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A cycle-based model to predict no usable blastocyst formation following cycles of in vitro fertilization in patients with normal ovarian reserve

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Abstract

Objective This study aimed to develop a predictive model for the risk of no usable blastocyst formation in patients with normal ovarian reserve undergoing IVF.

Methods The model was derived from 7,901 patients who underwent their first oocyte retrieval and subsequent blastocyst culture, of which 446 cases have no usable blastocysts formed. Univariate regression analyses, least absolute shrinkage and selection operator regression analysis were used to identify the association of patient and cycle characteristics with the presence of no available blastocyst and to create a nomogram. The performance of the nomogram was assessed using the receiver operating characteristic (ROC) curve and calibration curve, the net benefit threshold of prediction was determined using decision curve analysis (DCA).

Results Multivariate analysis identified three independent predictors: the number of day 3 (D3) embryos, the number of high-quality D3 embryos, and the number of embryos used for blastocyst culture. A nomogram model was developed and internally validated using bootstrapping, demonstrating good discriminative ability with an area under the receiver operating characteristic curve (AUC) of 0.879(95%CI: 0.861–0.890).

Conclusions The cycle-based nomogram can anticipate the probability of no available blastocyst formation in IVF/ICSI treatment. This can help doctors make appropriate clinical judgments and assist patients in managing their expectations effectively.

Keywords In vitro fertilization, Blastocyst formation, Predictive model, Nomogram, Ovarian reserve

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Introduction

In vitro fertilization (IVF) has emerged as a widely adopted assisted reproductive technology (ART) for addressing infertility in couples. A prevalent protocol in IVF involves culturing embryos to the blastocyst stage prior to uterine transfer. The selection of high-quality blastocysts for transfer is critical, as it can significantly enhance the success rate of IVF treatment [1, 2]. These optimal blastocysts demonstrate superior implantation potential [3] and are associated with improved live birth outcomes. Contemporary trends in ART are increasingly favoring elective single embryo transfer (eSET) and extended culture blastocyst transfer [4]. These approaches are being implemented to mitigate the incidence of multiple gestations, a common complication associated with assisted reproductive techniques. The shift towards eSET and blastocyst transfer represents a strategic evolution in IVF protocols, aimed at optimizing treatment outcomes while minimizing associated risks.

However, not all embryos are able to reach the blastocyst stage, and some may arrest at earlier stages of development. This can lead to the situation where after blastocyst culture, there are no viable blastocysts available for transfer. Recent studies have demonstrated that the rate of cultured embryos progressing to the blastocyst stage exhibits considerable variation, with reported ranges spanning from 28 to 55% [5]. Notably, at the individual patient level, blastocyst formation rates display even greater heterogeneity, ranging from complete failure (0%) to near-perfect success (approaching 100%). This marked inter-patient variability underscores the complex interplay of factors influencing embryonic development and highlights the need for personalized approaches in assisted reproductive technologies. Thus, understanding the factors that influence embryo development and predicting the likelihood of unsuccessful blastocyst formation is crucial for optimizing IVF outcomes.

Currently, reliable predictors of blastocyst development remain a subject of debate in the field of reproductive medicine [6, 7]. Limited efforts have been made to establish cycle-specific predictive models that assess the risk of blastocyst formation failure in individual patients. In clinical practice, such a model is highly desirable, particularly for patients with good ovarian reserve and heightened expectations regarding blastocyst culture outcomes. A robust prediction model would serve multiple purposes: Provide early warning of potential blastocyst culture failure, optimize cost-effectiveness of IVF procedures, mitigate psychological stress for patients, and facilitate personalized clinical management strategies.

In this study, we conducted a comprehensive analysis of patients undergoing IVF with normal ovarian reserve, incorporating a wide range of factors associated with blastocyst formation. We set out to establish a well-calibrated nomogram model to predict the likelihood of failed blastocyst culture following oocyte retrieval. This model could provide an accurate reference for both patients and clinicians, facilitating more informed decision-making and optimized management of IVF-ET cycles.

Materials and methods

Patient data

A retrospective cohort study was conducted to analyze the clinical data of patients who had undergone IVF treatment at the Reproductive Medicine Center of the Henan Provincial People's Hospital, Henan, China from September 1st, 2016 to March 1st, 2024. For this study, patients who underwent blastocyst culture after oocyte retrieval in all fresh cycles were included. Patients were excluded if they had received cycles with preimplantation genetic testing (PGT), sperm/oocyte donation, surgically retrieved sperm or had chromosomal abnormalities. Figure 1 shows a flowchart depicting the selection process and the inclusion criteria. All participants provided informed consent. This study was approved by the Reproductive Ethics Committee of Henan Provincial People's Hospital (SYSZLL-2019110401).

