Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2026**; 53(9): 52607
https://doi.org/10.31083/CEOG52607

Original Research

A Prediction Model for Pregnancy Outcomes in Frozen-Thawed High-Quality Single Blastocyst Transfer Cycles

Xiaohua Cai[1,2,†], Xinxin Li[2,†], Shuqi Ge[2], Yan Liu[2], Li Sun[2,*]

[1]Department of Laboratory Medicine, The Third Affiliated Hospital of Sun Yat-Sen University, 510630 Guangzhou, Guangdong, China
[2]Reproductive Medicine Center, The Third Affiliated Hospital of Sun Yat-Sen University, 510630 Guangzhou, Guangdong, China
*Correspondence: sunli@zssy.com.cn (Li Sun)
[†]These authors contributed equally.

Academic Editor: Paolo Ivo Cavoretto
Submitted: 5 April 2026 Revised: 18 June 2026 Accepted: 24 June 2026 Published: 11 September 2026

Abstract

Background: Frozen-thawed high-quality single blastocyst transfer has become a mainstream strategy to reduce multiple pregnancy risks in assisted reproductive technology, yet personalized prediction for pregnancy outcomes remains limited in clinical practice. Few validated models have incorporated key clinical and embryological factors for this specific population. The aim of this study was to identify predictors of clinical pregnancy following frozen-thawed, high-quality single blastocyst transfer, and to develop a nomogram for predicting pregnancy outcomes. **Methods:** We retrospectively analyzed 3398 cycles of frozen-thawed, high-quality single blastocyst transfer performed at the Third Affiliated Hospital of Sun Yat-sen University from July 2016 to December 2023. Cycles were randomly allocated to training (n = 2378) and validation (n = 1020) datasets at a 7:3 ratio. Predictors of clinical pregnancy were identified using univariate and multivariate logistic regression and were subsequently used to construct a nomogram. Model performance was assessed by area under the curve (AUC), calibration curves, and decision curve analysis (DCA). **Results:** Multivariate analysis identified four independent predictors of clinical pregnancy (all $p < 0.05$): maternal age at oocyte retrieval, endometrial thickness, fertilization method (conventional *in vitro* fertilization [IVF] vs. intracytoplasmic sperm injection [ICSI]), and blastocyst developmental stage (day-5 vs. day-6). Stratified analysis revealed that, compared with patients younger than 35 years, significantly lower odds of clinical pregnancy were first observed in the 35–37 years old subgroup (odds ratio [OR] = 0.753, 95% confidence interval [CI]: 0.612–0.928). Optimal pregnancy rates occurred at an endometrial thickness of 10.0–11.9 mm (OR = 1.973, 95% CI: 1.435–2.713). The model demonstrated moderate discriminative capacity, with AUC values of 0.63 (95% CI: 0.60–0.65) and 0.62 (95% CI: 0.59–0.66) in the training and validation sets, respectively. Calibration curves showed good agreement between predicted and observed probabilities, while DCA confirmed clinical utility across relevant threshold probabilities. **Conclusions:** Four clinical parameters independently predicted the clinical pregnancy rate: maternal age <35 years, endometrial thickness of 10.0–11.9 mm, ICSI fertilization, and day-5 blastocyst transfer. This validated nomogram shows moderate predictive capacity for the clinical pregnancy rate in frozen-thawed, high-quality single blastocyst transfer cycles and may serve as a useful tool for clinical application.

Keywords: frozen-thawed embryo transfer; prediction model; nomogram; single blastocyst transfer; clinical pregnancy

1. Introduction

Multiple pregnancies present relatively high risks in patients receiving double-embryo transfer (DET) within *in vitro* fertilization/Intracytoplasmic Sperm Injection (IVF/ICSI)-embryo transfer (ET) cycles [1]. They represent a common complication of IVF-ET, resulting in significantly increased rates of maternal and neonatal complications. Multiple pregnancies are associated with an elevated risk of adverse obstetric outcomes, including pregnancy loss, pre-eclampsia, gestational diabetes mellitus, and antepartum/postpartum hemorrhage [2]. Compared with singletons, IVF/ICSI-conceived multiples exhibit markedly inferior perinatal prognosis, such as preterm birth, small-for-gestational-age status, admission to the neonatal intensive care unit, and neonatal mortality [3]. Notably, even singleton pregnancies following DET demonstrate an increased risk of neonatal death, very preterm birth, and low birth weight compared with pregnancies achieved via elective single embryo transfer (eSET) [4].

To minimize the risks of multiple pregnancies and complications associated with DET, eSET has become an important strategy for achieving safe and effective outcomes from assisted reproductive technology (ART). Evidence suggests that eSET in women aged <38 years significantly reduces multiple birth rates without compromising live birth rates [5]. In frozen-thawed embryo transfer (FET) cycles, single blastocyst-stage transfer offers superior clinical pregnancy performance [6]. Cryopreservation followed by FET is recommended in cases of suboptimal conditions, such as elevated risk of ovarian hyperstimulation syndrome (OHSS), impaired endometrial receptivity, premature progesterone elevation, or the availability of supernumerary embryos [7]. A randomized controlled trial (RCT) provided further support that frozen single blastocyst transfer yields higher rates of singleton live birth than

fresh transfer in ovulatory women with favorable prognoses [8]. Consequently, FET offers distinct physiological advantages, primarily through improved embryo-endometrium synchronization and enhanced flexibility in cycle scheduling. These benefits have contributed to the growing adoption of frozen-thawed single blastocyst transfer in contemporary ART practice.

The IVF treatment process imposes considerable biopsychosocial stress on infertile couples, with particular anxiety surrounding treatment outcomes and prognosis. Multiple clinical and embryological factors significantly influence the implantation potential and reproductive outcomes in frozen-thawed single blastocyst transfer cycles [9]. The present study seeks to develop and validate a multivariate predictive model incorporating several key determinants to facilitate personalized prognosis. The model's predictive outputs will enable clinicians to provide patients with quantitative estimates of pregnancy probability, as well as data-driven embryo transfer strategy recommendations, thereby optimizing FET cycle outcomes.

2. Materials and Methods

2.1 Study Design and Populations

This study employed a retrospective cohort design. The Institutional Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University approved the study (approval number: II2023-212-01). Informed consent waiver was approved by the Institutional Ethics Committee, given the retrospective nature of the study. Patients undergoing FET cycles in the Center for Reproductive Medicine, the Third Affiliated Hospital of Sun Yat-sen University from July 2016 to December 2023 and who satisfied pre-defined inclusion and exclusion criteria were enrolled for retrospective analysis. The inclusion criteria were women who underwent high-quality single blastocyst transfer cycles. The exclusion criteria were: (1) male or female chromosomal abnormalities; (2) uterine malformation, adenomyosis, or submucous myoma of the uterus; (3) intrauterine adhesions or endometrial polyps; (4) recurrent miscarriage; (5) cycles with preimplantation genetic testing; (6) lost to follow-up or missing core data. A total of 3398 cycles were included in the final analysis for this study.

