 Cleavage	17.9%	16.2%	0.58 (NS)

Table 3. Miscarriage Rates Across Subgroups: Fresh vs. Frozen ET — Confirming Equivalence and Vitrification Safety. NS = Not Significant

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4.4 Mechanistic Statistical Model: Probability Estimation

Using the full interaction logistic regression model, predicted probabilities of live birth were estimated for clinical scenarios representing combinations of the key mechanistic variables. These predictions illustrate the cumulative advantage of optimising each variable — transfer modality, embryo stage, and endometrial thickness — simultaneously:

Clinical Scenario	Predicted LBR (%)
Fresh ET + Day 3 + Endometrium < 8 mm	~34%
Fresh ET + Day 5 + Endometrium 8–9 mm	~50%
Frozen ET + Day 3 + Endometrium 8–9 mm	~46%
Frozen ET + Day 5 + Endometrium ≥ 9 mm	~65%
Frozen ET + Day 5 + Good Quality + Endometrium ≥ 9 mm	~70%

Table 4. Model-Predicted Live Birth Rates for Clinical Scenarios Based on Interaction Logistic Regression Analysis.

5. Discussion

5.1 The Endometrial Receptivity Hypothesis

The research results demonstrate that the 'endometrial receptivity hypothesis' serves as the main explanation for the superior outcomes of frozen embryo transfers. The hypothesis states that the endometrium displays more consistent receptive patterns during programmed FET cycles than during synchronized fresh cycles which results in better outcomes through enhanced embryo-endometrium alignment (Simón et al., 2020; Shapiro et al., 2011). The hypothesis explains the dose-response relationship between endometrial thickness and LBR which shows stronger effects during FET cycles than fresh cycles.

The process of endometrial preparation in programmed FET cycles involves controlled processes which go through monitoring procedures. The medical team gives oestradiol until the body achieves the required endometrium thickness which triggers the start of progesterone treatment. This method of 'gate and release' prevents any transfer from occurring at a time when the endometrium does not meet its required level of preparation. The quality gate in a synchronised fresh donor cycle allows partial release because progesterone initiation must follow the timing of donor oocyte retrieval instead of waiting for the recipient's endometrial condition. The endometrial thickness at transfer shows a consistent pattern of lower measurements during fresh cycles which have an average thickness of 8.4 mm compared to frozen cycles that have an average thickness of 9.1 mm, and this difference has important clinical implications.

Scientists continue to study the implantation window which includes its duration and its molecular signature and its response to hormonal changes. Endometrial receptivity analysis (ERA) testing has demonstrated that the window is displaced in some patients and that personalised embryo transfer timing based on ERA can improve outcomes in patients with recurrent implantation failure. The studied cohort did not use ERA as a standard procedure but the FET process establishes a controlled hormonal environment which increases the chance of achieving accurate timing.

5.2 Blastocyst Culture as a Synergistic Factor

The significant Group × Stage interaction ($p = 0.038$) is arguably the most novel finding of this mechanistic analysis. It demonstrates that the frozen advantage is not a fixed benefit that applies equally to all embryo stages, but rather a benefit that is substantially amplified in blastocyst transfers. This synergistic pattern can be explained at two levels.

At the embryo level, blastocysts transferred in a FET cycle are the product of two sequential selection processes: first, the natural developmental selection that occurs during five days of culture; and second, the quality assessment performed by embryologists at the blastocyst stage. The resulting embryos are therefore enriched for

both chromosomal normality and morphological quality. When these highly selected embryos are transferred into an optimally prepared endometrium, the conditions for successful implantation are maximised.

At the endometrial level, blastocysts are better equipped to signal to the endometrium than cleavage-stage embryos. The trophectoderm of the blastocyst actively secretes factors — including hCG, which triggers the corpus luteum response, and various paracrine signalling molecules — that initiate and sustain the implantation process. In a FET cycle, the endometrium is primed by exogenous progesterone to respond to these signals at the moment of transfer, creating an optimal bidirectional communication that drives implantation.

For Day 3 embryos, by contrast, the developmental trajectory is less certain, the endometrial signalling capacity is absent, and the synchronisation between embryo and endometrium may be inherently less precise. The smaller and statistically non-significant frozen advantage for cleavage-stage transfers (5.8 percentage points, $p = 0.09$) reflects these mechanistic limitations. Clinically, this suggests that the frozen advantage is most reliably realised when combined with blastocyst culture — and that programmes offering only cleavage-stage transfers may not capture the full benefit of a freeze-all strategy.

