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DEVELOPMENT OF A PREDICTIVE MODEL FOR EMBRYO TRANSFER OUTCOMES BASED ON EEVA, MORPHOKINETIC PARAMETERS AND MORPHOLOGICAL PARAMETERS OF BLASTOCYSTS

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INTRODUCTION

1. Rationale for the Study

Selecting embryos with high developmental potential is crucial for In vitro fertilization (IVF) success [1]. Currently, embryo assessment is primarily based on morphology, but conventional morphology-based assessment has limited accuracy and predictive value and depends on embryologist expertise [2], while removing embryos from the incubator may disrupt environmental stability and development [3].

Time-lapse culture systems integrated with automated embryo assessment software enable continuous monitoring and morphokinetic data collection without removing embryos from the incubator [4]. Software such as EEVA (Early Embryo Viability Assessment) and iDAScore (Intelligent Data Analysis Score) may improve the objectivity of embryo assessment. However, models integrating morphokinetic parameters, automated embryo assessment software, and morphological characteristics to predict embryo developmental potential remain limited and have primarily undergone internal validation [5–16]. In Vietnam, evidence in this field remains limited [17,18]. Therefore, developing a predictive model that integrates morphokinetic parameters, blastocyst morphology, and automated embryo assessment software is necessary. On this basis, we conducted the study entitled "Development of a model for predicting embryo transfer outcomes based on EEVA, morphokinetic parameters and morphological blastocyst"

2. Research Objectives

1. To evaluate pregnancy outcomes following single frozen blastocyst transfer based on embryo quality assessed using the EEVA software alone or in combination with blastocyst morphology.
2. To develop a predictive model for the implantation rate following single frozen blastocyst transfer based on EEVA grades, morphokinetic parameters and morphological parameters of blastocyst.

3. Scope of the Study

The study was conducted at the Assisted Reproduction Center, Tam Anh General Hospital, Hanoi, from March 2023 to December 2025.

4. Research Content

- To evaluate the predictive performance of the EEVA automated embryo assessment software at day 3 in predicting blastocyst formation rate and blastocyst morphological quality.
- To evaluate the associations between blastocyst morphokinetic parameters and blastocyst morphological quality.
- To evaluate the association between blastocyst morphological quality and pregnancy outcomes.
- To evaluate the association between EEVA scores and pregnancy outcome.
- To evaluate the association between blastocyst morphokinetic parameters and pregnancy outcomes.
- To develop and evaluate a predictive model for embryo implantation following single frozen blastocyst transfer based on the EEVA automated embryo assessment software, blastocyst morphology, and selected blastocyst morphokinetic parameters.

Chapter 1. LITERATURE REVIEW

1.1. Preimplantation Development of the Human Embryo

Preimplantation development of the human embryo begins with the zygote following fertilization, when the two pronuclei fuse. On day 2, the embryo cleaves into 2–4 cells. It reaches 6–8 cells in day 3, with increasing cell-to-cell adhesion. On day 4, the embryo develops into a morula comprising approximately 16–32 cells and begins the process of compaction. On day 5, the blastocyst forms, consisting of the blastocoel, the inner cell mass (ICM), and the trophectoderm (TE), in preparation for implantation. In IVF, embryos are typically transferred to the uterus on day 3 or day 5.

1.2. Embryo Quality Assessment

Embryo quality assessment systems: conventional morphological assessment; embryo assessment using preimplantation genetic testing for aneuploidy (PGT-A); embryo assessment using embryo biomarkers; and embryo assessment using time-lapse embryo culture systems, including morphokinetic assessment and EEVA software.

1.3. Research Status

1.3.1. Global Research Status

a. Studies on predictive models based on morphokinetic parameters

Numerous studies have developed models to predict embryo implantation potential based on morphokinetic parameters. Meseguer et al. used t5 (the time to reach the 5-cell stage), cc2 (the duration of the second cell cycle), and s2 (the synchrony of the second cell division) to classify embryos according to their cleavage timings, achieving an area under the curve (AUC) of 0.72 [10]. Petersen et al. (2016) developed a decision-tree model using tPNf (the time of pronuclear fading), t2, t3, t5, and t8 (the times to reach the 2-, 3-, 5-, and 8-cell stages) based on multicentre data comprising 3.275 day-3 embryo transfers, with an adjusted AUC of approximately 0.64–0.65 [11]. Other models have combined morphokinetic parameters with conventional morphological assessment to improve embryo selection [12]. However, when validated using independent datasets, their performance declined (AUC, 0.52–0.63; positive predictive value [PPV], <45%) [13]. These findings indicate that the predictive value of such models remains limited and is influenced by differences among IVF centres.

b. Studies on prediction models combining embryo assessment software, morphokinetic parameters, and morphology

Numerous studies have developed embryo selection models combining embryo assessment software, morphokinetic parameters, and morphology. Adamson et al. (2016) demonstrated that a model combining EEVA and morphological assessment improved the implantation rate

compared with morphology-based embryo selection (30.2% vs. 19.0%, p = 0.003). However, the study was limited by differences between the groups [14]. Valera et al. (2023) developed three models (EEVA, ASEBIR morphological assessment; and a combination of both), with AUC values ranging from 0.61 to 0.63, indicating limited predictive performance and the presence of multiple confounding factors [9]. Bori et al. (2022) evaluated the KIDScore D5 model, which combines morphokinetic parameters and blastocyst morphology, and reported an AUC of 0.633 [84]. Overall, these models demonstrate potential, but their predictive value remains limited owing to confounding factors and differences among IVF centres. Therefore, a model suited to the conditions of each centre is needed to improve embryo selection.