2.2 Endometrial Preparation and Embryo Evaluation

Hormone replacement therapy (HRT) was used for endometrial preparation. Patients were treated orally with estradiol valerate (Progynova, 1 mg/tablet, Bayer, Berlin, Germany) starting on cycle day 2–3 at an initial daily dose of 4–6 mg. The dosage and treatment duration were modified individually based on serial endometrial thickness, as measured by transvaginal ultrasound. The maximum double-layer endometrial thickness measured on the day of progesterone initiation before progesterone administration was used for statistical analysis. Progesterone transformation was initiated when endometrial thickness exceeded 7.0 mm, or when thin endometrium reached its maximal achievable thickness for individual patients. Oral dydrogesterone (Dufaston, Abbott, Chicago, IL, USA) at a dose of 10 mg was given twice daily in combination with an optional progesterone formulation, including intramuscular progesterone at 40 mg daily, vaginal sustained-release progesterone gel (Crinone, Merck Serono, Darmstadt, Germany) at 90 mg daily, or vaginal micronized progesterone capsules (Utrogestan, BesinsHealthcare, Brussels, Belgium) at 400 mg daily. The first day of progesterone administration was set as transformation day-0 (D0), and blastocyst transfer was scheduled on transformation day-6 (D6).

Following embryo transfer, estradiol and progesterone were given at identical doses as during the transformation period. Serum beta-human chorionic gonadotropin (β-hCG) was tested on day 14 post-transfer. All hormone supplements were withdrawn immediately in non-conceived patients. Full-dose hormonal support was maintained for women who received positive pregnancy confirmation. Medication was gradually tapered after the detection of a fetal heartbeat by ultrasound, and all supplementary hormones were stopped entirely at gestational week 10.

Blastocysts were scored based on the Gardner and Schoolcraft scoring systems [10]. According to the standard operating procedure (SOP) of our center, grade 4BB or above is defined as a high-quality blastocyst, while grades below this are considered poor-quality blastocysts.

2.3 Outcome Measures

The primary study outcome was clinical pregnancy. This endpoint was confirmed when ultrasonography revealed the presence of one or more gestational sacs.

2.4 Statistical Analysis

SPSS 23.0 (IBM, Chicago, IL, USA) and R software (version 4.2, R Core Team, Vienna, Austria, http://www.r-project.org/) were used for statistical analysis. The samples were randomized into a training dataset and a validation dataset at a ratio of 7:3, respectively. Kolmogorov-Smirnov tests for normality were conducted, and non-normally distributed continuous variables were described by the median (25th percentile, 75th percentile) [M (Q1, Q3)], while categorical variables were described by counts and percentages (%). Differences between the two groups in baseline factors were compared by the Mann-Whitney U test and the χ^2 test. Univariate and multivariate logistic regression analyses were performed in the training dataset to determine the relevant factors that affect clinical pregnancy. Furthermore, we developed a nomogram to predict the probability of clinical pregnancy based on the regression coefficient of relevant variables. Three criteria were used to evaluate the model's performance: discrimination, calibration abilities, and clinical utility. Discrimination was assessed using receiver-operating characteristic (ROC) curves and area under the curve (AUC). Calibration was evaluated using cal-

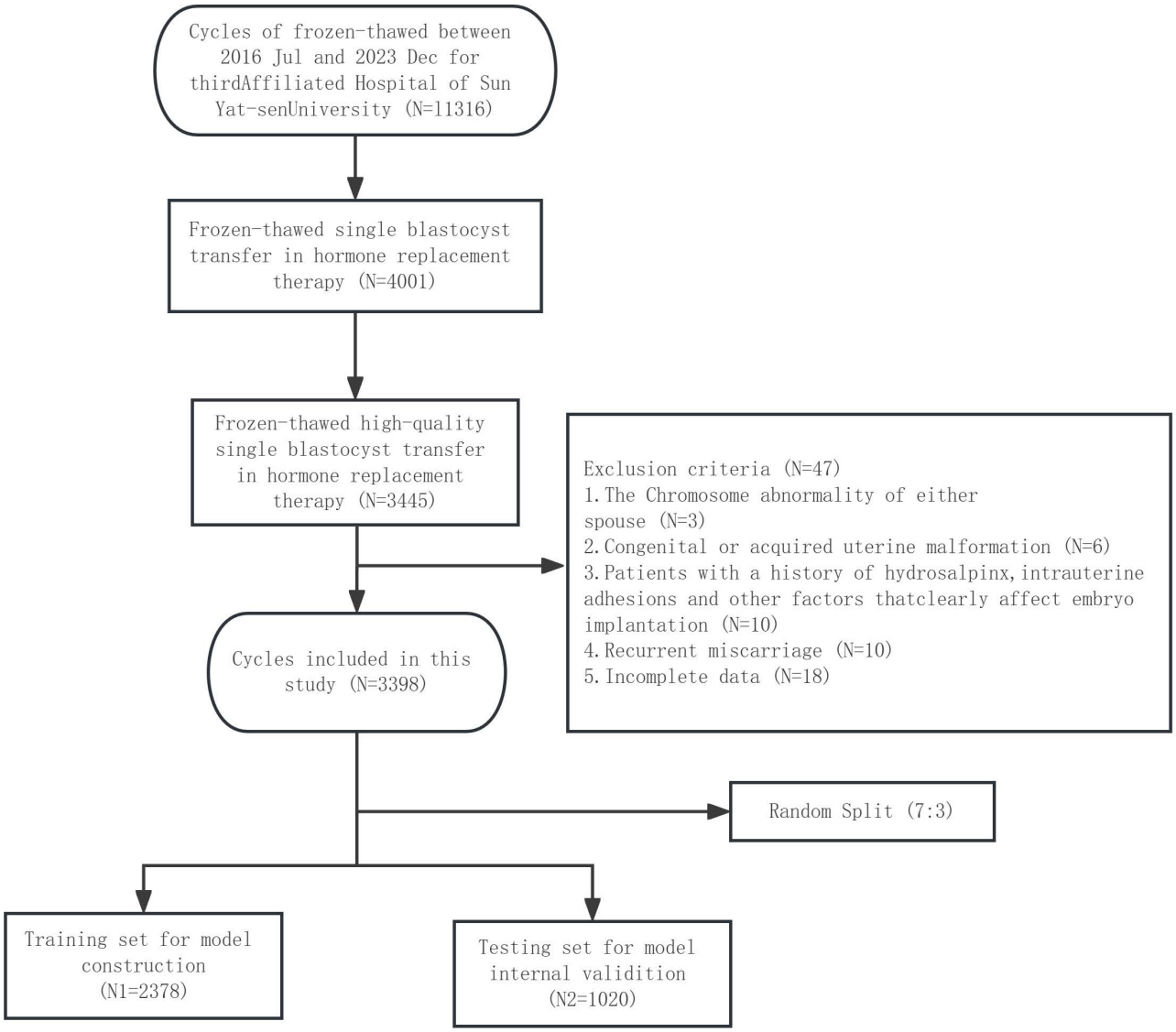

Fig. 1. The flowchart of participants.

