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Clinical validation of the early embryo viability assessment system: Analysis for the blastocyst morphology and pregnancy outcomes

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ABSTRACT

Objective: To determine the relationship between the early embryo viability assessment (EEVA) and blastocyst morphological parameters and pregnancy outcomes.

Methods: This retrospective cohort study was conducted on 291 intracytoplasmic sperm injection cycles including 2522 embryos with indications of prolonging embryo culture to the blastocyst stage in the Genea embryo review incubator, and 511 single vitrified-warmed blastocyst transfer cycles from January 2020 to June 2023. The EEVA system produced an EEVA score from E1 (best) to E5 (worse) for the potential of blastocyst formation. Blastocyst morphology was evaluated. The association between the EEVA score and each type of blastocyst morphology, implantation rate, clinical pregnancy, and ongoing pregnancy were assessed using generalized estimating equations.

Results: The inner cell mass A (ICM A), trophectoderm A (TE A), blastocoele expansion degree of 3, 4, 5, 6, 7 rates were higher with lower the EEVA score. The adjusted odd ratio (aOR) (E5 vs E1) was 0.3 for ICM A, 0.174 for TE A and 0.210 for BL3, 4, 5, 6, 7 (all $P < 0.001$), suggesting a significant association between lower EEVA scores and improved embryo quality. The implantation, clinical pregnancy, and ongoing pregnancy rate were also higher with lower the EEVA score. The aOR of E5 vs E1 was 0.245 for implantation, 0.185 for clinical pregnancy and 0.200 for ongoing pregnancy rate ($P < 0.001$).

Conclusions: There were associations between blastocyst morphology, pregnancy outcome and EEVA scores. The good blastocyst morphology and pregnancy outcomes are higher with lower the EEVA score.

KEYWORDS: Automated embryo assessment; Blastocyst; Early embryo viability assessment; Genea embryo review incubator; Morphology; Pregnancy outcomes; Timelapse

1. Introduction

In recent years, the trend of single embryo transfer to avoid multiple pregnancies has been applied to the embryo transfer policy of most assisted reproductive centers. Along with the priority selection of a transfer embryo, prolonging embryo culture to the blastocyst stage was also becoming more common[1]. However, there is still not a completely reliable method to select embryos with blastocyst development potential to apply worldwide[2].

To improve the culture and selection of embryos, the timelapse incubator system is a new technology that allows continuous observation and monitoring of embryos through a computer screen.

Significance

Automation of morphokinetic annotations such as early embryo viability assessment (EEVA) can be a potential approach that reduces subjectivity in embryo selection and improve the workflow in *in vitro* fertilization laboratories. This study determined the relationship between the EEVA score and blastocyst morphological parameters, and between the EEVA score and pregnancy outcomes. The study revealed that there were associations between blastocyst morphology, pregnancy outcomes and EEVA scores. The good blastocyst morphology and pregnancy outcomes are higher with lower the EEVA score.

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This provides a lot of information about the embryo's development process for embryologists without removing embryos outside of the incubator[3–6]. Many timelapse incubators have been introduced, such as the Embryoscope[7–9], Primo-Vision, ASTEC, etc[10]. However, the artificially intelligent early embryo viability assessment (EEVA) software with the latest version was created and adapted to be operated in only the Genea embryo review incubator (GERI incubator) to evaluate day 3 embryos automatically, supporting embryologists in predicting embryonic development ability[11].

EEVA is the first software approved by the US Food and Drug Administration for embryo assessment. EEVA software automatically detects the durations of the 2-cell (P2:t3–t2) and 3-cell (P3:t4–t3) stages and characteristics of the embryo during the first 3 days of embryonic development. This may avoid prolonging culture and save resources[2]. EEVA system assesses embryos automatically and classifies them into 5 degrees: E1, E2, E3, E4, and E5, with a decreasing ability to develop into the blastocyst stage.

There were many studies on the influence of timelapse incubators, but most of the studies were divided into three main categories: comparative studies of timelapse incubators and traditional incubators[8], studies on the support of timelapse on the traditional method of evaluating embryo morphology, and studies on the effectiveness of kinetics tracking and embryo selection of timelapse[12–14]. There have been many studies on the safety and effectiveness of the GERI incubator[15]. To date, there have been very few studies on the effectiveness of EEVA. The study showed that culturing embryos in a timelapse incubator using EEVA automatic embryo assessment software combined with traditional embryo assessment on day 3 predicts the likelihood of developing into blastocysts[16]. Another study showed that the combination of EEVA and traditional embryo evaluation helps to select embryos with a high implantation rate[2]. However, there were also some studies that showed no difference in the selection of embryos for implantation when using EEVA software alone or when EEVA was combined with morphological assessment[17–19].