1.3.2. Research in Vietnam

In Vietnam, studies on morphokinetic parameters have mainly focused on embryo development and aneuploidy, with limited research on implantation potential. Nguyen Thuy Tra My et al. (2021) reported low predictive performance using t5 (AUC = 0.511; n = 331) [17], while Phi Thi Tu Anh et al. (2023) developed a decision - tree based on 383 blastocysts, achieving an AUC of 0.641 [18]. These models have limited predictive value and have primarily undergone internal validation, highlighting the need for centre-specific models, as recommended by ESHRE [4]. Therefore, this study investigated the associations of EEVA, morphokinetic parameters, and blastocyst morphology with implantation potential and developed a predictive model integrating these factors.

Chapter 2. STUDY SUBJECTS AND METHODS

2.1. Study Period and Setting

2.1.1. Study Setting

The study was conducted at the Reproductive Medicine Center, Tam Anh General Hospital, Hanoi, Vietnam.

2.1.2. Study Period

The study was conducted from May 2023 to December 2025.

2.2. Study Subjects

2.2.1. Study Population

A total of 2.522 embryos from 291 ICSI cycles cultured to day 5 or 6 in the GERI time-lapse incubator were analyzed to investigate the association between EEVA scores and blastocyst outcomes. 511 single frozen–thawed blastocyst transfer cycles were used to develop predictive model, meeting the inclusion and exclusion criteria at Tam Anh General Hospital from March 2020 to December 2023.

2.2.2. Inclusion Criteria

- Patients indicated for blastocyst culture in the GERI time-lapse incubator in combination with EEVA.
- Single frozen–thawed blastocyst transfer cycles with known implantation outcomes.
- No decline in blastocyst quality after thawing
- Endometrial thickness (EMT) before embryo transfer of >7 mm. According to the meta-analysis by Kasius et al. [80], clinical pregnancy rates were significantly lower when pre-transfer EMT was ≤7 mm than when EMT was >7 mm.
- No uterine cavity abnormalities or concomitant uterine or tubal pathologies, including endometriosis, uterine fibroids, hydrosalpinx, intrauterine adhesions, fluid accumulation at the cesarean section scar, uterine malformations, or endometrial polyps.
- Sufficient information available for the study.

2.2.3. Exclusion Criteria

- Oocyte donation or gestational carrier cycles.
- Severe sperm abnormalities or use of surgically retrieved sperm.
- Embryos that had undergone preimplantation genetic testing (PGT).

2.3. Study Design

This study employed a retrospective cohort study design. The study procedures are presented in the study flowchart.

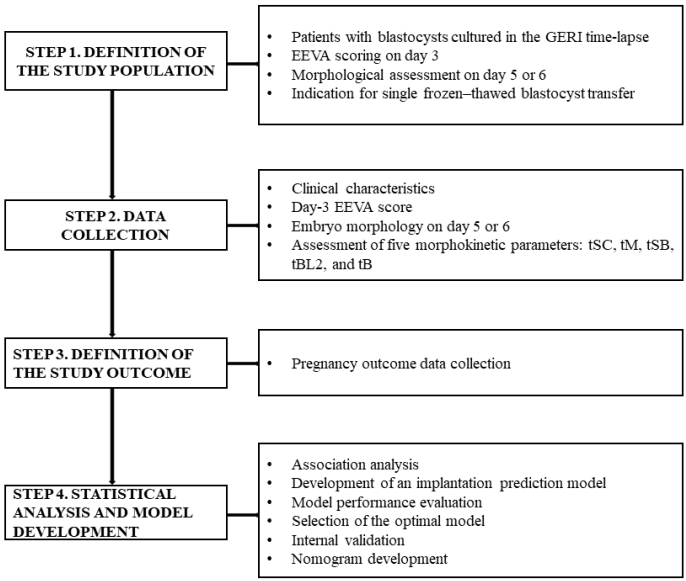


Figure 2.1. Summary Flowchart of the Study

Figure **2.1** presents a summary flowchart of the study, outlining the following steps:

Step 1: Identification of the Study Population

Patients whose embryos were cultured to the blastocyst stage (day 5 or day 6) in a GERI time-lapse incubator were identified for analysis of blastocyst outcomes. The EEVA software assigned a score to each day-3 embryo, while day-5 or day-6 blastocysts were assessed using conventional morphological criteria. From this patient population, the database was reviewed to identify all single frozen–thawed blastocyst transfer cycles (day 5 or day 6) performed at the Assisted Reproductive Technology Centre, Tam Anh General Hospital, from March 2020 through December 2023, for pregnancy outcome analysis and model development. Cases were included if they met the study inclusion criteria and none of the exclusion criteria.

Step 2: Collection of Clinical Characteristics and Embryo Parameters

Clinical characteristics were retrospectively collected from each patient's medical records. The day-3 EEVA scores and day-5 or day-6 morphological grades of the transferred blastocysts were obtained from the embryo monitoring records for each patient.

In addition, time-lapse videos recorded by the incubator were reviewed and analyzed to extract five embryo morphokinetic parameters: tSC (time at the start of compaction), tM (time at the completion of compaction), tSB (time at the onset of blastulation), tBL2 (time when the blastocoel reached half the blastocyst volume), and tB (time when the blastocoel was fully expanded).

Step 3: Determination of Study Outcomes

Pregnancy outcomes following embryo transfer were collected. These data were obtained from the system used by the Reproductive Medicine Center to manage and report monthly treatment outcomes. Pregnancy follow-up information for patients monitored at the center was documented in their medical records and entered the data management system. For patients who did not undergo pregnancy follow-up at the center, the call center contacted them monthly to collect information on pregnancy outcomes after embryo transfer. The information was then recorded in the center's data management system.