ibration curves. The clinical utility of the nomogram was assessed using decision curve analysis (DCA). A two-tailed *p*-value of <0.05 was considered statistically significant.

3. Results

3.1 Description of the Study Population

A total of 3398 cycles were included in the analysis based on the inclusion and exclusion criteria, with 2378 cycles in the training dataset and 1020 cycles in the validation dataset (Fig. 1). The baseline characteristics and pregnancy outcomes of the two groups are shown in Table 1. The two groups were comparable for baseline characteristics and pregnancy outcomes (all $p > 0.05$).

3.2 Construction of the Model

Univariate logistic regression was performed, and variables with $p < 0.05$ were screened for inclusion in the model. The factors of maternal age at oocyte retrieval, infertility duration, endometrial thickness, number of previous FET, fertilization method, and blastocyst development stage were significantly associated with clinical pregnancy (all $p < 0.05$). All six variables were simultaneously entered into a multivariate logistic regression model to adjust for confounding effects. After adjustment, multivariate logistic regression analysis revealed that maternal age at oocyte retrieval (OR = 0.942, 95% CI: 0.922–0.961, $p < 0.001$), endometrial thickness (OR = 1.166, 95% CI: 1.103–1.233, $p < 0.001$), fertilization method (OR = 1.445, 95% CI: 1.131–1.846, $p = 0.003$), and blastocyst development stage (OR = 0.533, 95% CI: 0.402–0.706, $p < 0.001$) were independent risk factors affecting clinical pregnancy (Table 2). Based on these four independent predictors, we developed a clinical pregnancy prediction model and constructed a customized nomogram (Fig. 2). Each parameter was individually assigned a vertical extension, as illustrated in the upper points bar. The total score was calculated by summing the values

Table 1. Comparison of baseline and clinical characteristics between the two groups.

Variables	Total (n = 3398)	Modeling group (n = 2378)	Validation group (n = 1020)	Z/χ^2	*p*-value
Maternal age at oocyte retrieval (years)	31.0 (28.0, 34.0)	31.0 (28.0, 34.0)	31.0 (28.0, 34.0)	–0.179	0.858
BMI (kg/m^2)	21.1 (19.3, 23.3)	21.0 (19.3, 23.3)	21.2 (19.4, 23.3)	–0.771	0.441
Infertility duration (years)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 5.0)	–1.496	0.135
Endometrial thickness (mm)	9.3 (8.4, 10.5)	9.3 (8.4, 10.5)	9.4 (8.4, 10.5)	–0.220	0.826
Number of FET times	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	–0.706	0.480
Infertility type, n (%)				1.416	0.234
Primary infertility	1736 (51.1)	1199 (50.4)	537 (52.6)		
Secondary infertility	1662 (48.9)	1179 (49.6)	483 (47.4)		
Fertilization method, n (%)				0.243	0.622
IVF	2805 (82.5)	1968 (82.8)	837 (82.1)		
ICSI	593 (17.5)	410 (17.2)	183 (17.9)		
Blastocyst development stage, n (%)				0.549	0.459
D5	3045 (89.6)	2137 (89.9)	908 (89.0)		
D6	353 (10.4)	241 (10.1)	112 (11.0)		
Clinical pregnancy, n (%)	2229 (65.6)	1554 (65.3)	675 (66.2)	0.217	0.064

BMI, body mass index; FET, frozen-thawed embryo transfer; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; D5, day-5 blastocyst; D6, day-6 blastocyst.

Table 2. Univariate and multivariate logistic regression analysis of factors affecting the clinical pregnancy rate.

Variables	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Maternal age at oocyte retrieval (years)	0.932 (0.914–0.950)	<0.001	0.942 (0.922–0.961)	<0.001
BMI (kg/m^2)	0.991 (0.972–1.010)	0.341		
Infertility duration (years)	0.955 (0.922–0.989)	0.01		
Endometrial thickness (mm)	1.176 (1.114–1.243)	<0.001	1.166 (1.103–1.233)	<0.001
Number of FET cycles	0.896 (0.804–0.998)	0.046		
Infertility type, n (%)				
Primary infertility = 0	ref.			
Secondary infertility = 1	1.034 (0.873–1.225)	0.696		
Fertilization method, n (%)				
IVF	ref.		ref.	
ICSI	1.508 (1.191–1.910)	<0.001	1.445 (1.131–1.846)	0.003
Blastocyst development stage, n (%)				
D5	ref.		ref.	
D6	0.514 (0.393–0.672)	<0.001	0.533 (0.402–0.706)	<0.001

OR, odds ratio.

assigned to each factor. The overall score, represented on the bottom scale, indicates the likelihood of clinical pregnancy.

Illustrative example:

For a 30-year-old woman with endometrial thickness = 12 mm, IVF origin embryo, and D5 blastocyst:

- Age 30 → 55 points
- Endometrial thickness 12 mm → 53 points
- IVF fertilization → 0 points
- D5 embryo development → 32 points

Total Points = 55 + 53 + 0 + 32 = 140 points.

A vertical line projected down from 140 of the Total Points axis yields a predicted clinical pregnancy rate of approximately 0.70.

3.3 Validation of the Model

The AUC was calculated to assess the discriminative ability of the nomogram (Fig. 3). The AUC of the training cohort was 0.63 (95% CI: 0.60–0.65), and for the validation cohort it was 0.62 (95% CI: 0.59–0.66). These values indicate the model has moderate discriminative performance. The calibration plot demonstrated good agreement between the actual and predicted probabilities, while the Hosmer-Lemeshow test revealed a nonsignificant difference (p = 0.430). This suggests the nomogram exhibited good calibration and acceptable overall predictive performance (Fig. 4). Furthermore, DCA revealed the prediction model was the highest line on the decision curve, suggesting it provides a greater net benefit and potential clinical utility (Fig. 5).