The introduction of any new prognostic test into the *in vitro* fertilization (IVF) laboratory requires rigorous validation and demonstration of reproducibility. So far, in Southeast Asia in general and Vietnam in particular, this is the first center that uses a GERI incubator with integrated EEVA. EEVA is used to support traditional morphology assessment to predict the potential of blastocyst formation, but the relationship between the EEVA score and blastocyst morphology, between the EEVA score and pregnancy outcomes after single vitrified-warmed blastocyst transfer are unknown. We conducted the study to determine the relationship between EEVA score and some blastocyst morphological characteristics, and pregnancy outcomes. Therefore, this study will give us more evidence regarding EEVA, which could also predict blastocyst morphology, pregnancy outcomes, helping doctors choose the best embryos for transfer.

2. Materials and methods

2.1. Study design

This study was conducted at a single Assisted Reproductive Center. The study was designed as retrospective research with 291 intracytoplasmic sperm injection (ICSI) cycles, including 2 522 embryos with indications of prolonging embryo culture to the blastocyst stage in the GERI incubator and 511 single vitrified-warmed blastocyst transfer cycles from January 2020 to June 2023. All embryos were assessed by the EEVA Test on day 3, resulting in classification into scores from E1 (best) to E5 (worse) to predict the ability of blastocyst formation based on the embryo's morphokinetic characteristics. Blastocysts were morphologically assessed. The blastocyst transfer quality was based on the morphology (according to the scoring blastocyst by the Gardner and Schoolcraft grading system) at 116 ± 1 (Gardner and Schoolcraft 1999a, b)[20]. We reviewed all the data and investigated a relationship between the EEVA score and blastocyst morphology including inner cell mass (ICM) morphology, trophectoderm (TE) morphology and blastocoele expansion degree (BL), and between the EEVA score and pregnancy outcomes including implantation, clinical pregnancy, and ongoing pregnancy rates.

2.2. Inclusion criteria

The study included all patients who were indicated for blastocyst culturing in the GERI incubator and transferred a single blastocyst in a vitrified-warmed cycle. After thawing, the blastocyst transfer's quality did not decrease. The embryo transfer patient had an endometrial thickness of 7 mm to 13 mm. There were no uterine abnormalities, and there was enough information for the research.

2.3. Exclusion criteria

Exclusion criteria included oocyte donation, surrogacy, severely abnormal sperm, and preimplantation genetic testing (PGT).

2.4. Oocytes and sperm handling

Oocytes after retrieval were collected, washed, and incubated in pre-equilibrated G-IVF PLUS culture medium (Vitrolife, Sweden) at a benchtop BT37 incubator at 37 °C using 6% CO₂ and 5% O₂ mixing gas. After incubation (approximately 3 h), oocyte denudation was carried out by mechanical pipetting in G-IVF PLUS medium containing the enzyme hyaluronidase 80 IU/mL. After 2 h of culture in IVF PLUS medium, ICSI was performed in gamete medium (Cook Medical, USA) supplemented with HEPES at 200× magnification using a Nikon inverted microscope with Hoffman optics.

Sperm used for ICSI was filtered and washed by a concentration

gradient method. All oocytes after ICSI were cultured in the continuous single culture NXC plate (Irvine Scientific).

2.5. Embryo culture and imaging

Embryos were cultured in the GERI Dishes® with a capacity for up to 16 embryos sharing a common medium droplet of 80 µL of pre-equilibrated continuous single culture-NXC plate (Irvine Scientific) covered in 3.5 mL of mineral oil in the GERI incubator (Genea Biomedx, Australia). Embryos were kept inside the incubator uninterruptedly from day 1 to 3. The fertilization, day 2, and day 3 scores were performed on the GERI incubator's screen. The embryos were assisted in hatching on day 3, and the culture medium was changed outside the incubator. After assisted hatching, the embryos were cultured uninterruptedly to day 5, and 6.

The EEVA system 3.0 (Progyny Inc., CA, USA) was used to image embryonic development and predict the potential of blastocyst formation. The system consists of a microscope (EEVA scope), which was placed in a GERI incubator. Every 5 min, a single, high-resolution, single-plane dark field illumination image was taken of each embryo.