Step 4: Statistical Analysis and Model Development

Analyses were performed to evaluate the association between EEVA scores and blastocyst outcomes, as well as the associations of EEVA scores, morphological characteristics, and morphokinetic parameters with pregnancy outcomes. A model was developed to predict the implantation potential of blastocysts based on these parameters. Model fit was assessed, and the optimal model was selected for internal validation. A nomogram was then constructed to provide a visual representation of the relationships between the predictors and the probability of implantation.

2.4. Sampling Method

The study employed a consecutive convenience sampling method, following the chronological order of eligible cases, as described below:

Step 1: Data were extracted from the database of the Reproductive Medicine Center, Tam Anh General Hospital, for treatment cycles performed between March 2020 and December 2023.

Step 2: Medical records were reviewed, and treatment cycles were screened according to the prespecified study inclusion and exclusion criteria.

Step 3: Cycles meeting all study eligibility criteria were selected, and the required information was collected, including data on embryo culture using the GERI time-lapse system and outcomes of single frozen–thawed embryo transfer cycles.

Step 4: Eligible cycles were included in the study until the target sample size specified in the study design was reached.

2.5. Sample Size Calculation

For the development of a model to predict the implantation rate:

The pmsampsize package [82] in R software was used to calculate the sample size required for developing a prognostic model with a binary outcome. The parameters used for sample size calculation included a shrinkage factor of 0.9 (the software's default value), a maximum of seven candidate predictors in the model (including EEVA, blastocyst morphology, and five morphokinetic parameters: tSC, time at the onset of compaction; tM, time at the completion of compaction; tSB, time at the onset of blastulation; tBL2, time when the blastocoel reached half the blastocyst volume; and tB, time when the blastocoel was fully expanded), a blastocyst implantation rate of 59% reported in the previous study by Korkidakis et al. [83], and an AUC of 0.7 for models predicting blastocyst implantation potential based on morphokinetic parameters [84].

Using R software, the required sample size for model development was calculated to be 428 embryos (Figure **2.2**). During the study, data were

collected for 511 embryos that met the eligibility criteria for analysis.

2.5. Sample Size Calculation Method

For the development of a model to predict the implantation rate:

```
Given input C-statistic = 0.7  & prevalence = 0.59
Cox-Snell R-sq = 0.132
NB: Assuming 0.05 acceptable difference in apparent & adjusted R-squared
NB: Assuming 0.05 margin of error in estimation of intercept
NB: Events per Predictor Parameter (EPP) assumes prevalence = 0.59

 Samp_size Shrinkage Parameter CS_Rsq Max_Rsq Nag_Rsq  EPP
 Criteria 1     428     0.900         7  0.132   0.742   0.172  36.0
 Criteria 2     188     0.805         7  0.132   0.742   0.172  15.9
 Criteria 3     374     0.900         7  0.132   0.742   0.172  31.7
 Final          428     0.900         7  0.132   0.742   0.172  36.0

Minimum sample size required for new model development based on user inputs = 428,
with 252 events (assuming an outcome prevalence = 0.59) and an EPP = 36.0
```

Figure 2.2. Sample size calculation results using R software

2.6. Data processing and statistical analysis

2.6.1. Software and principles of data analysis

After collection, the data were coded and managed using Microsoft Excel. Statistical analyses were performed using IBM SPSS Statistics version 26 and R statistical software version 4.5.2.

Statistical tests were conducted at a significance level of $\alpha = 0.05$. A p-value of less than 0.05 was considered statistically significant.

2.6.2. Statistical analysis plan

Step 1: Description of study participants

- Categorical variables, including binary, nominal, and ordinal variables, were presented as frequencies and percentages.
- Continuous variables were summarized as the mean ± standard deviation (SD) for normally distributed data, or as the median and interquartile range (IQR; Q1–Q3) for non-normally distributed data.

Step 2: Statistical testing

The distribution of continuous variables was assessed using the Kolmogorov–Smirnov or Shapiro–Wilk test.

- For normally distributed data, means between two groups were compared using the independent-samples t-test. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) was used.
- For non-normally distributed data, comparisons between two groups were performed using the Mann–Whitney U test, the nonparametric equivalent of the independent-samples t-test. For comparisons involving more than two groups, the Kruskal–Wallis test, the nonparametric equivalent of one-way ANOVA, was used. When statistically significant differences were identified, pairwise post hoc comparisons were performed using the Mann–Whitney U test with Bonferroni correction.
- For categorical variables, proportions were compared using the chi-square test or Fisher's exact test when the expected cell frequency was less than 5.

  A p-value of less than 0.05 was considered statistically significant.

Step 3: Assessment of variables before model development [88]

- Univariate generalized estimating equation (GEE) analyses were performed to identify variables associated with implantation outcomes. This approach accounted for the correlation between embryos from the same patient, as a patient could contribute multiple embryo transfer cycles to the study.
- Multicollinearity among morphokinetic variables was assessed using linear regression, based on the tolerance statistic and the variance inflation factor (VIF). A tolerance value of less than 0.2 or a VIF greater than 5 was considered indicative of substantial multicollinearity.
- Variables that were statistically significant or clinically relevant were considered for inclusion in the multivariable GEE models [88.89].

Step 4: Development of models to predict the implantation rate following single frozen–thawed blastocyst transfer using multivariable GEE [9.89]

- Five embryo-selection models were developed using multivariable GEE logistic regression: (1) Model 1, the morphology model; (2) Model 2, the EEVA model; (3) Model 3, the morphokinetic model; (4) Model 4, the combined EEVA and morphology model; and (5) Model 5, the combined EEVA, morphology, and morphokinetic model. GEE was used to account for clustering because a patient could undergo embryo transfer in two cycles, meaning that the observations were not independent [9].
- Model calibration: Model calibration was assessed using a calibration plot and calibration metrics to evaluate the agreement between the outcome probabilities predicted by the model and the observed outcome rates [90].
- Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC). AUCs were compared between models using Patient-level cluster bootstrap method. A p-value < 0.05 was considered statistically significant [91.92].

