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Original Research Article

Morphokinetic trajectory analysis for conservative embryo deselection in two-embryo transfer cycle: a pilot time-lapse imaging study

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ABSTRACT

Background: Time-lapse imaging enhances conventional morphological assessment by providing continuous developmental monitoring and novel morphokinetic parameters; however, its potential application as a conservative embryo deselection strategy remains inadequately explored. Therefore, this pilot study aimed to evaluate the feasibility of time-lapse morphokinetic analysis for identifying embryos with the lowest developmental viability in the in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycle.
Methods: A retrospective, observational, single-center, case-control, pilot study was conducted over a 3-month period at a tertiary care center in India. Morphokinetic parameters were compared between embryos associated with successful twin live births and failed pregnancies using generalized estimating equation models with a Gaussian distribution. A reference morphokinetic trajectory was derived from successful embryos using empirical 5th and 95th percentile limits for each developmental parameter.
Results: Twenty-nine IVF/ICSI cycles contributed 58 embryos, including 32 embryos from 16 cycles resulting in successful twin live birth and 26 embryos from 13 cycles resulting in pregnancy failure. Women in the successful outcome group were younger and had higher anti-Mullerian hormone concentrations than those with failed pregnancies. Embryos from successful cycles exhibited significantly greater morphokinetic progression and a more consistent developmental trajectory than embryos from failed cycles. About 81.3% of embryos from successful cycles remained within the reference developmental window, compared with only 34.6%of embryos from failed cycles.
Conclusions: The study suggests that time-lapse morphokinetic trajectory analysis may provide a feasible framework for a conservative embryo deselection by identifying embryos with markedly deviant developmental kinetics. These findings are hypothesis-generating and require validation in larger, independent cohorts before clinical implementation.

Keywords: Compaction, Embryo, IVF, ICSI, Morphokinetics, Time-lapse imaging

INTRODUCTION

IVF is a key assisted reproductive technique used in infertility treatment, involving ovarian stimulation, oocyte retrieval, fertilization and subsequent embryo culture under controlled conditions to achieve a successful pregnancy.[1] Although IVF techniques have achieved notable advancements, the overall success rate remains modest, since in-vitro embryo development remains suboptimal, with many high-quality embryos failing to implant, resulting in average live birth rates of approximately 30% per embryo transfer.[1-3] Therefore, optimizing embryo selection is critical for improving the efficacy of IVF treatment and increasing success rates.[1] Precise embryo selection aims to shorten the time to gestation and a healthy live birth by choosing the embryo with the highest implantation potential, thereby minimizing the number of transfer cycles required to achieve successful gestation and accelerating pregnancy. Moreover, accurate embryo selection allows single-embryo transfer, thereby reducing the risk of multiple pregnancies.[4] Traditionally, the embryo selection

approach has been based on static morphological assessment at specific developmental stages, including pronuclear appearance (PNA), early cleavage patterns, multinucleation and zona pellucida characteristics, which is a subjective method with considerable inter-observer variability. Moreover, it provides limited information on embryo viability.[1,3,5,6] Additionally, embryo development is a dynamic process and status can change dramatically within hours, potentially causing key events to be missed between observation points.[6]

Over the past two decades, time-lapse monitoring (TLM) has emerged as a promising tool for improving embryo selection.[7] TLM uses digital imaging and specialized software to enable continuous, non-invasive monitoring of embryo morphokinetics, including PNA, pronuclear disappearance (PND), cell divisions and blastocyst formation (at 5-20 min time interval) without disturbing the culture conditions.[1,5,8-10] Through real-time imaging of embryo development, TLM allows the identification of specific morphokinetic markers associated with embryo implantation potential and provides deeper insights into preimplantation embryo development.[5,6,10] A major advantage of TLM is its ability to apply predictive algorithms that integrate morphological and morphokinetic parameters for embryo selection or deselection.[10,11] Current embryo selection approaches predominantly emphasize positive selection of the 'best' or healthy, euploid embryos over the unhealthy, aneuploid embryos, with the greatest potential to achieve a viable pregnancy.[7] TLM studies have demonstrated that euploid embryos exhibit a much tighter division pattern and developmental kinetics, whereas aneuploid embryos frequently deviate from these developmental patterns.[12,13] However, increasingly aggressive embryo selection strategies may inadvertently exclude embryos with retained developmental competence, potentially contributing to suboptimal reproductive outcomes. A conservative embryo deselection strategy based on qualitative morphokinetic parameters rather than quantitative measurements may provide a safer approach by identifying embryos with the lowest developmental viability while minimizing the risk of excluding potentially competent embryos.