2.6. Embryo assessment

Evaluation and grading of embryo morphology were according to the Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011. Fertilization was assessed at (17±1) h post-ICSI by the presence of two pronuclei and two polar bodies. The number of blastomeres on embryo day 3 was assessed at (68±1) h post-ICSI. Blastocyst morphology was evaluated on day 5 and 6 based on cavity expansion, quality of TE, and ICM (according to the scoring blastocyst by grading system of Gardner and Schoolcraft) at 116±1 (Gardner and Schoolcraft 1999a, b)[20].

The EEVA assessment was based on day 3 embryo morphokinetics. EEVA software automatically recognized the durations of the 2-cell (P2; t3–t2) and 3-cell (P3; t4–t3) stages and characteristics of the embryo during the first 3 days of embryonic development. In addition, the number of blastomeres in the day 3 embryo of each embryo was put into the EEVA software by the embryologist. Then, EEVA software produced the EEVA grade automatically for each embryo. All embryos were classified by the EEVA software (with Version 3.0) and classified with a numeric score of E1, E2, E3, E4 and E5 according to their likelihood of reaching the blastocyst stage. The chances of forming the blastocyst are higher with lower the EEVA score. E1 will have the highest potential to form the blastocyst and E5 will have the lowest potential to form the blastocyst. The EEVA score is calculated based on the age of oocyte at the retrieval day; some morphokinetics including the duration of the 2 cells (P2:t3–t2), the duration of the 3 cells (P3:t4–t3); the cell number of embryo day 3 at 68 h; and some characteristics observed up to 68 h

post insemination, which are proprietary to the manufacturer.

2.7. Blastocyst vitrification and warming

For vitrification on day 5 or 6, blastocysts were required to attain a blastocyst expansion degree of 3 (>BL2). These blastocysts were vitrified immediately according to the cryotop method (Cryotech vitrification, Vitrolife, Sweden). If the developing embryo did not fulfill the criteria, it was cultured for a maximum of 7 days.

When the patient is indicated for single vitrified-warmed blastocyst transfer, the blastocyst will be warmed on the day of embryo transfer using the Cryotop method (Cryotech warming, Vitrolife, Sweden).

2.8. Post-warming embryo culture and vitrified-warmed blastocyst transfer procedure, and luteal support

Embryos were cultured in the continuous single culture NXC plate (Irvine Scientific) after warming for at least 1 h before embryo transfer. Blastocyst quality was assessed before transferring. Only blastocysts that maintain the same quality or develop better compared to blastocyst quality before vitrification were used in this study. A decreased-quality blastocyst will not be included in this study.

The hormonal replacement therapy (HRT) protocol was initiated on the second day of the menstrual cycle for all patients. To ensure optimal endometrial thickness, patients were required to undergo ultrasound check-ups of the endometrium, uterine, and adnexal on day 10 and 14 of the menstrual cycle. Once the endometrial thickness reached a minimum of 7 mm, endometrial transformation was achieved using vaginal micronized progesterone at a daily dosage of 600–800 mg (Utrogestan, Besins, Thailand, or Cyclogest, Actavis U.K. Limited, United Kingdom), in addition to orally administered dydrogesterone at a daily dose of 30 mg (Duphaston, Abbott Biologicals B.V., Netherlands). Blastocyst embryos were transferred after approximately (120±3) h. The embryo transfer procedure adhered to the 2017 guidelines by the American Society for Reproductive Medicine (ASRM). In situations where the catheter encountered difficulty passing through the cervical canal into the uterine cavity, resulting in prolonged transfer time and requiring the use of a catheter with a malleable mandrel or cervical clamp ponzi, the procedure was recorded as a difficult embryo transfer[21].

2.9. Pregnancy outcomes

For clinical outcomes, we used the following definitions: pregnancy was confirmed [serum human chorionic gonadotropin (hCG) >5 mIU/mL]; implantation was confirmed at the 8 weeks of pregnancy by observation of gestational sac by ultrasound; clinical pregnancy, with a confirmed gestational sac at 6–7 weeks of pregnancy; ongoing pregnancy: defined as pregnancy that continued beyond 12 weeks of gestation.