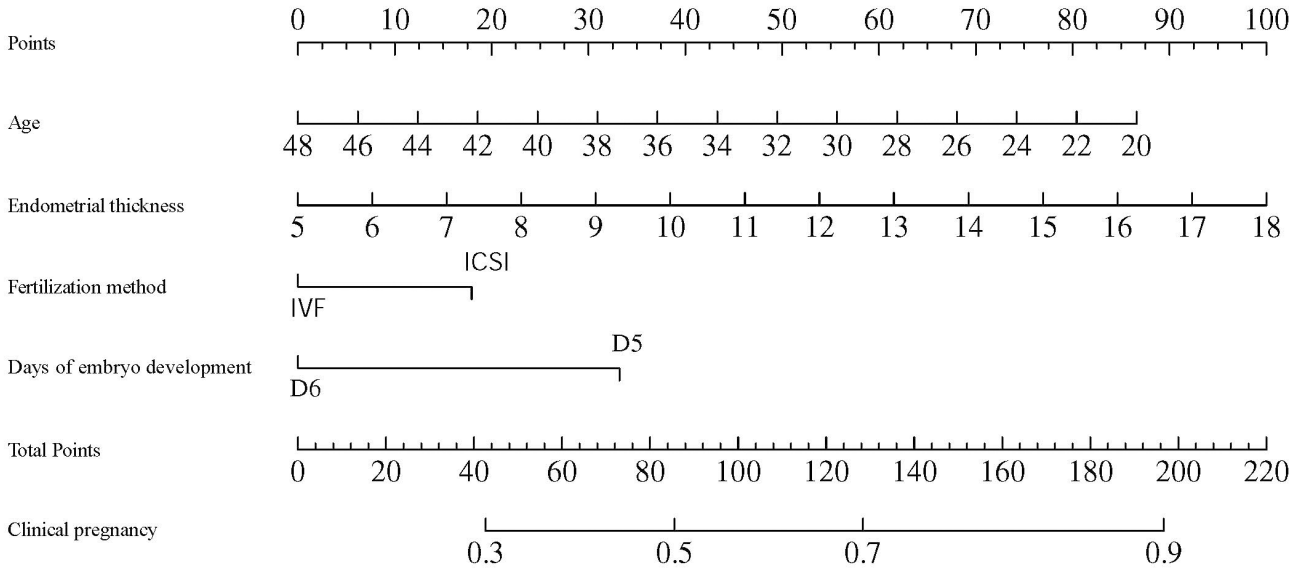

Fig. 2. Nomogram for predicting clinical pregnancy probability after frozen-thawed single high-quality blastocyst transfer. Note: when using the line chart for patients, find the point on the axis corresponding to the value of each variable and project a vertical line upward to the score axis (points). This shows the number of points for that variable. The scores obtained for each variable are then added to give the total number of points. Subsequently, a vertical line is projected down from the value in the total points axis onto the clinical pregnancy probability axis, which predicts the probability of clinical pregnancy by the patient.

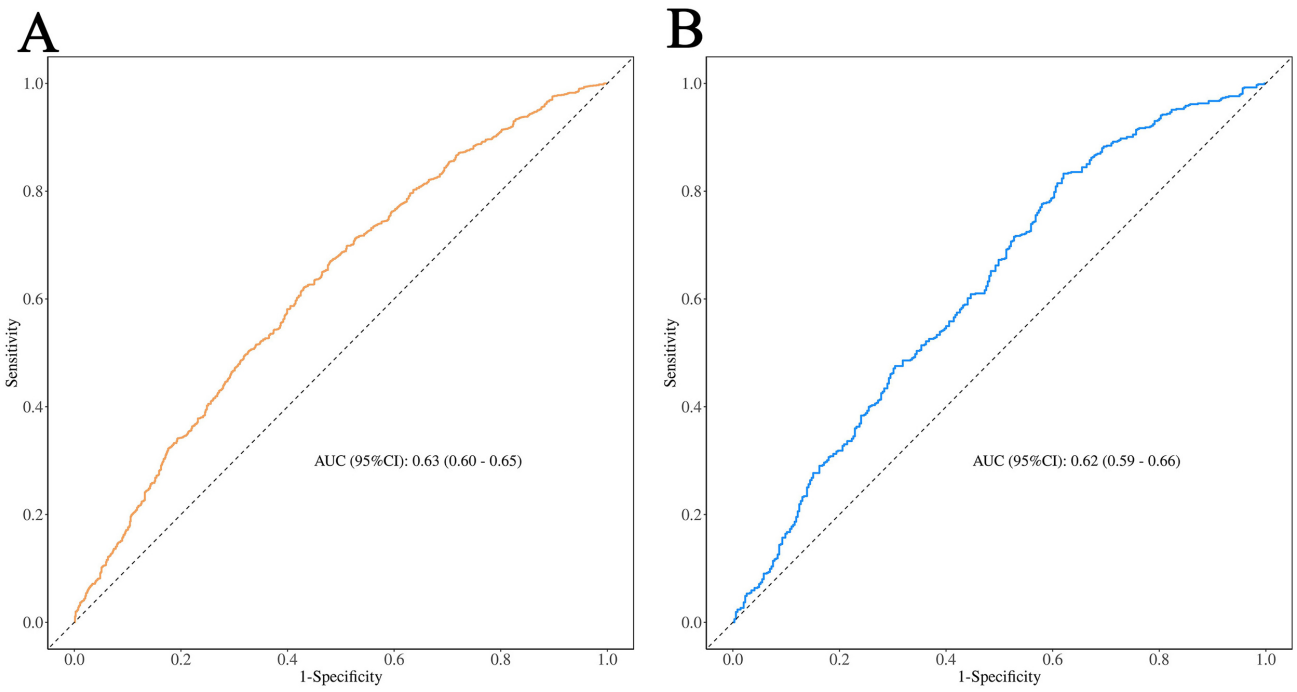

Fig. 3. ROC curves for the modeling group (A) and the validation group (B).

In the training cohort, the nomogram exhibited a favorable clinical net benefit when the predicted clinical pregnancy probability ranged from 30–90%. Only patients within this range presented obvious heterogeneity in terms of maternal age, endometrial thickness, fertilization method, and blastocyst development stage. Risk stratification and individualized endometrial preparation as well as luteal support guided by this nomogram can achieve superior clinical net benefit compared with universal intervention or non-stratified management. Patients with a predicted probability of <30.0% or >90.0% calculated by this model do not require personalized treatment.