In vitro fertilization procedures

Controlled ovarian stimulation and blastocyst culture based on the patient's age, ovarian function, and other factors, a suitable controlled ovarian stimulation protocol was selected. On the day of oocyte retrieval, sperm and oocytes were inseminated. On day 2 post-retrieval, the fertilization status of the embryos was observed. On day 3 post-retrieval, the embryos were scored based on parameters such as cell number, embryo fragmentation, and blastomere uniformity. Considering the embryo growth, the patient's physical condition, and their preference, the decision was made whether to proceed with blastocyst culture. Embryos selected for blastocyst culture were transferred to blastocyst culture dishes and cultured until days 5 and 6 post-retrieval, when blastocyst scoring was performed. The Gardner scoring system was used to evaluate the blastocysts [8, 9]. Blastocysts scored 4BB or higher (with neither inner cell mass nor trophectoderm scored as C) were regarded as high-quality blastocysts. Blastocysts that could not be used for transfer or cryopreservation because of poor quality were identified as failures of blastocyst formation. There was no change in our laboratory protocols during our study period.

Statistical analysis

The endpoint of our study was that no blastocyst formation is available. Data analysis was implemented using EmpowerStats statistical software (X&Y Solutions). Non-normal data were presented as median (interquartile

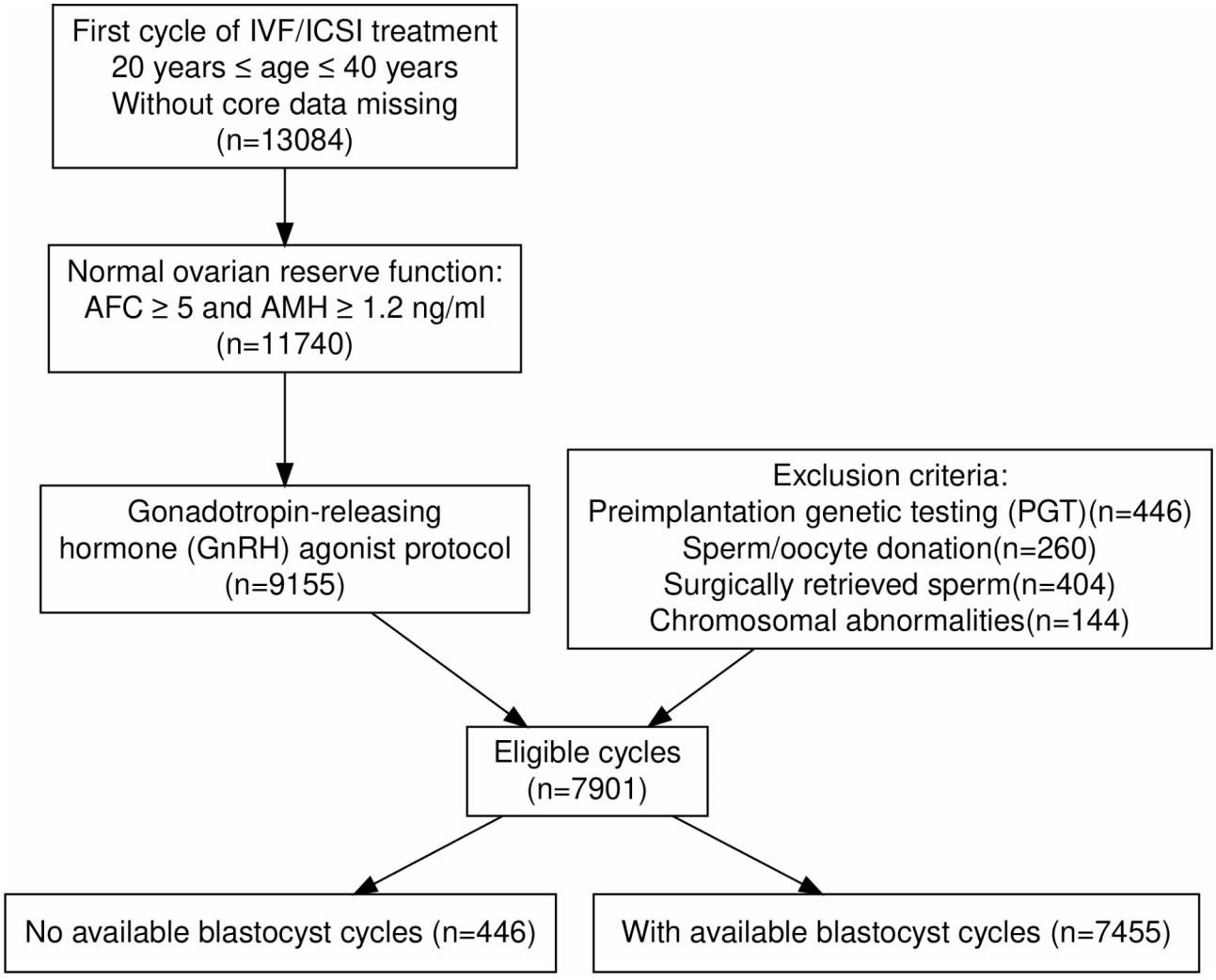

Fig. 1 Flowchart of the data collection process. Notes: AMH, anti-Müllerian hormone; AFC, antral follicle count

ranges). In the univariate analysis, chi-square test or Fisher's exact test was used to analyze the categorical variables, while the Student's t-test or rank-sum test was used to examine the continuous variables. The least absolute shrinkage and selection operator (LASSO) binary logistic regression analyse was used for multivariate analysis to screen the independent risk factors and build a prediction nomogram for no available blastcoyst.

LASSO was implemented by the "glmnet" package in R for variable selection and nomogram was created with the "rms" package. The "glmnet" package was utilized for efficient variable selection using LASSO and ridge regression techniques, helping simplify the model and improve prediction accuracy. The "rms" package was then employed to create a prediction nomogram, translating complex statistical models into user-friendly graphical tools to estimate probabilities of events like no blastocyst formation. The prediction nomogram can offer graphical representations of the selected factors in the model and to facilitate users in calculating probabilities [10].