2.10. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences 26 (SPSS Inc.). To describe the data, descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean and standard deviation (mean±SD) was used for continuous variables. Blastocyst rates were compared between different EEVA scores by *Chi*-square test. The association between the EEVA score and each type of blastocyst morphology including ICM, TE, and BL were assessed using generalized estimating equations (GEE). The association between the EEVA score and pregnancy outcomes were also assessed using GEE. The GEE model for blastocyst morphology included age of oocyte provider, body mass index (BMI) of the oocyte provider as confounders. The GEE model for pregnancy outcomes included maternal age, BMI of the oocyte provider, the day of embryo transfer (day 5 *vs* day 6) and blastocyst morphology as confounders. The GEE model considers multiple embryos from a single patient as an intra-subject variable, standardizing patient-related confounders. The adjusted odds ratios (aORs) of the effect of the variables included in the GEE on the outcome variables were expressed as 95% confidential interval (CI). Statistical significance was considered for *P*-values <0.05. In the GEE models, the odds of achieving a negative outcome were compared between embryos within each EEVA score with that of the embryos in the best score (Score E1).

Receiver operating characteristic (ROC) curves were graphed from the probability values obtained by the GEE models and the areas under the ROC curves (AUC) were calculated as model evaluation metrics.

2.11. Ethics statement

This study was approved by the Internal Committee for Ethics of the Tam Anh General Hospital, Hanoi, Vietnam (approval number: IRB.TAHN.055). Informed and written consents were obtained from all participants.

3. Results

3.1. Demographic and clinical characteristics of patients

Figure 1 shows our embryo culture, assessment, and transfer flowchart. The mean age of the participants were (30.49±4.90) years; overall age range were (20–49) years. We analyzed 511 single vitrified-warmed blastocyst transfer cycles, 2522 embryos from 291 ICSI cycles culturing to blastocyst in the GERI incubator. The blastocyst rate was 61%. Women's characteristics of the embryo transfer cycle in this study were described in Table 1. In addition, the blastocyst rate according to the EEVA score were 86.6% (401/463) for E1, 82.3% (387/470) for E2, 67.7% (314/464) for E3, 51.5% (268/520) for E4, 27.6% (167/605) for E5 (*P*<0.001).

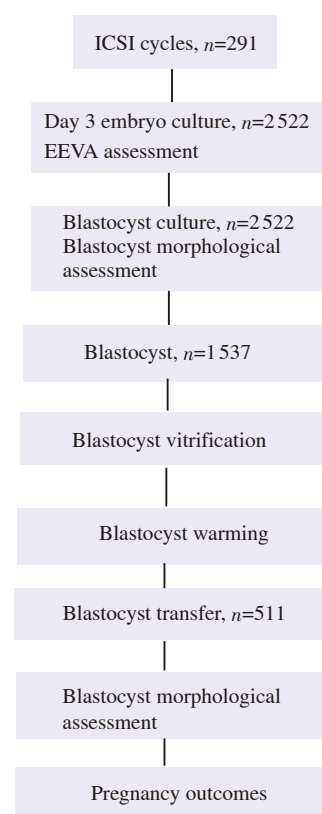


Figure 1. Flowchart of embryo culture, assessment, and transfer. ICSI: intracytoplasmic sperm injection, EEVA: Early embryo viability assessment.

3.2. ICM morphology grading on EEVA score

We analyzed the correlation between the quality of ICM morphology and each EEVA score. Figure 2A presents the distribution of the quality of ICM morphology within each EEVA score. The association between EEVA score and ICM morphology was confirmed by a GEE model and adjusted with confounding factors including maternal age, and BMI. The GEE model showed that the adjusted *OR* of each category was decreasingly lower for ICM A (good morphology), and increasingly higher for ICM C (bad morphology), as presented in Table 2. The associations were statistically significant when E1 was used as the reference group (*P*<0.05).

3.3. TE morphology grading on EEVA score

We analyzed the correlation between the quality of TE morphology and each EEVA score. Figure 2B presents the distribution of the quality of TE morphology within each EEVA score. The association between EEVA score and TE morphology was confirmed by a GEE model and adjusted with confounding factors including maternal age, and BMI. The GEE model showed that the adjusted *OR* of each category was decreasingly lower for TE A (good morphology), and increasingly higher for TE C (bad morphology), as presented in Table 3. The associations were statistically significant when E1 was used as the reference group (*P*<0.05).

Table 1. General descriptive and demographic characteristics of transfer cycles.