The present study is a pilot study aimed at exploring a conservative embryo deselection framework based on time-lapse morphokinetic analysis of IVF/ICSI-generated embryos. This framework provides preliminary evidence for evaluating the feasibility of embryos with the lowest developmental viability, rather than focusing exclusively on selecting the 'best' embryo for transfer.

METHODS

Study design

This retrospective, observational, single-center, case-control, pilot study was designed to develop a conservative, non-invasive embryo deselection model. A total of 120 cycles were screened before exclusion and 33 cases were considered. Data were collected over a 3 months period from January 2020 to March 2020 at a tertiary care center in Visakhapatnam, India (Krishna IVF Clinic), using the clinic's obstetric medical record system, maintained in a relational database application and development platform called FileMaker Pro.

Sample selection

Records of female patients undergoing IVF/ICSI treatment cycles in which two Grade 1 compacted embryos were transferred on Day 4 post insemination were retrospectively reviewed. The study included only fresh embryo transfer cycles. Cases were selected only when the transfer of two embryos resulted in healthy twin live births, thereby ensuring successful implantation and development of both embryos. The control group consisted of female patients who underwent transfer of two Grade 1 compacted embryos but failed to achieve clinical conception.

Embryos were grouped by pregnancy outcome. The case group included embryos from cycles resulting in twin live births, while the control group included embryos from cycles with a negative serum β-human chorionic gonadotropin (β-hCG) test 10 days after embryo transfer, indicating implantation failure or absence of pregnancy. Fetal viability was confirmed by the first antenatal ultrasound examination performed two weeks after a positive serum hCG test, which showed two gestational sacs.

Frozen-cycle transfers and donor-cycle transfers were excluded from the study. Additionally, patients with extensive uterine adenomyosis or other uterine anomalies, patients in whom the transfer of two embryos resulted in a singleton live birth or in whom the transfer of three embryos resulted in the development of two gestational sacs were excluded from the study.

The time-lapse images of the corresponding embryo culture dishes for both successful and failed pregnancy groups were subsequently evaluated. Demographic details of the corresponding patients were retrieved from the FileMaker Pro database.

Ovarian stimulation, oocyte retrieval and embryo culture

Controlled ovarian stimulation was performed following standard protocol using a gonadotropin-releasing hormone (GnRH) agonist regimen. Final oocyte maturation was induced by hCG when adequate follicular development was achieved, followed by transvaginal oocyte retrieval approximately 36 hours later. Follicles were aspirated and oocytes were washed and cultured at 37°C for 4 hours before denudation by mechanical pipetting.

ICSI was subsequently performed using conventional micromanipulation techniques. Oocytes were placed in pre-equilibrated slides and following microinjection,

presumptive zygotes were individually cultured in pre-equilibrated microwells within a time-lapse incubation system. Embryos were cultured in an integrated embryo culture time-lapse incubator, the Geri+ Time-lapse System (Genea Biomedx; Serial no: GERI03113 and GERI03155; Software Version: 6.2.0cr3) containing G-TL single-step culture medium (Genea Biomedx) from Day 0 (post-insemination) through Day 5, enabling continuous embryo development and monitoring without disturbance.

Evaluation of time-lapse images of morphokinetic parameters

Blastocysts were morphologically evaluated according to the Gardner and Schoolcraft grading system, based on the degree of blastocoel expansion and hatching status (scores 1–6), inner cell mass (ICM) quality (grades A–C) and trophectoderm (TE) quality (grades A–C).[14] A detailed description of blastocyst scoring is presented in Appendix 1. Blastocyst quality was recorded using the standard nomenclature (e.g., 4AA, 5AB, 3BB).

To ensure consistency across embryos, all developmental events were temporally standardized relative to the post-insemination time point and expressed as hours post insemination (hpi). Embryos with acceptable morphology were analyzed for morphokinetic parameters. Geri Connect and Assess software (Serial No. OTY3NTYY, Software Version 2.2 4409.6135b49) was used to log and record the precise timing of PNA, time of pronuclear fading (tPNf), time of PND, time of first cell division producing 2-cell stage (t2), time to 3-cell stage (t3), time to 4-cell stage (t4), time to 5-cell stage (t5), time to 6-cell stage (t6), time to 8-cell stage (t8) and time to morula/compaction stage (TM). Time-lapse images were captured at 5-minute intervals across 11 focal planes for all embryos throughout preimplantation development from post-insemination until the Day 4 compaction stage (92-96 hours), after which embryo transfer was performed.