For validation, we used internal validation with bootstrapping (500 repetitions) to reduce the overfit of the model and to obtain relatively unbiased estimates [11]. The same "rms" analysis was performed in the 500 data sets and a shrinkage factor was calculated by analyzing the variability of the models. It was used to correct the final model and the prediction formula was extracted from the data. The performance of the nomogram was assessed using the receiver operating characteristic (ROC) curve and calibration curve, with the area under the ROC curve (AUC) ranging from 0.5 (no discriminant) to 1 (complete discriminant). Calibration curves were also constructed to validate the model. A decision curve analysis (DCA) was also performed to determine the net benefit threshold of prediction [12].

Results

Patient characteristics

The study included a total of 7,901 patients, of whom 446 (5.64%) were in the no available blastocyst group. A total of 4,090 fresh embryo transfer (ET) cycles were performed. Baseline characteristics: Basal follicle-stimulating hormone (bFSH), basal luteinizing hormone (LH), anti-Müllerian hormone (AMH), antral follicle count (AFC), mean gonadotropin dosage, human chorionic gonadotropin (hCG) trigger day estradiol (E2) level, progesterone (P) level, and number of dominant follicles were significantly different between the two groups ($p < 0.05$). However, age, body mass index (BMI), duration of infertility, cause of infertility, and hCG trigger day LH levels were not significantly different ($p > 0.05$) (Table 1).

In the blastocyst group, the number of oocytes retrieved, mature (MII) oocytes, 2-pronuclear (2PN) embryos, cleavage-stage embryos, Day 3 (D3) embryos, D3 embryos available for blastocyst culture, 2PN rate, cleavage rate, D3 high-quality embryo rate, and fresh cycle clinical pregnancy rate were all significantly higher than the non-blastocyst group ($p < 0.05$). However, there was no significant difference in the fresh cycle live birth rates between the two groups ($p > 0.05$) (Table 2).

Feature selection and parameter building

The candidate predictors included in the original model were FemaleAge, MaleAge, Fertilizationtype, BMI, Infertility type, Infertility factor, Infertile time, bFSH, bLH, HCGdayE2, HCGdayLH, HCGdayP, HCGdayLargeFollicle, AMH, AFC, Gndosage, Gndays, oocytesNumber, MII, 2PN, cleavagenumber, D3embryo, HQ-D3embryo, and embryoforBC. Using LASSO regression analysis performed on the entire patient cohort, these were reduced to the three most significant predictors: D3embryo, HQ-D3embryo, and embryoforBC. The LASSO regression path diagram and coefficient profiles of these characteristics are illustrated in Fig. 2A and B. The most regularized and parsimonious model, with a cross-validated error within one standard error of the minimum, included these three variables. Further multivariate logistic analyses were conducted across the entire cohort, with results shown in Table 3.