Characteristics	Values
Transfer cycles, <i>n</i>	511
Maternal age, years	30.49±4.90
Endometrial thickness, mm	9.50±1.20
BMI, kg/m ²	21.07± 2.54
Embryo day 5	90% (460/511)
Embryo day 6	10% (51/511)
Blastocoele expansion degree	
BL3	3.91% (20/511)
BL4	10.37% (53/511)
BL5	47.55% (243/511)
BL6	31.51% (161/511)
BL7	6.66% (34/511)
Inner cell mass (ICM) grade	
ICM A	68.88% (352/511)
ICM B	22.12% (113/511)
ICM C	9% (46/511)
Trophectoderm (TE) grade	
TE A	61.06% (312/511)
TE B	30.71% (157/511)
TE C	8.23% (42/511)
Blastocyst transfer	
E1	43.05% (220/511)
E2	26.22% (134/511)
E3	14.87% (76/511)
E4	10.18% (52/511)
E5	5.68% (29/511)
Implantation rate, %	67.1
Clinical pregnancy rate, %	54.6
Ongoing pregnancy rate, %	49.7

Continuous data are expressed as mean±SD; categorical data are expressed as percentage (%). BMI: body mass index of the oocyte provider; BL: blastocyst expansion degree; E: early embryo viability (EEVA) assessment score. The EEVA system produces an EEVA score from E1 (best) to E5 (worse) for the potential of blastocyst formation.

3.4. Blastocyst expansion degree on EEVA score

We analyzed the correlation between the blastocyst expansion

degree and each EEVA score. Figure 2C presents the distribution of blastocyst expansion degree morphology within each EEVA score. The association between EEVA score and blastocyst expansion degree morphology was confirmed by a GEE model and adjusted with confounding factors including maternal age, and BMI. The GEE model showed that the adjusted *OR* of each category was decreasingly lower for BL3, 4, 5, 6, 7 (good morphology), as presented in Table 4. The associations were statistically significant when E1 was used as the reference group ($P<0.05$).

3.5. Pregnancy outcomes rates on EEVA score

The maternal age, BMI, the day of embryo transfer (day 5 *vs* day 6) and the blastocyst morphology have their clinical importance role for

pregnancy outcomes. These variables were considered as possible confounders in our study. The association between EEVA score and pregnancy outcomes including implantation, clinical pregnancy, and ongoing pregnancy were confirmed by a GEE model and adjusted with confounding factors. The GEE model showed the adjusted *OR* of each category was decreasingly lower for all implantation, clinical pregnancy, and ongoing pregnancy as presented in Table 5. The associations were statistically significant when E1 was used as the reference group ($P<0.05$).

To account for the value of the EEVA score predicting pregnancy outcomes including implantation, clinical pregnancy and ongoing pregnancy, the ROC curve was performed. The results showed that AUC were 0.641 (95% *CI* 0.598-0.683) for implantation, 0.652 (95% *CI* 0.609-0.694) for clinical pregnancy, 0.645 (95% *CI* 0.601-0.686) for ongoing pregnancy which were valid in predicting pregnancy outcomes ($P<0.05$) (Figure 3).

Table 2. Association of early embryo viability assessment scores and inner cell mass grading.

Variables	ICM A			ICM C		
	aOR	95% CI	P value	aOR	95% CI	P value
E2 <i>vs</i> E1	0.725	0.547-0.959	0.025	1.480	0.979-2.238	0.063
E3 <i>vs</i> E1	0.598	0.444-0.806	0.001	1.680	1.098-2.570	0.017
E4 <i>vs</i> E1	0.351	0.254-0.486	<0.001	2.962	1.965-4.465	<0.001
E5 <i>vs</i> E1	0.300	0.203-0.443	<0.001	3.781	2.414-5.921	<0.001

ICM: inner cell mass; E: early embryo viability assessment (EEVA) score; aOR: adjusted odds ratio; CI: confidential interval; $P<0.05$ statistical significance of variable–outcome association.

Table 3. Association of early embryo viability assessment scores and trophectoderm cell grading.

Variables	TE A			TE C		
	aOR	95% CI	P value	aOR	95% CI	P value
E2 <i>vs</i> E1	0.699	0.527-0.927	0.013	2.139	1.375-3.328	0.001
E3 <i>vs</i> E1	0.566	0.418-0.767	<0.001	2.142	1.352-3.395	0.001
E4 <i>vs</i> E1	0.312	0.221-0.440	<0.001	3.861	2.446-5.953	<0.001
E5 <i>vs</i> E1	0.174	0.108-0.279	<0.001	4.482	2.762-7.274	<0.001

TE: trophectoderm cell; E: early embryo viability assessment (EEVA) score; aOR: adjusted odds ratio; CI: confidential interval; $P<0.05$ statistical significance of variable–outcome association.