Step 5: Internal validation of the selected model [93.94]

Internal validation was performed using 1.000 bootstrap resamples to estimate optimism and obtain optimism corrected performance measures [93.94].

Model performance was evaluated in three domains:

- Discrimination: assessed using the AUC.
- Calibration: assessed using a calibration plot.
- Overall predictive accuracy (Brier score): defined as the mean squared difference between predicted probabilities and observed outcomes.

Step 6: Nomogram development

A nomogram was developed using R statistical software version

4.5.2, based on the final multivariable logistic regression model, to provide a visual representation of the relationships between prognostic factors and the probability of implantation.

Chapter 3. RESULTS AND DISCUSSION

3.1. General Characteristics of the Study Population

Table 3.1. General Descriptive and Demographic Characteristics of Patients Undergoing Intracytoplasmic Sperm Injection (ICSI)

Characteristic	Value
Number of cycles	291
Maternal age, year, mean±SD	30.49±4.90
Min – max maternal age, years	20-49
BMI (kg/m^2) (mean ± SD)	21.11±2.4
Number of oocytes retrieved, Median (IQR, Q1-Q3)	17 (13-23)
Number of oocytes ICSI, Median (IQR, Q1-Q3)	13 (9-17)
Fertilazation rate, *%*	83.8
Number of blastocyst cultured embryos	2522
Blastocyst rate, % (number)	61 (1537/2522)

** Continuous data are presented as the mean ± standard deviation (mean ± SD) or median (interquartile range, IQR; Q1–Q3); categorical data are presented as percentages (%).*

Table 3.2. Patient characteristics in embryo transfer cycles

Characteristic	Value
Number of embryo transfer	511
Maternal age, year, mean±SD	30.5±4.9
AMH (mean ± SD)	5.9 ± 4.2
AFC (mean ± SD)	30.9 ±16.8
BMI (kg/m^2) (mean ± SD)	21.07 ± 2.54
Endometrial thickness (mm) (mean ± SD)	9.5±1.2
- Primary infertility (n, %) - Secondary infertility (n, %)	287 (56.2%) 224 (43.8%)

AMH, anti-Müllerian hormone, a marker of ovarian reserve; AFC, antral follicle count; BMI, body mass index

Table 3.3. Characteristics of embryos in transfer cycles

Characteristic		Value: n (%)
Age of embryo	**Day 5** **Day 6**	460 (90%) 51 (10%)

Characteristic		Value: n (%)
Morphological quality	**Rất tốt (RT)**	293 (57.3%)
	Tốt (T)	167 (32.7%)
	Trung bình (TB)	51 (10%)
Blastocyst expansion	**BL3**	20 (3.91%)
	BL4	53 (10.37%)
	BL5	243 (47.55%)
	BL6	161 (31.51%)
	BL7	34 (6.66%)
Quality of ICM	**ICM A**	352 (68.88%)
	ICM B	113 (22.12%)
	ICM C	46 (9%)
Quality of TE	**TE A**	312 (61.06%)
	TE B	157 (30.71%)
	TE C	42 (8.23%)
Score of EEVA	**E1**	220 (43.05%)
	E2	134 (26.22%)
	E3	76 (14.87%)
	E4	52 (10.18%)
	E5	29 (5.68%)
Implantation rate		343 (67.1 %)
Clinical pregnancy rate		279 (54.6 %)
Ongoing pregnancy rate		254 (49.7 %)
Live birth rate		230 (45 %)

3.2. Embryo Assessment Results Using the EEVA Software

The predictive value of EEVA for blastocyst formation (Table **3.5**) and morphological quality (Tables **3.6–3.9**) was evaluated using GEE models adjusted for maternal and embryo age. GEE model was used to evaluate EEVA's predictive value while accounting for the correlation between embryos from the same patient [9,123]. aOR, adjusted odds ratio; 95% CI, 95% confidence interval. Statistical significance was set at $p < 0.05$.

Table 3.5. GEE Analysis of the association between EEVA scores and blastocyst formation

	Blastocyst		
Variable	**a*OR***	**95% CI**	**p**
E2 vs E1	0.653	0.452 – 0.942	0.023
E3 vs E1	0.300	0.214 – 0.423	<0.001
E4 vs E1	0.151	0.109 – 0.210	<0.001
E5 vs E1	0.053	0.038 – 0.074	<0.001

Table 3.6. GEE analysis of the association between EEVA scores and the formation of excellent and good quality blastocysts

	Excellent and good quality blastocysts		
Variable	**a*OR***	**95% CI**	**p**
E2 vs E1	0.584	0.410 – 0.831	0.003
E3 vs E1	0.496	0.344 – 0.713	<0.001
E4 vs E1	0.314	0.218 – 0.452	<0.001
E5 vs E1	0.207	0.138 – 0.309	<0.001

Table 3.7. GEE analysis of the association between EEVA scores and ICM morphological quality (grade A and grade C)

	ICM A			ICM C		
Variable	**a*OR***	**95% CI**	**p**	**a*OR***	**95% CI**	**p**
E2 vs E1	0.725	0.547-0.959	0.025	1.480	0.979-2.238	0.063
E3 vs E1	0.598	0.444-0.806	0.001	1.680	1.098-2.570	0.017
E4 vs E1	0.351	0.254-0.486	<0.001	2.962	1.965-4.465	<0.001
E5 vs E1	0.300	0.203-0.443	<0.001	3.781	2.414-5.921	<0.001