Post-embryo transfer luteal phase support was provided with vaginal administration of Crinone gel twice daily. No difficulties were encountered during embryo transfer in any of the cases. Endometrial thickness was uniformly above 7 mm, with a trilaminar appearance and adequate cervical secretions.

Morphokinetic trajectory development

For each embryo, morphokinetic trajectories were reconstructed as sequential temporal profiles representing dynamic developmental progression. The information from the TLM system was exported as a CSV file and then imported into Microsoft Excel (Microsoft Corp., Redmond, WA, USA) based on the timings of different embryonic events (t2, t3, t4 and TM compacted stages). Embryo developmental trajectories using an automated plotting process the minimum (Tmin) and maximum (Tmax) developmental times were generated using an automated plotting process rather than artificial intelligence (AI). A canonical morphokinetic trajectory was developed as a conservative approach to embryo deselection rather than as a direct embryo-ranking model. Embryos from successful twin live birth cycles were used to create reference morphokinetic trajectories for ideal developmental kinetics. Embryos showing significant temporal deviations from these reference trajectories were categorized as low-competence and appropriate for conservative deselection.

Statistical analysis

Continuous variables were summarized using the mean and standard deviation (SD) and categorical variables as counts (n) and percentages. Baseline characteristics were presented descriptively by pregnancy outcome; no formal statistical comparisons were performed due to the exploratory nature of the study and small sample size. Morphokinetic parameters were compared between embryos from successful and failed cycles using generalized estimating equation (GEE) models with a Gaussian distribution, identity link function and an exchangeable correlation structure to account for clustering of embryos within the same IVF cycle. Results are reported as β coefficients (mean differences), 95% confidence intervals (CIs) and p-values.

A reference morphokinetic trajectory was derived from successful cycles using empirical 5th and 95th percentile limits for each developmental parameter. Embryos were classified as being within or outside the reference trajectory based on these predefined windows. The association between trajectory classification and pregnancy outcome was assessed at the cycle level using Fisher's exact test. Odds ratios (ORs) and 95% CIs were calculated using contingency tables. A two-sided p-value <0.05 was considered statistically significant.

Ethical approval

This retrospective study was conducted in accordance with the routine IVF consent protocol, which included participants' approval for the use of their images and demographic data for research purposes. No separate Institutional Ethics Committee/Institutional Review Board approval was required. The study adhered to the ethical standards while promoting transparency and reproducibility. All patient and embryonic data were anonymized prior to analysis. No identifiable patient information was used at any stage.

RESULTS

Baseline clinical characteristics

Data from 29 patients were included in the study, comprising 16 patients with successful twin live births (cases) and 13 patients with failed pregnancy outcomes (controls). Female age, body mass index (BMI), anti-Mullerian hormone (AMH) level and cause of infertility

are reported in table 1. Women achieving successful twin live birth tended to be younger and had higher AMH levels than those experiencing pregnancy failure, whereas BMI appeared broadly similar between groups.

A total of 58 embryos, including 32 embryos derived from 16 IVF/ICSI cycles resulting in twin live births and 26 derived from 13 IVF/ICSI cycles with failed pregnancy outcomes, were analyzed. Implantation of transferred embryos was confirmed by ultrasound of the gestational sacs. In successful cycles, two gestational sacs were observed after dual embryo transfer, enabling reliable attribution of embryo outcomes.