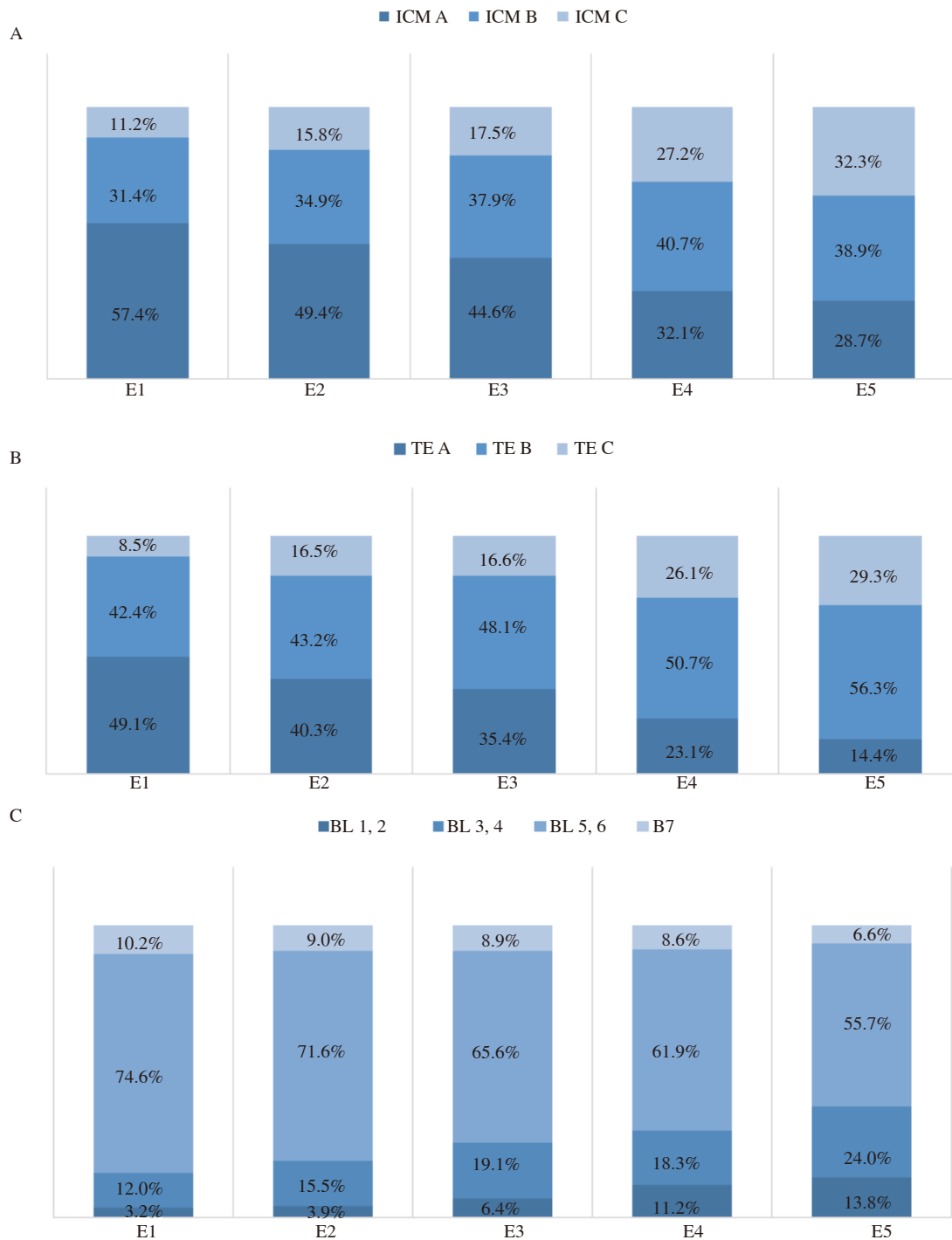


Figure 2. **A:** Distribution of quality of inner cell mass (ICM) morphology in each EEVA score. **B:** Distribution of trophoblast cell (TE) morphology in each EEVA score. **C:** Distribution of expansion degree morphology in each EEVA score. E: early embryo viability assessment (EEVA) score.

4. Discussion

Prolonging culture to the blastocyst stage allows to have embryos with higher developmental potential, thereby maximizing pregnancy outcomes[22,23]. However, this can lead to the cancellation of the cycle because the embryo does not develop to the blastocyst stage, causing emotional stress for both the patient and the embryologist. Therefore, the selection of suitable embryos for prolonged culture can help embryologists make better

predictions regarding embryonic development[3]. Currently, most IVF Centers rely on morphological features and growth rate as the main indicators for embryo selection. EEVA artificial intelligence software is a new step in predicting embryos from day 3 with the potential to develop into blastocysts. The objective of our study was to determine the association between the EEVA score and blastocyst morphology in terms of ICM quality, TE quality, and blastocyst cavity expansion degree. Furthermore, we took one more step to determine the association between the EEVA score and the

Table 4. Association of early embryo viability assessment scores and blastocyst expansion degree.