Table 3.8. GEE analysis of the association between EEVA scores and TE morphological quality (grade A and grade C)

	TE A			TE C		
Varible	**a*OR***	**95% CI**	**p**	**a*OR***	**95% CI**	**p**
E2 vs E1	0.699	0.527-0.927	0.013	2.139	1.375-3.328	0.001
E3 vs E1	0.566	0.418-0.767	<0.001	2.142	1.352-3.395	0.001
E4 vs E1	0.312	0.221-0.440	<0.001	3.861	2.446-5.953	<0.001
E5 vs E1	0.174	0.108-0.279	<0.001	4.482	2.762-7.274	<0.001

Table 3.9. GEE analysis of the association between EEVA scores and blastocyst expansion (BL > 2)

	BL 3, 4, 5, 6, 7		
Variable	**a*OR***	**95% CI**	**p**
E2 vs E1	0.831	0.390-1.770	0.631
E3 vs E1	0.493	0.241-1.006	0.050
E4 vs E1	0.266	0.136-0.520	<0.001
E5 vs E1	0.210	0.103-0.425	<0.001

3.3. Association between morphokinetic parameters and blastocyst morphological quality

Table 3.3 presents the characteristics and numbers of transferred embryos by morphological grade, ICM quality, TE quality, and blastocyst expansion. Associations between morphokinetic parameters and overall

morphological grade, ICM grade, TE grade, and blastocyst expansion were investigated by comparing the median morphokinetic values across the respective subgroups.

Data are presented as medians (IQR; Q1–Q3); groups were compared using the Kruskal–Wallis test followed by Bonferroni-corrected Mann–Whitney U tests (p1, excellent vs. good; p2, excellent vs. fair; p3, good vs. fair), with $p < 0.05$ considered significant. tSC, time at the onset of compaction; tM, time at the completion of compaction; tSB, time at the onset of blastulation; tBL2, time when the blastocoel reached half the blastocyst volume; tB, time when the blastocoel was fully expanded (Tables **3.10 - 3.13**.)

Table 3.10. Association between morphokinetic parameters and embryo morphological grade

	Execllent (n=293)	**Good (n=167)**	**Fair (n=51)**	**(p1, p2, p3)**
tSC	65.00 (59.00-72.00)	72.00 (62.00-79.40)	76.05 (66.50-85.00)	0.001, 0.001, 0.012
tM	81 (75.35-85.75)	85.00 (80.10-91.05)	90.15 (83.20-98.15)	0.001, 0.001, 0.002
tSB	94.55 (90.15-98.10)	96.45 (93.45-101.30)	102.30 (96.05-108.45)	0.001, 0.001, 0.001
tBL 2	100.25 (96.30-104.18)	104.00 (99.25-109.10)	109.00 (102.00-118.30)	0.001, 0.001, 0.001
tB	104.00 (99.55-108.15)	108.00 (102.30-112.40)	116.00 (105.00-124.55)	0.001, 0.001, 0.001

Table 3.11. Association between morphokinetic parameters and ICM morphology

	ICM A (n=352)	**ICM B (n=113)**	**ICM C (n=46)**	**(p1, p2, p3)**
tSC	65.02 (59.27-73.15)	73.00 (62.77-81.18)	74.73 65.35-85.03)	0.001, 0.001, 0.147
tM	81.22 (76.00-86.88)	86.00 (81.00-92.00)	90.08 (83.15-96.80)	0.001, 0.001, 0.058
tSB	95.03 (90.41-98.15)	98.1 (94.03-103.10)	101.40 (95.88-108.18)	0.001, 0.001, 0.147
tBL2	101.00 (96.55-104.39)	105.40 (100-110.20)	109.63 (101.39-116.65)	0.001, 0.001, 0.004
tB	104.25 (100.20-108.4)	109 (102.17-114.1)	115.50 (103.93-123.58)	0.001, 0.001, 0.002

Table 3.12. Association between morphokinetic parameters and TE morphology

	TE A (n=312)	TE B (n=157)	TE C (n=42)	(p1, p2, p3)
tSC	65.03 (59.04-72.50)	72.05 (62.18-80.40)	73.28 (66.24-84.59)	0.001, 0.001, 0.099
tM	81.00 (75.67-86.19)	86.00 (80.78-91.43)	88.70 (82.79-97.03)	0.001, 0.001, 0.084
tSB	94.53 (90.11-98.09)	97.40 (94.08-102.78)	101.23 (95.88-107.54)	0.001, 0.001, 0.017
tBL2	100.20 (96.26-104.13)	105.00 (100.30-109.73)	107.89 (101.39-117.95)	0.001, 0.001, 0.009
tB	103.53 (99.51-108.13)	108.25 (103.53-113.40)	113.05 (103.93-124.69)	0.001, 0.001, 0.015

Table 3.13. Association between morphokinetic parameters and blastocyst expansion

	BL 3.4 (n=73)	BL 5.6,7 (n=438)	p
tSC	72.00 (62.00-83.00)	66.43 (60.04-75.44)	0.002
tM	85.40 (78.35-92.15)	82.32 (77.23-88.06)	0.020
tSB	97.35 (93.50-105.05)	95.35 (91.25-99.21)	0.003
tBL2	105.10 (100.08-112)	101.30 (97.30-106.30)	0.001
tB	108.05 (103.15-115.48)	105 (100.44-110.06)	0.001

3.4. Pregnancy outcomes following single frozen–thawed blastocyst transfer

The clinical characteristics of embryo transfer cycles were compared between the implantation and non-implantation groups (Table **3.14**). The independent predictive value of each parameter for implantation outcomes was assessed using GEE univariable [95].