Development of an individualized prediction model

The developed model for estimating the lack of usable blastocysts used the selected variables, incluiding three independent predictors (D3embryo, HQ-D3embryo, and embryoforBC) as indicators. The nomogram for prediction is depicted in Fig. 3. Each parameter was assigned a vertical extension (shown in the top points

Table 1 Patient demographics and baseline characteristics

	AVAILABLE.BLASTOCYST group	NO.AVAILABLE.BLASTOCYST group	*P*-value
N	7455	446	
Female Age(Years)	30.4 ± 3.9	30.7 ± 4.2	0.132
Male Age(Years)	31.2 ± 4.5	31.6 ± 4.8	0.141
BMI	23.3 ± 3.7	23.4 ± 3.7	0.493
Infertile Duration	3.4 ± 2.6	3.6 ± 2.6	0.123
Basal FSH(IU/L)	6.2 ± 1.6	6.4 ± 1.7	0.001
Basal LH(IU/L)	6.2 ± 4.0	5.7 ± 3.4	0.009
AMH(ng/ml)	5.0 ± 3.5	4.5 ± 3.1	0.001
AFC	16.5 ± 5.8	15.0 ± 5.6	< 0.001
Gonadotropin Dosage(IU)	2268.0 ± 945.9	2385.1 ± 934.3	0.011
Gonadotropin Days(days)	11.7 ± 2.5	11.5 ± 2.6	0.173
E2 on hCG trigger day(pg/ml)	2519.0 ± 1499.6	1942.3 ± 1112.5	< 0.001
LH on hCG trigger day(IU/L)	1.0 (0.5–1.8)	1.0 (0.5–1.8)	0.352
P on hCG trigger day(ng/ml)	0.6 (0.4–0.9)	0.5 (0.3–0.8)	0.023
Dominant follicles on hCG trigger day	12.1 ± 5.1	9.4 ± 4.6	< 0.001
Infertility Type			0.28
Primary	3665 (49.2%)	231 (51.8%)	
Secondary	3790 (50.8%)	215 (48.2%)	
Infertility Factor			0.74
Tubal factor	5099 (68.4%)	309 (69.3%)	
Ovulation disturbance	1378 (18.5%)	73 (16.4%)	
Endometriosis	411 (5.5%)	29 (6.5%)	
Male factor	567 (7.6%)	35 (7.8%)	

Values are the mean ± standard deviation or number (percentage).

hCG, human chorionic gonadotropin; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing.

hormone; PRL, prolactin; E2, estrogen; P, progesterone; AFC, total antral follicle count; Gn, gonadotropin; CI, confidence interval

Table 2 Patients laboratory index and clinical outcomes

	AVAILABLE.BLASTOCYST group	NO.AVAILABLE.BLASTOCYST group	*P*-value
N	7455	446	
Oocyte	14.3 ± 6.3	9.9 ± 4.8	< 0.001
No. of MII	12.4 ± 5.6	8.0 ± 3.9	< 0.001
No. of 2PN	9.1 ± 4.3	5.1 ± 2.7	< 0.001
No. of Cleavage	8.9 ± 4.2	4.9 ± 2.6	< 0.001
No. of D3 embryo	7.8 ± 3.9	4.0 ± 1.7	< 0.001
No. of high quality D3 embryo	4.0 (2.0–6.0)	1.0 (0.0–2.0)	< 0.001
No. of embryo used for blastocyst culture	7.8 ± 4.3	3.6 ± 2.5	< 0.001
2PN rate	0.7 ± 0.2	0.6 ± 0.2	< 0.001
Cleavage rate	1.0 ± 0.1	0.9 ± 0.2	< 0.001
Top day 3 embryo rate	0.5 (0.3–0.7)	0.2 (0.0-0.5)	< 0.001
Embryo type transfered in fresh cycle(%)			< 0.001
D2	1 (0.0%)	0 (0.0%)	
D3	2340 (61.3%)	269 (99.3%)	
D4	2 (0.1%)	2 (0.7%)	
D5	1351 (35.4%)	0 (0.0%)	
D6	126 (3.3%)	0 (0.0%)	
Embryo number transfered in fresh cycle			< 0.001
single embryo transfer	2853 (74.7%)	79 (29.2%)	
double embryo transfer	967 (25.3%)	192 (70.8%)	
Clinical pregnancy rate of fresh cycle(%)	2458 (64.3%)	138 (50.9%)	< 0.001
Fresh embryo transfer cycles	3820	271	
Live birth rate of fresh cycles(/ET cycles)*	55.80%(1972/3534)	43.04% (102/237)	0.15

Values are the mean ± standard deviation or number (percentage).