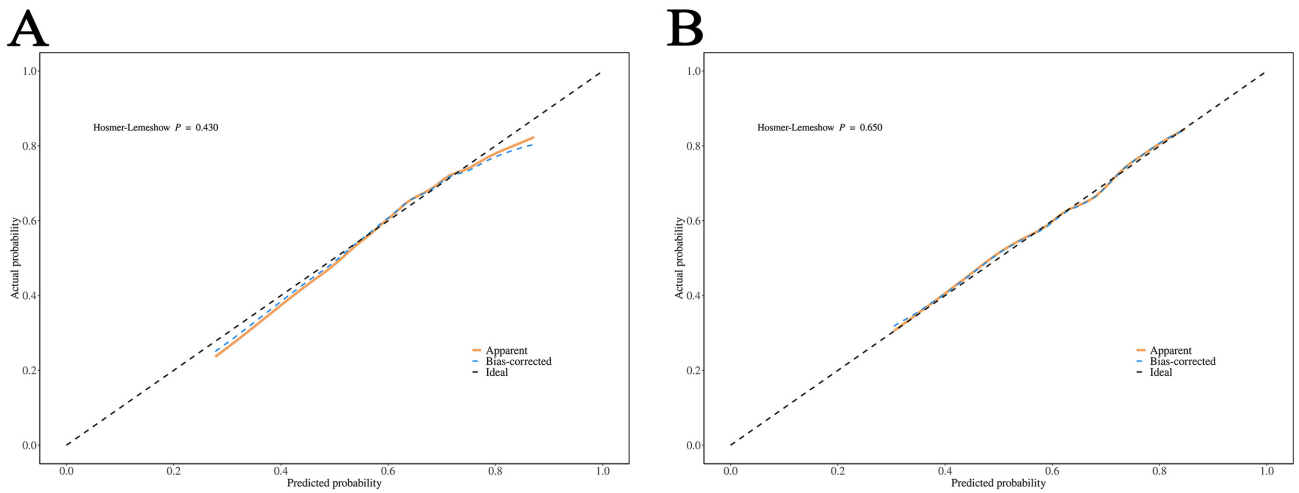

Fig. 4. Calibration curves for the modeling group (A) and the validation group (B).

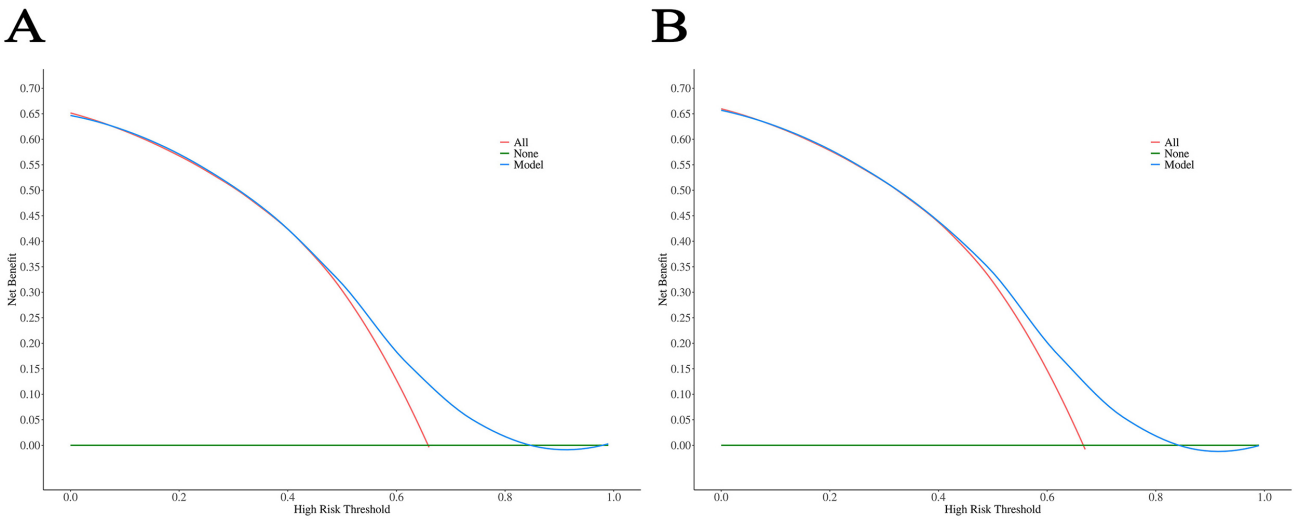

Fig. 5. Decision curves for the modeling group (A) and the validation group (B).

3.4 Subgroup Analysis of Factors Influencing the Clinical Pregnancy Rate

We next conducted categorical multivariate logistic regression analysis to optimize the clinical pregnancy probability of frozen single high-quality blastocyst transfer (Table 3). Multivariate models were adjusted for confounding variables screened from the preceding univariate analysis ($p < 0.05$), and the stratified OR values represent the adjusted results. Stratified analysis revealed that, compared with patients younger than 35 years, significantly lower odds of clinical pregnancy were first observed in the 35–37 years old subgroup (OR = 0.753, 95% CI: 0.612–0.928). Optimal pregnancy rates occurred at an endometrial thickness of 10.0–11.9 mm (OR = 1.973, 95% CI: 1.435–2.713), indicating this range was an independent favorable predictor for clinical pregnancy after adjustment for confounders.

4. Discussion

Prospective cohort analyses have confirmed that compared with fresh blastocyst transfer, frozen-thawed blastocyst transfer yields greater first-trimester crown-rump length, higher estimated fetal weight and birth weight, indicating that frozen embryo transfer exerts beneficial effects on both embryo implantation and subsequent fetal developmental trajectories [11,12]. Frozen single blastocyst transfer presents distinct clinical advantages in assisted reproductive practice. Nevertheless, clinical pregnancy outcomes remain markedly heterogeneous among individuals receiving this treatment. This retrospective cohort study analyzed 3398 frozen-thawed, single high-quality blastocyst transfer cycles in infertile patients. Multivariate logistic regression identified four independent predictors of clinical pregnancy: maternal age at oocyte retrieval, endometrial thickness, fertilization method (conventional IVF vs. ICSI), and blastocyst developmental stage (D5 vs. D6).

Table 3. Stratified analysis in different subgroups.