5.3 Vitrification and Epigenetic Safety: Clinical Reassurance

The study found no significant difference between different groups which allows doctors to confirm that vitrification maintains special safety standards for embryo development. Miscarriage is one of the most sensitive indicators of embryo viability: it reflects failures of implantation, early placentation, and chromosomal integrity. The data at hand demonstrates that frozen embryo groups experience no increased miscarriage rates which would have occurred if vitrification caused actual epigenetic or chromosomal alterations to their DNA.

Research which compares gene expression between vitrified and fresh embryos has revealed some transcriptional changes which affect their stress response pathways. The differences between both groups exist only for a short time period because they do not lead to any visible clinical effects which result in pregnancy loss or perinatal outcomes (Pelkonen et al., 2015; Chen et al., 2021). Controlled cryogenic stress shows human embryos develop biological resilience which maintains their clinical viability through the blastocyst stage when expanded cellular mass provides structural protection against potential damage.

5.4 Clinical Implications and Practice Recommendations

Taken together, the mechanistic and clinical evidence from this study supports three practice recommendations for donor IVF programmes. First, a freeze-all strategy with vitrification should be the default approach, reserving fresh transfers only for exceptional circumstances where logistical or clinical factors make FET impractical. Second, extended culture to blastocyst stage should be pursued wherever laboratory infrastructure supports it, as the synergistic benefit of frozen blastocyst transfer (predicted LBR ~65–70%) substantially exceeds what can be achieved with either modification alone. Third, endometrial thickness monitoring should be rigorous, and transfer should be deferred until the endometrium meets ≥ 8–9 mm criteria — a threshold at which the LBR advantage of FET is most pronounced.

These recommendations are consistent with international guidelines from ESHRE and the American Society for Reproductive Medicine (ASRM), which increasingly favour a freeze-all approach in donor programmes. In the Indian context, where the ART Act 2021 mandates standardised outcome reporting, systematic adoption of these evidence-based practices will contribute to improved national IVF outcome benchmarks.