Table 3.14. Clinical characteristics of embryo transfer cycles in the two groups

Characteristic	Implantation	Non- implantation	p value
Maternal age (yeare)	30.1 ± 4.2	30.9 ± 4.6	0.06[a]
Primary infertility Secondary infertility	198 (57.7%) 145 (42.3%)	93 (55.4%) 75 (44.6%)	0.694[c]
AMH	6.1 ± 4.2	5.8 ± 4.1	0.07[b]
AFC	31.6 ± 16.7	30.5 ± 16.9	0.07[b]
BMI	21.1 ± 2.5	20.9 ± 2.5	0.44[a]
Endometrial thickness	9.5 ± 1.2	9.4 ± 1.4	0.456[a]
Characteristic	**Implantation**	**Non- implantation**	**p value**
Maternal age (yeare)	29.7 ± 4.05	30.9 ± 4.5	0.052[a]

Primary infertility Secondary infertility	290 (56.8%) 221 (43.2%)	225 (54.7%) 186 (45.3%)	0.69[c]
AMH	6.0 ± 4.2	5.9 ± 4.1	0.055[b]
AFC	31.7 ± 16.7	30.4 ± 16.9	0.062[b]
BMI	21.1 ± 2.5	21.1 ± 2.5	0.953[a]
Endometrial thickness	9.6 ± 1.2	9.4 ± 1.3	0.059[a]

Note: Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as frequencies and percentages (%). P-values were calculated using the following tests: a: independent-samples t-test, b: Mann–Whitney U test, and c: chi-square test. AMH: anti-Müllerian hormone, a marker of ovarian reserve; AFC: antral follicle count; BMI: body mass index.

Table 3.21. Univariable analysis of factors associated with implantation

	Univariable Analysis		
Charactertistic	**aOR**	**95% CI**	**p value**
Maternal age (year)	-		0.069
Endometrial thickness	-		0.455
BMI	-		0.437
Embryo age (Day 5 and day 6)	0.393	0.219-0.705	0.002
BL4 vs BL3	-		0.631
BL5 vs BL3	-		0.423
BL6 vs BL3	-		0.456
BL7 vs BL3	-		0.768
ICM B vs ICM A	0.315	0.202-0.492	< 0.001
ICM C vs ICM A	0.131	0.067-0.257	< 0.001
TE B vs TE A	0.313	0.206-0.475	< 0.001
TE C vs TE A	0.070	0.032-0.155	< 0.001
E2 vs E1	0.791	0.484-1.293	0.350
E3 vs E1	0.335	0.194-0.580	< 0.001
E4 vs E1	0.259	0.138-0.485	< 0.001
E5 vs E1	0.245	0.111-0.544	0.001
tSC	0.977	0.961-0.994	0.008

tM	0.976	0.957-0.996	0.019
tSB	0.966	0.936-0.989	0.036
tBL2	0.957	0.935-0.980	<0.001
tB	0.955	0.935-0.989	0.034

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation

3.5. Development and evaluation of a model for predicting embryo implantation outcomes

Multivariable GEE was used to estimate implantation probability, including variables with $p < 0.05$ or clinical significance to assess their independent associations with the outcome

Five multivariable GEE models were developed: (1) morphology model (Table **3.24**); (2) EEVA model (Table **3.25**); (3) morphokinetic model (Table **3.26**); (4) combined morphology and EEVA model (Table **3.27**); and (5) combined morphology, EEVA, and morphokinetic (Table **3.28**).

The area under the receiver operating characteristic curve (AUC) was used to compare the discriminative ability of the predictive models. Patient-level cluster bootstrap method was applied to determine whether differences between two AUCs were statistically significant, with $p < 0.05$ considered statistically significant in this study (Table **3.30**) [99].

Bootstrap resampling with 1.000 repetitions was used for internal model validation and to construct calibration plots [97], aiming to evaluate the model's discrimination and degree of overfitting (Table **3.31**) [97].

The calibration plot of the predictive model (Model 4) after bootstrap correction (n = 511) is presented in Figure **3.7** [98.99].

A nomogram was constructed based on the final multivariable logistic regression model to visualize the relationships between predictive factors and the probability of implantation (Figure **3.8**). The nomogram was developed using R software, version 4.5.2.

Table 3.24. GEE Model assessing the association between embryo morphology and implantation outcomes, adjusted for maternal age and embryo Age (M1)

Characteristic	aOR	95% CI	p
Embryo Day 5 vs Day 6	-		0.759
ICM B vs ICM A	-		0.073
ICM C vs ICM A	-		0.929
TE B vs TE A	0.405	0.240-0.683	0.001
TE C vs TE A	0.072	0.017-0.293	< 0.001

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation.

Table 3.25. GEE model assessing the association between EEVA scores and implantation outcomes, adjusted for maternal age and embryo Age (M2)

	Multivariable		
Chracteristic	**aOR**	**95% CI**	**p value**
Embryo Day 5 vs Day 6	-		0.074
E2 vs E1	0.795	0.486-1.301	0.362
E3 vs E1	0.350	0.201-0.607	< 0.001
E4 vs E1	0.288	0.151-0.546	< 0.001
E5 vs E1	0.282	0.125-0.636	0.002

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation.