Variables	OR (95% CI)	*p*-value
Maternal age at oocyte retrieval (years)		
<35	ref.	
35–37	0.753 (0.612–0.928)	0.008
38–39	0.537 (0.398–0.725)	<0.001
≥40	0.341 (0.244–0.475)	<0.001
Endometrial thickness (mm)		
<8.0	ref.	
8.0–9.9	1.134 (1.006–1.790)	0.046
10.0–11.9	1.973 (1.435–2.713)	<0.001
≥12.0	1.898 (1.276–2.825)	0.002
Fertilization method, n (%)		
IVF	ref.	
ICSI	1.445 (1.131–1.846)	0.003
Blastocyst development stage, n (%)		
D5	ref.	
D6	0.533 (0.402–0.706)	<0.001

The derived prediction nomogram demonstrated moderate discriminative performance and favorable calibration.

Extensive evidence has consistently demonstrated an inverse relationship between advancing maternal age and clinical pregnancy outcomes across ART cycles [13,14,15]. With advancing maternal age, the ovarian reserve progressively declines, accompanied by a reduction in both the quantity and quality of oocytes. This is characterized by decreased mitochondrial content in oocytes and increased incidence of embryonic aneuploidy [16]. Concurrently, age-related morphological and functional changes in the endometrium contribute to impaired endometrial receptivity. These cumulative effects result in reduced clinical pregnancy rates and elevated rates of miscarriage [17]. Cohort studies have demonstrated that embryonic aneuploidy increases significantly with advancing maternal age, and that implantation failure of chromosomally abnormal embryos is the leading cause of reduced pregnancy rates in older patients [18]. Our subgroup analysis revealed a marked decline in clinical pregnancy rates among women aged ≥35 years, consistent with existing literature documenting age-related reproductive decline.

The receptive property of the endometrium is another core contributor to final pregnancy results, with endometrial thickness being one of the clinically significant determinants of endometrial receptivity. However, there is currently no consensus regarding the optimal threshold for implantation. Several studies have indicated that a thin endometrium is correlated with reduced implantation and pregnancy rates, thereby resulting in less favorable IVF outcomes [19]. A large retrospective cohort study of 10,165 HRT-FET cycles identified an optimal endometrial thickness cutoff of 8.7 mm for embryo transfer. For patients with an endometrium thinner than this threshold, each 1 mm increase in thickness markedly enhanced the implantation, clinical pregnancy, and live birth rates [20]. The correlation between endometrial thickness and live birth rate was not linear, and no consistent cut-off value was found. Another study of patients receiving FET or preimplantation genetic testing (PGT) cycles found no adverse thickness range, and the predictive efficacy of endometrial thickness remained modest (AUC 0.56–0.60) [21]. In the present study, we used categorical multivariate logistic regression analysis to identify an optimal endometrial thickness range. Significantly higher clinical pregnancy rates were found in all three comparison groups versus controls, with the rates plateauing after reaching a saturation threshold rather than showing continuous improvement with increasing thickness. Our data revealed that 10.0–11.9 mm was the optimal range of endometrial thickness for frozen single blastocyst transfers, providing clinically actionable guidance for cycle management.

Although the ICSI technique was initially developed to treat severe male-factor infertility, the scope of its clinical application has undergone continuous expansion in routine practice. Current evidence from two multicenter RCTs and a Cochrane systematic review demonstrates that ICSI provides no significant improvement in clinical pregnancy rates or live birth outcomes compared to conventional IVF in non-male factor infertility populations [22,23,24]. Moreover, an RCT indicated that for couples with non-severe male-factor infertility, ICSI did not improve the live birth rate compared with conventional IVF [25,26]. According to the SOP of our institute, ICSI is primarily indicated for severe male-factor infertility. Our results demonstrated a significantly higher clinical pregnancy rate in the ICSI group compared to the conventional IVF group. Although the current study excluded known factors impacting embryo implantation, the conventional IVF group may harbor other underlying female infertility factors. These unidentified

factors could potentially compromise pregnancy outcomes following blastocyst transfer in conventional IVF patients by adversely affecting endometrial receptivity and/or embryo quality.

Blastocyst quality is a significant determinant of embryonic implantation potential. Advanced maternal age impairs blastocyst quality via oocyte meiotic errors and increased embryonic aneuploidy [18]. Evidence regarding the comparative efficacy of D5 versus D6 blastocyst transfer remains complex and occasionally conflicting. Retrospective analyses demonstrated significantly higher euploidy rates in D5 blastocysts compared to D6 counterparts, yet found no significant differences in pregnancy or live birth rates following transfer of euploid blastocysts between these two developmental stages [27]. Conversely, other studies have found that D5 blastocyst transfer yields higher clinical pregnancy rates and live birth rates compared to D6 blastocyst transfer in both fresh and/or frozen embryo transfer cycles [28]. Moreover, a meta-analysis published in 2010 reported comparable clinical pregnancy and ongoing pregnancy/live birth rates between D5 and D6 frozen-thawed blastocyst transfers, suggesting similar reproductive potential [29]. In contrast, a subsequent meta-analysis published in 2019 indicated superior clinical pregnancy and live birth rates with D5 blastocyst transfers compared to D6 transfers in both fresh and frozen cycles [30]. Notably, when examining eSET involving euploid or low-level mosaic blastocysts, D5 blastocysts (particularly those with high morphological grade) yielded significantly higher clinical pregnancy and live birth rates than D6 blastocysts [31,32]. Supporting the potential impact of developmental timing, Hashimoto et al. [33] reported an increased incidence of abnormal spindle morphology and diminished implantation competence in developmentally delayed embryos relative to normally developing embryos. Consistent with these latter findings, our results demonstrated a markedly higher clinical pregnancy rate following the transfer of high-quality D5 blastocysts compared to high-quality D6 blastocysts. In conclusion, the optimal developmental stage (D5 vs. D6) for blastocyst transfer requires further elucidation through rigorous investigation.

This study has several notable strengths. First, our analysis incorporated a substantial cohort of treatment cycles. Furthermore, the nomogram developed here integrates four critical prognostic variables: maternal age at oocyte retrieval, endometrial thickness, fertilization method (conventional IVF vs. ICSI), and blastocyst developmental stage (D5 vs. D6). This model provides a personalized probability assessment of pregnancy following frozen single high-quality blastocyst transfer. While these predictive factors have been identified previously, the construction and validation of the current predictive instrument represent a novel contribution. Given the increasing utilization of IVF, this nomogram serves as a valuable clinical tool to optimize patient counseling and manage expectations. Patients increasingly prefer personalized risk stratification over population-based estimates when making informed treatment decisions. Personalized counseling can deliver tailored prognostic information based on patient-specific characteristics, aligning fundamentally with the principles of precision medicine.