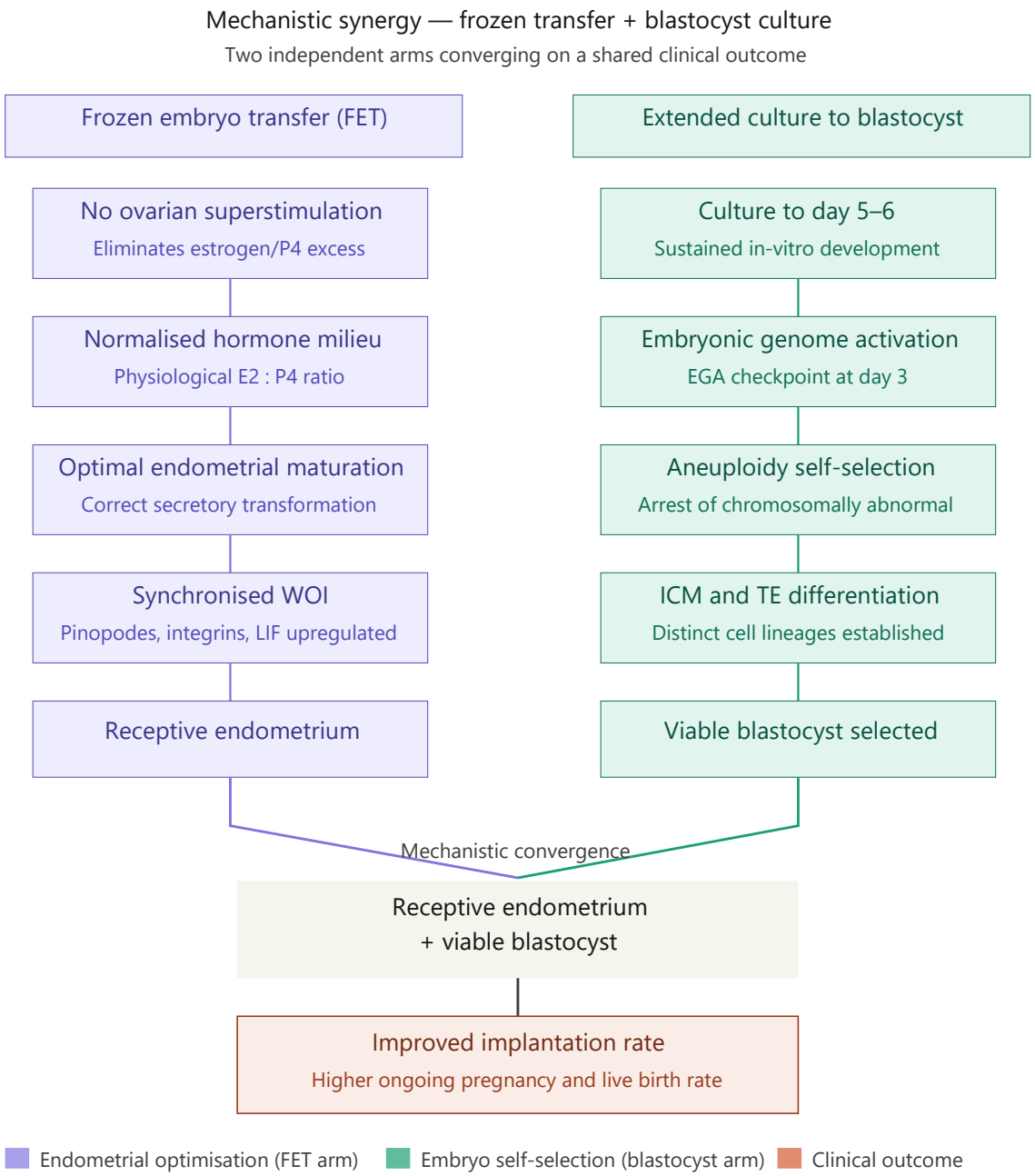

Figure 2: Conceptual Diagram: Mechanistic Synergy Between Frozen Transfer and Blastocyst Culture — Convergence of Endometrial Optimisation and Embryo Self-Selection, Source: Author Generated

6. Limitations and Future Research Directions

This study has several limitations that contextualise its findings. The retrospective design limits causal inference, and unmeasured confounders — including donor age, laboratory technician skill, and cycle-to-cycle variability in vitrification protocols — cannot be fully accounted for. The single-centre nature of the dataset may limit generalisability to programmes with different laboratory standards, patient demographics, or donor pool characteristics.

The absence of endometrial receptivity array (ERA) testing data is a significant limitation for mechanistic interpretation. Future studies should prospectively incorporate ERA assessment in FET cycles to directly test the hypothesis that displaced implantation windows account for a portion of the fresh cycle disadvantage observed

here. Similarly, preimplantation genetic testing for aneuploidy (PGT-A) data would allow a more precise characterisation of the chromosomal selection advantage of blastocyst culture, independent of morphological grading.

Longitudinal follow-up of children born from these cycles — examining perinatal outcomes, birthweight, and long-term health parameters — would provide the most definitive evidence regarding the epigenetic safety of vitrification in the context of donor embryo programmes. Multicentre prospective trials, ideally with randomisation between fresh and frozen transfer in donor cycles where both are clinically feasible, represent the highest-priority future research direction.

7. Conclusion

This mechanistic and clinical analysis of a five-year donor IVF cohort demonstrates that the superiority of frozen embryo transfer is not a uniform phenomenon but one that is substantially shaped by embryo developmental stage and endometrial preparation quality. The statistically significant interaction between transfer modality and embryo stage confirms a synergistic benefit for frozen blastocyst transfers, which achieve predicted live birth rates of 65–70% under optimal conditions. The safety of vitrification is confirmed by the equivalence of miscarriage rates across all subgroups.

The mechanistic explanation for these findings integrates endometrial receptivity physiology — specifically the superior synchronisation between embryo and endometrium in programmed FET cycles — with the self-selecting biological advantage of blastocyst culture. Together, these factors create a clinical environment in which the probability of successful implantation is maximised. For donor IVF programmes globally, and particularly in the rapidly evolving Indian ART landscape, these findings provide a mechanistic evidence base for the recommendation of frozen blastocyst transfer as the gold standard approach.

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