Variables	BL 3, 4, 5, 6, 7		
	aOR	95% CI	P value
E2 <i>vs</i> E1	0.831	0.390-1.770	0.631
E3 <i>vs</i> E1	0.493	0.241-1.006	0.050
E4 <i>vs</i> E1	0.266	0.136-0.520	<0.001
E5 <i>vs</i> E1	0.210	0.103-0.425	<0.001

BL: blastocyst expansion; E: early embryo viability assessment (EEVA) score; aOR: adjusted odds ratio; CI: confidence interval; $P < 0.05$ statistical significance of variable–outcome association.

implantation rate, clinical pregnancy, and ongoing pregnancy rate.

To our knowledge, at the time of the study, there were some studies that validated the correlation between the EEVA score and blastocyst formation rates[11,24]. In our study, we not only determined the correlation between the EEVA score and blastocyst formation rates but we also determined the relationship between the EEVA score and the morphological characteristics of blastocysts. Our study showed that embryos with high EEVA scores developed into blastocysts on day 5 and 6 with lower morphological rates of ICM A and TE A, blastocoele expansion of BL3, 4, 5, 6, 7 (good morphology), and with higher morphological rates of ICM C, TE C, and BL1, 2. The OR was decreasingly lower for ICM A, TE A, blastocoele expansion of BL3, 4, 5, 6, 7 (good morphology) and increasingly higher for ICM C, TE C (bad morphology) when comparing embryos with E2, E3, E4, and E5 *versus* E1, respectively. The associations were statistically significant.

The blastocoele expansion degree is important to clinical outcomes. Many studies have shown a significant association between blastocoele expansion and clinical and biochemical pregnancy

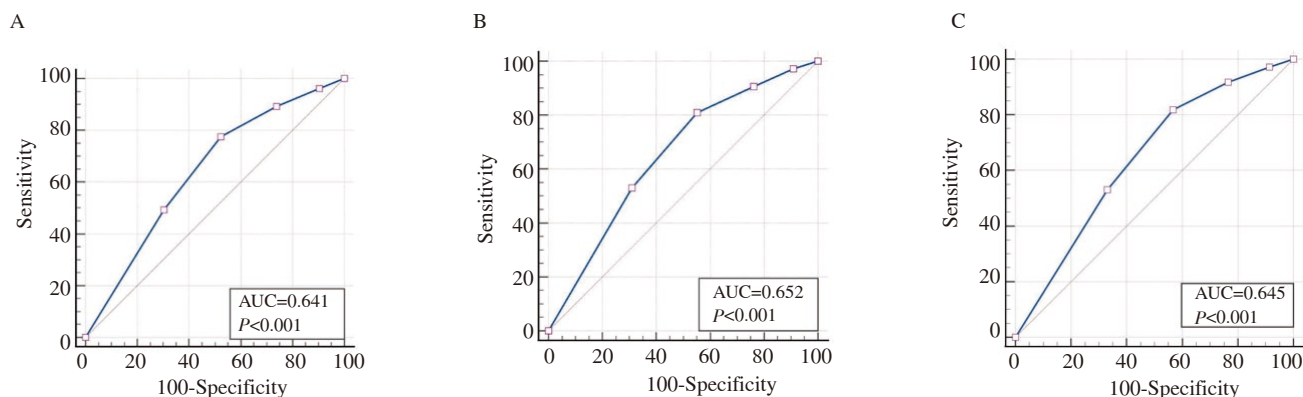
rates when transferring one or two frozen blastocysts[25]. In 2013, a study also showed that the blastocoele expansion degree was an independent and significant predictor of the live birth rate in the cycle of single blastocyst transferring. The blastocoele expansion degree has many important functions in the early stages of blastocyst implantation. These functions involve hatching from the Zona pellucida, signaling through hCG levels, adhesion and invasion of the endometrium, and communication with the mother. These are essential for a successful pregnancy as well as miscarriage prevention[26]. Therefore, the blastocoele expansion degree is thought to be more predictive information than that of TE and ICM quality[26].