Limitations

This study has several limitations. First, as a retrospective cohort investigation, the study covered a relatively long-term observational window. Gradual improvements in assisted reproductive practice, laboratory settings, and clinical protocols during this period may have contributed to time-associated confounding effects. Furthermore, external validation of our cohort is warranted prior to clinical implementation of the nomogram. Additionally, other potential predictors, including blastocyst morphological grade, developmental kinetics, and pre-transfer serum progesterone level, were not incorporated into the model. Clinical pregnancy was selected as the primary endpoint, and live birth and neonatal outcomes were not evaluated. Future studies that also assess obstetric outcomes and perinatal endpoints are essential. Incorporation of these additional prognostic factors could enhance the model's discriminatory power and clinical applicability.

5. Conclusions

This retrospective cohort study developed a clinically calibrated predictive model that reasonably estimates the probability of clinical pregnancy, offering potential utility for fertility treatment planning. The nomogram serves as a useful predictive reference for frozen-thawed, single high-quality blastocyst transfer cycles. By integrating four key parameters—maternal age at oocyte retrieval, endometrial thickness, fertilization method (IVF/ICSI), and blastocyst developmental stage (D5/D6), this model provides individualized outcome probability, enabling optimized patient counseling and clinical decision-making. Further validation and refinement will facilitate broader implementation of this precision medicine tool to guide treatment strategies and enhance outcomes in FET cycles.

Abbreviations

IVF/ICSI-ET, *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer; DET, double embryo transfer; eSET, elective single embryo transfer; ART, assisted reproductive technology; OHSS, ovarian hyperstimulation syndrome; FET, frozen embryo transfer; RCT, randomized controlled trial; HRT, hormone replacement therapy; SOP, standard operating procedure; ROC, the receiver operating characteristic; DCA, decision curve analysis; AUC, area under the receiver operating characteristic curve.

Availability of Data and Materials

The datasets generated during this study are available from the corresponding author upon reasonable request.

Author Contributions

XC performed the formal analysis and investigation, and wrote and revised the manuscript. XL contributed to the conceptualization and methodology. SG and YL provided study materials and clinical data, assisted with data curation. LS supervised the whole study, participated in conceptualization, and contributed to manuscript review and revision. All authors commented on previous versions of the manuscript, read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (Ethics Approval Number: II2023-212-01). Due to the retrospective design, informed consent was waived.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT-3.5 in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG52607.

References

[1] Dietrich JE, Cáceres Valcárcel I, Capp E, Strowitzki T, Germeyer A. High multiple pregnancy rates after double embryo transfers in human: a retrospective cohort study. Reproduction & Fertility. 2025; 6: e240078. https://doi.org/10.1530/RAF-24-0078

[2] ESHRE Guideline Group on the Number of Embryos to Transfer, Alteri A, Arroyo G, Baccino G, Craciunas L, De Geyter C, et al. ESHRE guideline: number of embryos to transfer during IVF/ICSI†. Human Reproduction (Oxford, England). 2024; 39: 647–657. https://doi.org/10.1093/humrep/deae010

[3] van Heesch MMJ, Evers JLH, Dumoulin JCM, van der Hoeven MAHBM, van Beijsterveldt CEM, Bonsel GJ, et al. A comparison of perinatal outcomes in singletons and multiples born after in vitro fertilization or intracytoplasmic sperm injection stratified for neonatal risk criteria. Acta Obstetricia et Gynecologica Scandinavica. 2014; 93: 277–286. https://doi.org/10.1111/aogs.12328

[4] Rodriguez-Wallberg KA, Palomares AR, Nilsson HP, Oberg AS, Lundberg F. Obstetric and Perinatal Outcomes of Singleton Births Following Single- vs Double-Embryo Transfer in Sweden. JAMA Pediatrics. 2023; 177: 149–159. https://doi.org/10.1001/jamapediatrics.2022.4787

[5] Mancuso AC, Boulet SL, Duran E, Munch E, Kissin DM, Van Voorhis BJ. Elective single embryo transfer in women less than age 38 years reduces multiple birth rates, but not live birth rates, in United States fertility clinics. Fertility and Sterility. 2016; 106: 1107–1114. https://doi.org/10.1016/j.fertnstert.2016.06.017

[6] Li Y, Liu S, Lv Q. Single blastocyst stage versus single cleavage stage embryo transfer following fresh transfer: A systematic review and meta-analysis. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2021; 267: 11–17. https://doi.org/10.1016/j.ejogrb.2021.10.004

[7] Blockeel C, Campbell A, Coticchio G, Esler J, Garcia-Velasco JA, Santulli P, et al. Should we still perform fresh embryo transfers in ART? Human Reproduction. 2019; 34: 2319–2329. https://doi.org/10.1093/humrep/dez233

[8] Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. Lancet (London, England). 2019; 393: 1310–1318. https://doi.org/10.1016/S0140-6736(18)32843-5

[9] Li X, Jia J, Zhang X, Liu W, Luo Y, Zhen X, et al. Analysis of key factors influencing clinical pregnancy outcomes in high-quality single blastocyst frozen-thawed transfer cycles. Zygote. 2026; 34: 117–122. https://doi.org/10.1017/S0967199426100471

[10] Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertility and Sterility. 2000; 73: 1155–1158. https://doi.org/10.1016/s0015-0282(00)00518-5

[11] Cavoretto PI, Farina A, Gaeta G, Seidenari A, Pozzoni M, Spinillo S, et al. Greater estimated fetal weight and birth weight in IVF/ICSI pregnancy after frozen-thawed vs fresh blastocyst transfer: prospective cohort study with novel unified modeling methodology. Ultrasound in Obstetrics & Gynecology: the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2022; 60: 76–85. https://doi.org/10.1002/uog.24806

[12] Cavoretto PI, Farina A, Girardelli S, Gaeta G, Spinillo S, Morano D, et al. Greater fetal crown-rump length growth with the use of in vitro fertilization or intracytoplasmic sperm injection conceptions after thawed versus fresh blastocyst transfers: secondary analysis of a prospective cohort study. Fertility and Sterility. 2021; 116: 147–156. https://doi.org/10.1016/j.fertnstert.2020.11.035

[13] Jiang W, Zheng Z, Yan N, Yao S, Xie Q, Ni D, et al. Maternal age-related declines in live birth rate following single euploid embryo transfer: a retrospective cohort study. Journal of Ovarian Research. 2025; 18: 24. https://doi.org/10.1186/s13048-025-01602-9