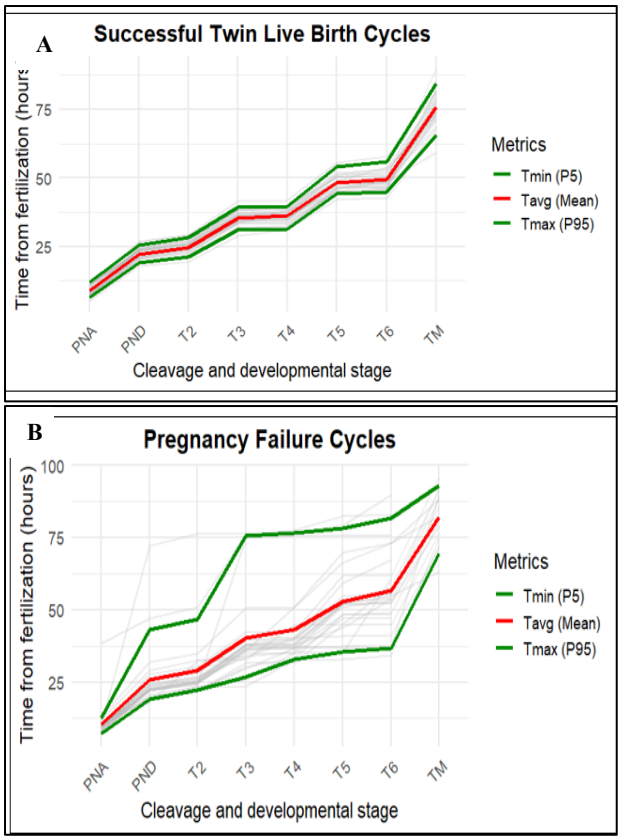

Figure 1 (A, B): Morphokinetic trajectories of embryos, successfully implanted embryos leading to live birth, embryos associated with failed pregnancies.

Morphokinetic parameter evaluation of embryos

Morphokinetic analysis revealed that embryos from successful cycles exhibited consistently earlier morphokinetic development than embryos from failed cycles at all assessed time points.

Using GEE models accounting for clustering of embryos within cycles, statistically significant differences (β=Successful − Failed) were observed at the t4stage (β=-6.93, 95% CI -12.05 to -1.81; p=0.008), t-6 stage (β=-7.58, 95% CI -14.50 to -0.65; p=0.032) and TM stage (β=-6.20, 95% CI -10.97 to -1.42; p=0.011), while t2 and t3 stages showed borderline statistical difference (t2: p=0.054; t3: p=0.054) (Table 2). These findings suggest that embryos leading to successful pregnancy reach key developmental milestones approximately 6–8 hours earlier than those associated with pregnancy failure.

Canonical morphokinetic trajectory development

A reference morphokinetic trajectory was constructed using embryos that resulted in successful twin live birth (n=32). For each developmental milestone, the acceptable developmental window was defined by the empirical 5th and 95th percentiles of the observed timing distribution. The resulting reference intervals were: PNA, 5.52-12.15 hpi; PND, 18.37-26.75 hpi; t2, 20.48-29.20 hpi; t3, 30.08-40.28 hpi; t4, 31.01-40.45 hpi; t5, 43.30-55.26 hpi; t6, 44.13-56.70 hpi; and TM, 63.39-87.35 hpi (Table 1).

Embryos were classified as being within the reference trajectory when all morphokinetic parameters fell within these predefined developmental windows; embryos with one or more parameters outside these limits were classified as outside the reference trajectory. When this trajectory was applied to all embryos, 81.3% of successful embryos remained within the reference developmental window compared with only 34.6% of embryos associated with failed pregnancy outcomes.

Conversely, trajectory deviation was observed in 65.4% of embryos with failed pregnancy outcomes but only 18.7% embryos from successful cycles. Cycle-level analysis demonstrated a strong association between trajectory deviation and pregnancy failure. Cycles containing at least one embryo outside the reference trajectory exhibited 23.03-fold higher odds of pregnancy failure (OR 23.03, 95% CI 2.32–1217.51; Fisher's exact p=0.0018) (Table 3).

Embryos resulting in successful twin live births exhibited a consistent morphokinetic trajectory (as shown in the graphs), with most Tmin and Tmax values clustered closely around the cohort mean. In contrast, embryos associated with failed pregnancy outcome exhibited heterogeneous, significantly deviated morphokinetic trajectories, with broad confidence intervals for Tmin and Tmax (Figure 1).

Apparent classification performance of the reference morphokinetic trajectory

Exploratory assessment of classification performance demonstrated a sensitivity of 65.4% (95% CI 44.3–82.8), specificity of 81.2% (95% CI 63.6–92.8), positive predictive value of 73.9% (95% CI 51.6–89.8), negative predictive value of 74.3% (95% CI 56.7–87.5) and apparent overall accuracy of 74.1% (95% CI 61.0–84.7).

Table 1: Baseline characteristics of the patients.

Patient characteristics and causes of infertility	Patients with successful twin live birth (n=16)	Patients with failed pregnancy outcome (n=13)
Female characteristics (Mean±SD)		
Age (in years)	29.81±3.53	33.23±5.54
BMI	24.98±2.78	23.73±3.62
AMH (mg/ml)	2.83±2.03	1.15±0.81
Cause of infertility N (%)		
Unexplained	NA	0 (0.00%)
Endometriosis	3 (18.75%)	0 (0.00%)
Male factors	16 (100.00%)	13 (100.00%)
Tubal factors	1 (6.25%)	3 (23.08%)
Reduced ovarian reserve	4 (25.00%)	7 (53.85%)
Reduced ovarian reserve, endometriosis+tubal	NA	4 (30.77%)

Table 2: Morphokinetic differences between embryos resulting in successful twin live birth and associated with pregnancy failure.