Table 3.26. GEE model assessing the association between morphokinetic parameter values and implantation outcomes, adjusted for maternal age and embryo age (M3)

	Multivariable		
Chracteristic	**aOR**	**95% CI**	**p value**
tSC	0.985	0.958-1.012	0.268
tM	1.016	0.980-1.052	0.394
tSB	1.002	0.994-1.010	0.590
tBL2	0.951	0.917-0.986	0.006
tB	1.004	0.987-1.021	0.662

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation

Table 3.27. GEE model assessing the added value of integrating EEVA with embryo morphology for predicting implantation outcomes, adjusted for maternal age and embryo age (M4)

	Multivariable		
Chracteristic	**aOR**	**95% CI**	**p value**
Embryo Day 5 vs Day 6	-		0.559
ICM B vs A	-		0.214
ICM C vs A	-		0.699
TE B vs A	0.436	0.256-0.742	0.002
TE C vs A	0.065	0.016-0.267	< 0.001
E2 vs E1	0.928	0.552-1.563	0.780
E3 vs E1	0.514	0.282-0.935	0.029
E4 vs E1	0.402	0.199-0.810	0.011
E5 vs E1	0.338	0.258-0.591	0.017

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation.

The corresponding regression equation was derived as follows:

Logit (p) *= 2,108 – 0,011 (maternal age) – 0,244 (embryo day 6) – 0,362 (ICM B) – 0,785 (ICM C) – 0,838 (TE B) – 2,715 (TE C) – 0,078 (E2) – 0,656 (E3) – 0,929 (E4) – 1,085 (E5)*

Table 3.28. GEE model assessing the added value of integrating EEVA, blastocyst morphology, and morphokinetic parameters for predicting implantation outcomes, adjusted for maternal age and embryo age (M5)

	Multivariable		
Chracteristic	**aOR**	**95% CI**	**p value**
Embryo Day 5 vs Day 6	1.296	0.540–3.113	0.562
E2 vs E1	0.928	0.549 – 1.568	0.780
E3 vs E1	0.515	0.282 – 0.940	0.031
E4 vs E1	0.404	0.199 – 0.821	0.012
E5 vs E1	0.399	0.274 – 0.785	0.030
ICM B vs A	0.682	0.378 – 1.229	0.203
ICM C vs A	1.294	0.329 – 5.093	0.712
TE B vs A	0.432	0.251 – 0.743	0.002
TE C vs A	0.064	0.016 – 0.267	< 0.001
tSC	0.991	0.962 – 1.021	0.559
tM	1.024	0.985 – 1.064	0.231
tSB	1.001	0.992 – 1.010	0.794
tBL2	0.976	0.893 – 1.067	0.595
tB	1.008	0.928 – 1.095	0.850

Note: OR, odds ratio; 95% CI, 95% confidence interval. p-value < 0.05 indicated a statistical significance. Analyses were performed using generalized estimating equation.

Table 3.30. Comparison of AUC differences between pairs of models for predicting blastocyst transfer outcomes using cluster bootstrap resampling

Cặp mô hình	ΔAUC	95% CI	p bootstrap
M1 vs M2	0.048	-0.005 vs 0.104	0.100
M1 vs M3	**0.079**	**0.013 vs 0.145**	**0.016**
M1 vs M4	**-0.027**	**-0.052 vs -0.001**	**0.048**
M1 vs M5	**-0.034**	**-0.068 vs -0.009**	**0.050**
M2 vs M3	0.030	-0.023 vs 0.084	0.278
M2 vs M4	**-0.075**	**-0.116 vs -0.034**	**0.001**
M2 vs M5	**-0.083**	**-0.126 vs -0.039**	**0.001**
M3 vs M4	**-0.105**	**-0.165 vs -0.045**	**0.001**
M3 vs M5	**-0.113**	**-0.162 vs -0.065**	**0.001**
M4 vs M5	-0.007	-0.029 vs 0.013	0.447

Note: ΔAUC, difference in the area under the receiver operating characteristic (ROC) curves between two models; 95% CI, 95% confidence interval. Differences in AUC and their 95% CIs were estimated using patient-level cluster bootstrap resampling with 1.000 repetitions to account for the dependence between embryo transfer cycles from the same patient. A p-value < 0.05 was considered statistically significant. Model 1 (M1): morphology; Model 2 (M2): EEVA; Model 3 (M3): morphokinetics; Model 4 (M4): combined EEVA and morphology; Model 5 (M5): combined EEVA, morphology, and morphokinetic parameters.

Table 3.31. Internal validation results for Model 4, integrating EEVA and morphology, using bootstrap resampling (B = 1.000)

Parameter	value	Interpretation
AUC (apparent)	0.73	Discriminative ability of the original model
AUC (optimism-corrected)	0.72	Discriminative ability after bootstrap correction
Optimism in AUC	0.01	Difference between the original and optimism corrected AUCs
95% CI for AUC	0.69–0.78	95% confidence interval for discriminative ability

Note: Internal model validation was performed using bootstrap resampling (B = 1.000). AUC, area under the receiver operating characteristic (ROC) curve. Bootstrap (B =

1.000), a resampling method involving 1.000 iterations of sampling with replacement to assess model stability and correct for optimism. Optimism, the degree to which model performance is overestimated in the training data, used to assess overfitting. 95% CI, 95% confidence interval for the AUC, indicating the precision of the estimate.