[14] Gao W, Tang W, Li C, Deng Y, Chen Y, Zhang Y, et al. Effect of maternal age, embryo number and quality on pregnancy outcome during frozen embryo transfer cycle. Frontiers in Endocrinology. 2025; 16: 1596178. https://doi.org/10.3389/fendo.2025.1596178

[15] Huang W, Li X, Yang H, Huang H. The impact of maternal age on aneuploidy in oocytes: Reproductive consequences, molecular mechanisms, and future directions. Ageing Research Reviews. 2024; 97: 102292. https://doi.org/10.1016/j.arr.2024.102292

[16] Matorras R, Sierra S, Pérez-Fernández S, Malaina I, Santos-Zorrozua B, Prieto B, et al. Influence of parental age on chromosomal abnormalities in PGT-A embryos: exponentially increasing in the mother and completely null in the father. Journal of Assisted Reproduction and Genetics. 2025; 42: 1833–1844. https://doi.org/10.1007/s10815-025-03462-0

[17] Wang Y, Zhou P, Shan H, Liu X, Cheng M, Ye Z, et al. Endometrial aging is accompanied by H3K27ac and PGR loss. Nature Aging. 2025; 5: 816–830. https://doi.org/10.1038/s43587-025-00859-5

[18] Iwasa T, Kuwahara A, Takeshita T, Taniguchi Y, Mikami M, Irahara M. Preimplantation genetic testing for aneuploidy and chromosomal structural rearrangement: A summary of a nationwide study by the Japan Society of Obstetrics and Gynecology. Reproductive Medicine and Biology. 2023; 22: e12518. https://doi.org/10.1002/rmb2.12518

[19] Liao Z, Liu C, Cai L, Shen L, Sui C, Zhang H, et al. The Effect of Endometrial Thickness on Pregnancy, Maternal, and Perinatal Outcomes of Women in Fresh Cycles After IVF/ICSI: A Systematic Review and Meta-Analysis. Frontiers in Endocrinology. 2022; 12: 814648. https://doi.org/10.3389/fendo.2021.814648

[20] Shaodi Z, Qiuyuan L, Yisha Y, Cuilian Z. The effect of endometrial thickness on pregnancy outcomes of frozen-thawed embryo transfer cycles which underwent hormone replacement therapy. PloS One. 2020; 15: e0239120. https://doi.org/10.1371/journal.pone.0239120

[21] Huang W, Tang J, Wei L, Nong L, Tang N, Wei X, et al. Association of endometrial thickness with live birth rates among women undergoing fresh IVF, FET, and PGT cycles. Frontiers in Cell and Developmental Biology. 2025; 13: 1530953. https://doi.org/10.3389/fcell.2025.1530953

[22] Dang VQ, Vuong LN, Luu TM, Pham TD, Ho TM, Ha AN, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial. Lancet (London, England). 2021; 397: 1554–1563. https://doi.org/10.1016/S0140-6736(21)00535-3

[23] Berntsen S, Zedeler A, Nøhr B, Rønn Petersen M, Grøndahl ML, Andersen LF, et al. IVF versus ICSI in patients without severe male factor infertility: a randomized clinical trial. Nature Medicine. 2025; 31: 1939–1948. https://doi.org/10.1038/s41591-025-03621-x

[24] Cutting E, Horta F, Dang V, van Rumste MM, Mol BWJ. Intracytoplasmic sperm injection versus conventional in vitro fertilisation in couples with males presenting with normal total sperm count and motility. The Cochrane Database of Systematic Reviews. 2023; 8: CD001301. https://doi.org/10.1002/14651858.CD001301.pub2

[25] Wang Y, Li R, Yang R, Zheng D, Zeng L, Lian Y, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation for couples with infertility with non-severe male factor: a multicentre, open-label, randomised controlled trial. Lancet (London, England). 2024; 403: 924–934. https://doi.org/10.1016/S0140-6736(23)02416-9

[26] Pham TD, Dang VQ, Ho VNA, Tran CT, Nguyen DTP, Vuong LN, et al. Intracytoplasmic sperm injection versus conventional in vitro fertilization in infertile couples with normal total sperm count and motility: does sperm morphology matter? Human Reproduction (Oxford, England). 2025; 40: 23–29. https://doi.org/10.1093/humrep/deae252

[27] Taylor TH, Patrick JL, Gitlin SA, Wilson JM, Crain JL, Griffin DK. Comparison of aneuploidy, pregnancy and live birth rates between day 5 and day 6 blastocysts. Reproductive Biomedicine Online. 2014; 29: 305–310. https://doi.org/10.1016/j.rbmo.2014.06.001

[28] Li Z, Fang X, Cheng L, Ou Z, Huo J, Yu W, et al. Evaluation of day 5 versus day 6 blastocyst biopsy in preimplantation genetic testing: clinical and neonatal outcomes. Frontiers in Endocrinology. 2025; 16: 1544009. https://doi.org/10.3389/fendo.2025.1544009

[29] Sunkara SK, Siozos A, Bolton VN, Khalaf Y, Braude PR, El-Toukhy T. The influence of delayed blastocyst formation on the outcome of frozen-thawed blastocyst transfer: a systematic review and meta-analysis. Human Reproduction (Oxford, England). 2010; 25: 1906–1915. https://doi.org/10.1093/humrep/deq143

[30] Bourdon M, Pocate-Cheriet K, Finet de Bantel A, Grzegorczyk-Martin V, Amar Hoffet A, Arbo E, et al. Day 5 versus Day 6 blastocyst transfers: a systematic review and meta-analysis of clinical outcomes. Human Reproduction (Oxford, England). 2019; 34: 1948–1964. https://doi.org/10.1093/humrep/dez163

[31] Chen CH, Lee CI, Huang CC, Chen HH, Chang CY, Cheng EH, et al. Increased incidence of live births in implanted day 5 versus day 6 blastocysts following single embryo transfers with PGT-A. Scientific Reports. 2023; 13: 12725. https://doi.org/10.1038/s41598-023-40052-5

[32] Yin B, Li S, Sun L, Yao Z, Cui Y, Zhang C, et al. Comparing Day 5 versus Day 6 euploid blastocyst in frozen embryo transfer and developing a predictive model for optimizing outcomes: a retrospective cohort study. Frontiers in Endocrinology. 2024; 14: 1302194. https://doi.org/10.3389/fendo.2023.1302194

[33] Hashimoto S, Amo A, Hama S, Ito K, Nakaoka Y, Morimoto Y. Growth retardation in human blastocysts increases the incidence of abnormal spindles and decreases implantation potential after vitrification. Human Reproduction (Oxford, England). 2013; 28: 1528–1535. https://doi.org/10.1093/humrep/det059