Parameter	Embryos from successful cycles (n=32) Mean±SD	Embryos from failed cycles (n=26) Mean±SD	β (95% CI)	P value
PNA	9.00±1.81	10.27±5.93	-1.27 (-3.62, 1.08)	0.291
PND	22.22±2.21	25.97±10.93	-3.75 (-8.21, 0.71)	0.099
t2	24.65±2.27	29.15±11.24	-4.50 (-9.07, 0.07)	0.054
t3	35.42±2.77	40.18±14.07	-4.77 (-9.61, 0.07)	0.054
t4	36.18±2.73	43.11±13.17	-6.93 (-12.05, -1.81)	0.008
t5	48.16±3.17	52.83±13.13	-4.67 (-10.26, 0.93)	0.102
t6	49.42±3.46	56.52±14.15	-7.58 (-14.50, -0.65)	0.032
TM	75.85±6.39	82.06±8.87	-6.20 (-10.97, -1.42)	0.011

Table 3: Pregnancy outcomes according to adherence to the reference morphokinetic trajectory.

Outcome	Within reference trajectory (P5-P95)	Outside reference trajectory (P5-P95)	Odds ratio (95% CI), P value
Embryo-level classification			
Embryos from successful cycles (n=32)	26 (81.3%)	6 (18.7%)	—
Embryos from failed cycles(n=26)	9 (34.6%)	17 (65.4%)	—
Cycle-level association analysis			
Embryos from successful cycles (n=16)	11	5	Reference
Embryos from failed cycles (n=13)	1	12	23.03(95% CI 2.32–1217.51),0.0018)

Table 4: Successful embryos using empirical percentile limits.

Parameter	Mean (hpi)	SD	T_{min} (P5)	T_{max} (P95)
PNA	9.00	1.81	5.52	12.15
PND	22.22	2.21	18.37	26.75
t2	24.65	2.27	20.48	29.20
t3	35.42	2.77	30.08	40.28
t4	36.18	2.73	31.01	40.45
t5	48.16	3.17	43.30	55.26
t6	49.42	3.46	44.13	56.70
TM	75.85	6.39	63.39	87.35

DISCUSSION

The current pilot study demonstrates that embryos associated with successful pregnancies follow relatively synchronized, tightly clustered morphokinetic trajectories, with a narrow confidence interval between minimum and maximum timings of developmental events, especially during early cleavage, suggesting lower developmental asynchrony and greater morphokinetic stability.

In contrast, the embryos associated with failed pregnancy outcomes show broader confidence intervals, indicating greater temporal variability and asynchronous developmental patterns. Confidence interval-based thresholds at the 5th percentile may facilitate the identification and deselection of embryos that exhibit significant developmental deviations, potentially supporting the deselection of embryos with lower developmental competence. Women with unsuccessful pregnancy outcomes were older and had lower AMH levels compared to the women with successful twin live births. The observed differences in age and AMH are clinically relevant and may represent potential confounding factors when interpreting associations between morphokinetic parameters and pregnancy outcomes. This may reflect poorer baseline reproductive prognosis and may contribute to differences in embryo development and implantation outcomes. Consequently, the observed morphokinetic differences should be interpreted as exploratory and hypothesis-generating.

Continuous image acquisition by TLM helps detect subtle developmental abnormalities and enables detailed assessment of dynamic cleavage synchrony, which are often missed by conventional morphological assessment methods.[15,16] The findings of the present study are consistent with those of Maghier et al and Liu et al studies, which demonstrated that continuous TLM provides additional morphokinetic information that may support embryo selection and deselection strategies in IVF practice. However, both studies also observed that not all developmental timing parameters were consistently associated with implantation or live birth outcomes, indicating that the clinical utility of individual morphokinetic markers remains variable and requires further validation.[13,15] Accordingly, morphokinetic thresholds should be validated within individual IVF laboratories before broader clinical application, given the influence of laboratory-specific conditions (such as culture media, incubator systems, oxygen tension, insemination methods, annotation practices and patient populations) on embryo developmental kinetics.