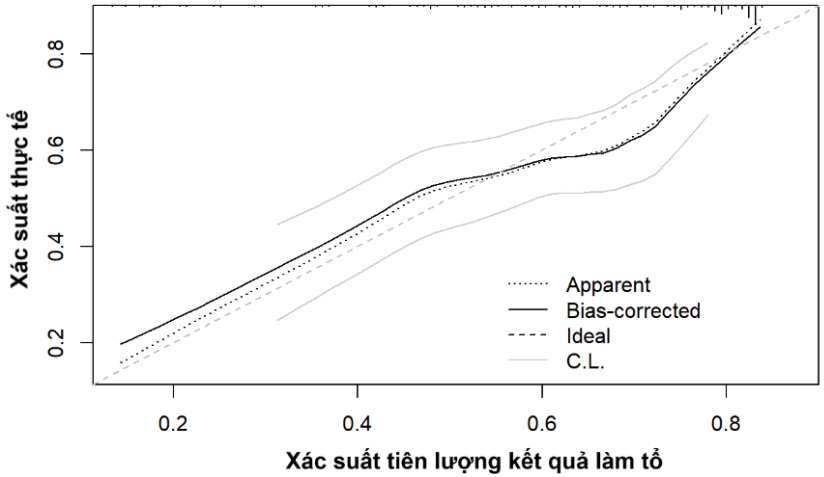


Figure 3.7. Calibration plot of the predictive model (Model 4) after bootstrap correction (n = 511)*. The plot illustrates the agreement between predicted probabilities and observed outcome probabilities for the predictive model, internally validated using bootstrap resampling. The dashed line (Ideal) represents perfect calibration (45° line). The dotted line (Apparent) represents calibration in the original dataset. The solid line (Bias-corrected) represents the calibration curve after correction for optimism using 1.000 bootstrap resamples. The gray lines (confidence limits, C.L.) indicate the 95% confidence intervals. Mean absolute error (MAE) = 0.028. Calibration slope = 0.91. Mean squared error (MSE) = 0.001. The 90th percentile of the absolute error = 0.053. The Brier score, used to assess the overall predictive accuracy of the model, = 0.18.*

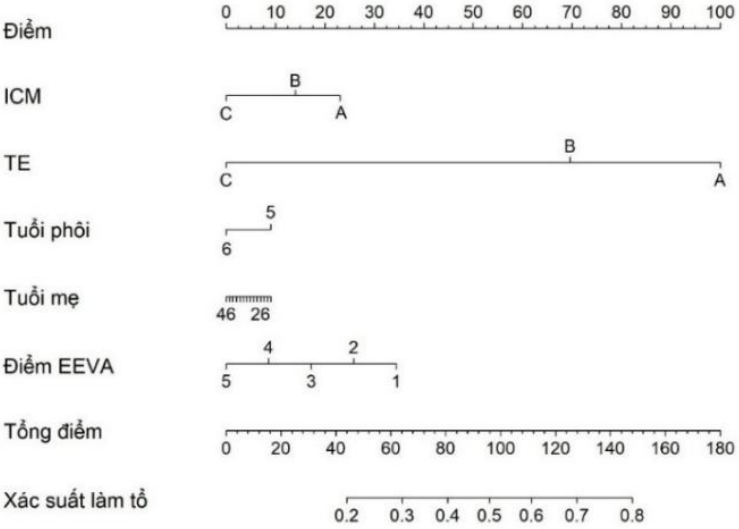


Figure 3.8. Nomogram for predicting the probability of implantation following single frozen–thawed blastocyst transfer based on Model 4.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. The value of the automated embryo assessment software EEVA in predicting culture outcomes and pregnancy outcomes

- EEVA has predictive value for blastocyst development and morphological quality, with higher scores associated with lower blastocyst formation rates and a greater likelihood of poor-quality blastocysts.
- EEVA scores are associated with implantation outcomes following single frozen–thawed blastocyst transfer, Higher EEVA scores are associated with lower implantation probability after single frozen–thawed blastocyst transfer, while combining EEVA with conventional morphology improves pregnancy outcome prediction.

2. Development and evaluation of a model for predicting implantation following frozen–thawed blastocyst transfer

- This study evaluated the predictive value of EEVA, conventional morphology, and blastocyst-stage morphokinetic parameters, developing models based on individual methods and their combinations. The optimal model combined EEVA scores with conventional morphological assessment
- The predictive model was developed using data from Vietnamese patients undergoing single frozen–thawed blastocyst transfer, integrating EEVA with morphological assessment based on current international consensus criteria. The model demonstrated good predictive performance, with a bootstrap-corrected AUC of 0.72
- Model performance was assessed in terms of discrimination and calibration, with internal validation using 1,000 bootstrap resamples. The model remained stable after internal validation and was presented as a nomogram to support embryo selection and counseling on single-embryo transfer in clinical practice.

RECOMMENDATIONS

Based on the study findings, we propose the following applications and directions for future research:

1. The embryo selection model combining EEVA assessment with morphological evaluation demonstrates good and stable predictive performance for implantation and can be applied to embryo selection for transfer.
2. Before the model can be widely implemented at other centers, external validation using data from each center is necessary.
3. Further studies with larger sample sizes and longer follow-up periods are needed to evaluate additional important clinical outcomes, including live birth rates and perinatal outcomes, thereby further improving predictive models in assisted reproduction.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. **Hop, V. D.**, Cuong, A. M., Anh, P. T., Huong, N. T., Hoang, L., & Hanh, N. V. (2024). Clinical validation of the early embryo viability assessment system: Analysis for the blastocyst morphology and pregnancy outcomes. *Asian Pacific Journal of Reproduction*, *13*(5), 219-227.
2. **Hop, V. D.**, Cuong, A. M., Anh, P. T. T., Huong, N. T. L., Hoang, L., & Van Hanh, N. (2025). Correlation between human embryo morphokinetics and pregnancy outcomes on Asian infertility women: A retrospective cohort study. *Asian Pacific Journal of Reproduction*, *14*(3), 140-142.
3. **Vu, D. H.**, Phi, T. T. A., An, M. C., Nguyen, T. M. T., Nguyen, T. L. H., Le, H., & Nguyen, V. H. (2025). Prediction of pregnancy outcomes of single vitrified-warmed blastocyst transfer using a combination of the Early Embryo Viability Assessment and morphological assessment. *Vietnam Journal of Biotechnology*, *23*(3), 275-295.