In the study 2019, it was also shown that the majority of blastocysts with a blastocoele expansion degree of BL5, 6 and type of ICM, TE, were highly associated: ICM and TE types A and B were more likely to be correlated with TE and ICM types A and B; for example, blastocyst quality grades of AA, AB, BA, BB, or CC were more likely to appear than with other combinations (*i.e.*, types AC, CA, BC, and CB)[25]. These observations suggest a dependence between ICM and TE in the development of blastocyst stages. The blastocoele expansion is associated with the cell number and the adhesive characterization of the TE. That means TE cells have the function of helping a blastocyst to fully expand, and this function may have more association with the molecular quality of the TE cells than the number and adhesive characterization of TE cells[25]. The results of our study also show that the EEVA score can support the evaluation of embryo morphology. The embryos with a high EEVA score have a lower ratio of ICM A, TE A, and blastocoele expansion of BL3, 4, 5, 6, 7.

Table 5. Association of early embryo viability assessment scores and pregnancy outcomes.

Variables	Implantation			Clinical pregnancy			Ongoing pregnancy		
	aOR	95% CI	P value	aOR	95% CI	P value	aOR	95% CI	P value
E2 <i>vs</i> E1	0.791	0.484-1.293	0.350	0.678	0.435-1.056	0.086	0.753	0.488-1.164	0.202
E3 <i>vs</i> E1	0.335	0.194-0.580	<0.001	0.268	0.155-0.464	<0.001	0.309	0.178-0.535	<0.001
E4 <i>vs</i> E1	0.259	0.138-0.485	<0.001	0.258	0.136-0.487	<0.001	0.232	0.119-0.453	<0.001
E5 <i>vs</i> E1	0.245	0.111-0.544	0.001	0.185	0.078-0.439	<0.001	0.200	0.082-0.489	<0.001

E: early embryo viability assessment (EEVA) score; aOR: adjusted odds ratio, CI: confidence interval. $P < 0.05$ statistical significance of variable–outcome association.

**Figure 3.** Performance metrics for the generalized estimating equations (GEE) for implantation (A), clinical pregnancy (B), and ongoing pregnancy (C). Receiver operating characteristic (ROC) curves and area under the ROC curves (AUCs) of the GEE modelled for assessing the performance of EEVA Test.

As for predicting pregnancy outcomes, this study was to determine the correlation between the EEVA score and pregnancy outcomes, including implantation rate, clinical pregnancy, and ongoing pregnancy, applied to only single vitrified-warmed blastocyst transfers. We found a correlation between EEVA and those pregnancy outcomes. The aOR was decreasingly lower for those pregnancy outcomes when comparing embryos with E2, E3, E4, and E5 *versus* E1, respectively. The associations were statistically significant. Indeed, in this study, the AUC for implantation, clinical pregnancy, and ongoing pregnancy were 0.641, 0.652, and 0.645, respectively. Although the AUC values obtained in our study may not be considered high (typically, an AUC above 0.7 is considered acceptable), they do indicate a discernible correlation between the EEVA score and the pregnancy outcomes. This suggests that despite the modest AUC values, the EEVA score can still serve as a useful predictive tool in the realm of single vitrified-warmed blastocyst transfers. Our study is in line with the findings of the study in 2023, which reported a similar AUC of 0.61 applied on the population of blastocysts including single, double, and fresh, frozen-thawed transfer[11].

The limitation of the study was that the details of the algorithm and some morphological features used in the EEVA model were hidden by the manufacturer, which prevented our later conclusions because we did not know how to calculate the score. The EEVA score is automatically obtained from the software. In addition, it should be noted that our study was a retrospective cohort study and therefore may have limitations.

In conclusion, our results suggest that the blastocyst morphological parameters and pregnancy outcomes were associated with the EEVA score. The rate of blastocyst with ICM A, TE A, BL3, 4, 5, 6, 7 tended to decrease, and the rate of blastocyst with ICM C and TE C tended to increase for day 3 embryos with a high EEVA score. The rate of implantation, clinical pregnancy, and ongoing pregnancy tended to decrease for day 3 embryos with a high EEVA score.

Conflict of interest statement

The authors declare no competing interests.

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Data availability

The datasets analyzed during the current study are available in the main text, tables, and figures.

Authors' contributions

Vu D Hop, Nguyen T L Huong, Nguyen V Hanh, and Le Hoang conceived, designed, interpreted the data, and coordinated the work. Vu D Hop wrote the manuscript. An M Cuong and Phi T T Anh collected, double checked the data, and participated in interpreting the data. Nguyen T L Huong, Nguyen V Hanh, and Le Hoang reviewed the manuscript. All authors jointly contributed to the final version of the manuscript.

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