The morphokinetic trajectories served as the reference framework for the proposed conservative embryo-deselection model, which represents a preliminary approach for identifying embryos whose marked deviations from these trajectories may be associated with reduced developmental competence and viability. The findings suggest that adherence to the reference morphokinetic developmental windows is associated with successful reproductive outcomes and may represent a useful adjunctive marker of embryo competence. However, given the exploratory nature of the present study, this concept requires validation in larger and more diverse patient populations. If validated, it may provide a biologically interpretable approach to assessing developmental competence using morphokinetic parameters. Moreover, future integration of the proposed trajectory into AI-based tools may enable more standardized and reproducible embryo selection and deselection in IVF practice.

Blastomere compaction is the first morphogenetic event, typically occurring on Day 4 of fertilization, when the embryo proceeds to the morula stage.[17] Compaction initiation varies among embryos; however, it is typically confirmed at 74-85 hpi or just after the 8-cell stage. In about 10% of embryos, compaction occurs during early cleavage, before the 8-cell stage.[17,18] The timing and completeness of compaction are linked to subsequent embryonic developmental potential and quality.[18] Embryos that achieve complete compaction are more likely to develop into high-quality blastocysts with favorable chromosomal profiles, whereas partial or delayed morula formation is associated with lower blastocyst formation rates, increased aneuploidy and lower implantation rates.[19] Building on these findings, the present study used a routine Day 4 compaction-stage transfer protocol, combined with TLM, for all embryos to investigate the relationship between compaction dynamics and subsequent embryo viability. Although compaction timing is regarded as a significant morphokinetic marker of embryo developmental ability, in the present study, TM values in both groups showed considerable variability in compaction dynamics and substantial overlap between embryos with divergent developmental outcomes. The findings suggest that compaction timing alone may not adequately distinguish embryos with differing developmental potential, highlighting the multifactorial nature of embryo competence. Since compaction occurs relatively late in preimplantation development and close to the embryo transfer decision, its use in predictive models warrants careful consideration. Otherwise, it may result in temporal information leakage if the intended prediction time point is not defined clearly.

Consequently, model performance may be overestimated if predictor variables are obtained at or after the clinical decision point, resulting in temporal information leakage. Consistent with Davis et al such timing-related bias can lower the validity and clinical applicability of prediction models.[20]

Consequently, late-stage morphokinetic parameters should be interpreted within a clearly defined prediction horizon to minimize temporal information leakage and support clinical applicability. The exploratory predictive analysis demonstrated moderate-to-good discriminatory performance, with an overall accuracy of 74.1%,

suggesting that adherence to a successful morphokinetic trajectory may be associated with favorable reproductive outcomes and may serve as a useful adjunctive tool for embryo selection/deselction in IVF practice. However, the predictive performance estimates should be interpreted as exploratory due to the limited sample size and potential within-cycle correlations among embryos.

The study has several limitations. First, the sample size was relatively small and the findings were derived from a pilot dataset from a single center, limiting their generalizability. Second, embryo-level outcomes were derived from cycle-level clinical outcomes rather than being directly linked to individual embryos' fates, potentially leading to misclassification. Third, late-stage morphokinetic parameters, such as the timing of morula compaction, which often occurs near the embryo transfer decision point, may inadvertently incorporate post-decision information, thereby introducing bias into predictions of embryo selection.

Moreover, since the study intentionally compared outcome extremes, such as confirmed twin live birth versus failed pregnancy after double embryo transfer, the derived morphokinetic boundaries may overestimate the ability to discriminate and require testing in a broader clinical population, including singleton pregnancies, biochemical pregnancies, miscarriages and single-embryo transfer cycles.

Furthermore, some developmental events may have occurred outside the predefined observation period and, therefore, may not be captured. This censoring may affect trajectory estimation and the interpretation of morphokinetic patterns. A key strength of this study is that the proposed model can serve as a foundational framework for the development of clinically interpretable AI-based models for embryo deselection in IVF.

CONCLUSION

This retrospective pilot study suggests that time-lapse morphokinetic trajectory analysis may provide a feasible framework for a conservative embryo deselection by identifying embryos with markedly deviant developmental kinetics. Early cleavage parameters appeared more informative than late compaction timing, which showed substantial overlap between outcome groups. Because the study was small, single-center and based on outcome-extreme double embryo transfer cycles, the proposed framework should be considered hypothesis-generating. Validation in larger, independent cohorts, particularly single embryo transfer cycles with embryo-specific outcomes, is required before clinical implementation.

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