ET, embryo transfer. 2PN Rate = Number of 2PN Oocytes/Number of MII Oocytes; Cleavage Rate = Number of Cleaved Embryos/Number of Fertilized Oocytes; Top Day 3 Embryo Rate = Number of Top Quality Embryos on Day 3/Total Number of Embryos on Day 3.

* Patients who were more than 3 months pregnant but had not yet delivered were not included (14 patients in the No Avilable Blastocyst group and 146 patients in the Avilable Blstocyst group)

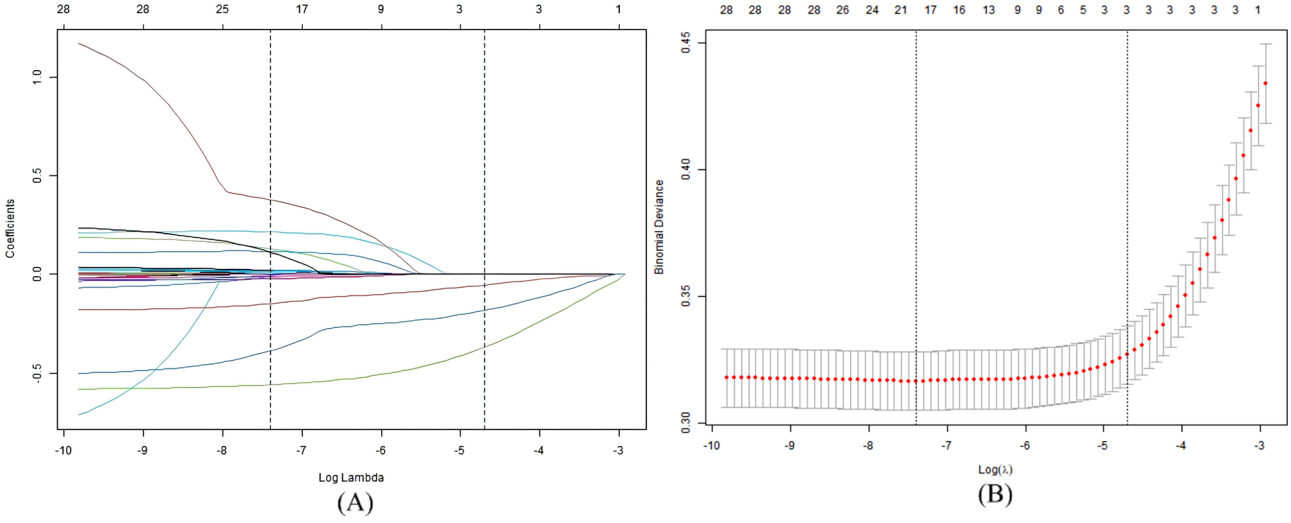

Fig. 2 Variable selection using the least absolute shrinkage and selection operator (LASSO) regression algorithm. **(A)** Lasso regression path diagram. **(B)** LASSO coefficient profiles of the characteristics. Parameters were screened out by 10-fold cross-validation and using lambda.1se as the criteria

Table 3 Results of Multivariate Logistic regression

Characteristic	*N*	Event *N*	OR	95% CI	*p*-value
No. of D3 embryo	7901	446	0.86	0.78, 0.95	0.004
No. of high quality D3 embyo	7901	446	0.58	0.53, 0.65	< 0.001
No. of embryo used for blastocyst culture	7901	446	0.78	0.72, 0.84	< 0.001

OR = Odds Ratio, CI = Confidence Interval

bar) individually. The total score was determined by summing up the scales for each factor. The overall point projected on the bottom scale suggests the likelihood of lack of available blasyst. The equation for the nomogram is as follows: logit (NO. AVAILABLE.BLASTCYST. RCD) = 0.59400-0.11383*D3EMBRYO-0.57934*HQ. D3EMBRYO – 0.28251*EMBRYOFORBC.

Validation of the nomogram

To investigate the forecast value of the model, we performed analyses of the ROC curves. The results indicated that our model demonstrated good discriminative potential, with an AUC of 0.879 (95%CI = 0.862–0.892). The AUC confidence interval and significance test were obtained using the bootstrap method (bootstrap resampling times = 500) (Fig. 4A and B). The calibration plots of the nomogram model are plotted in Fig. 4C, which demonstrate a good correlation between the observed and predicted no usable blastocyst. The calibration curve was relatively close to the ideal curve, which indicates that the predicted results were consistent with the actual findings. The Fig. 4D displays the Decision Curve Analysis (DCA) curves related to the nomogram. A high-risk threshold probability indicates the chance of significant discrepancies in the model's prediction when clinicians encounter

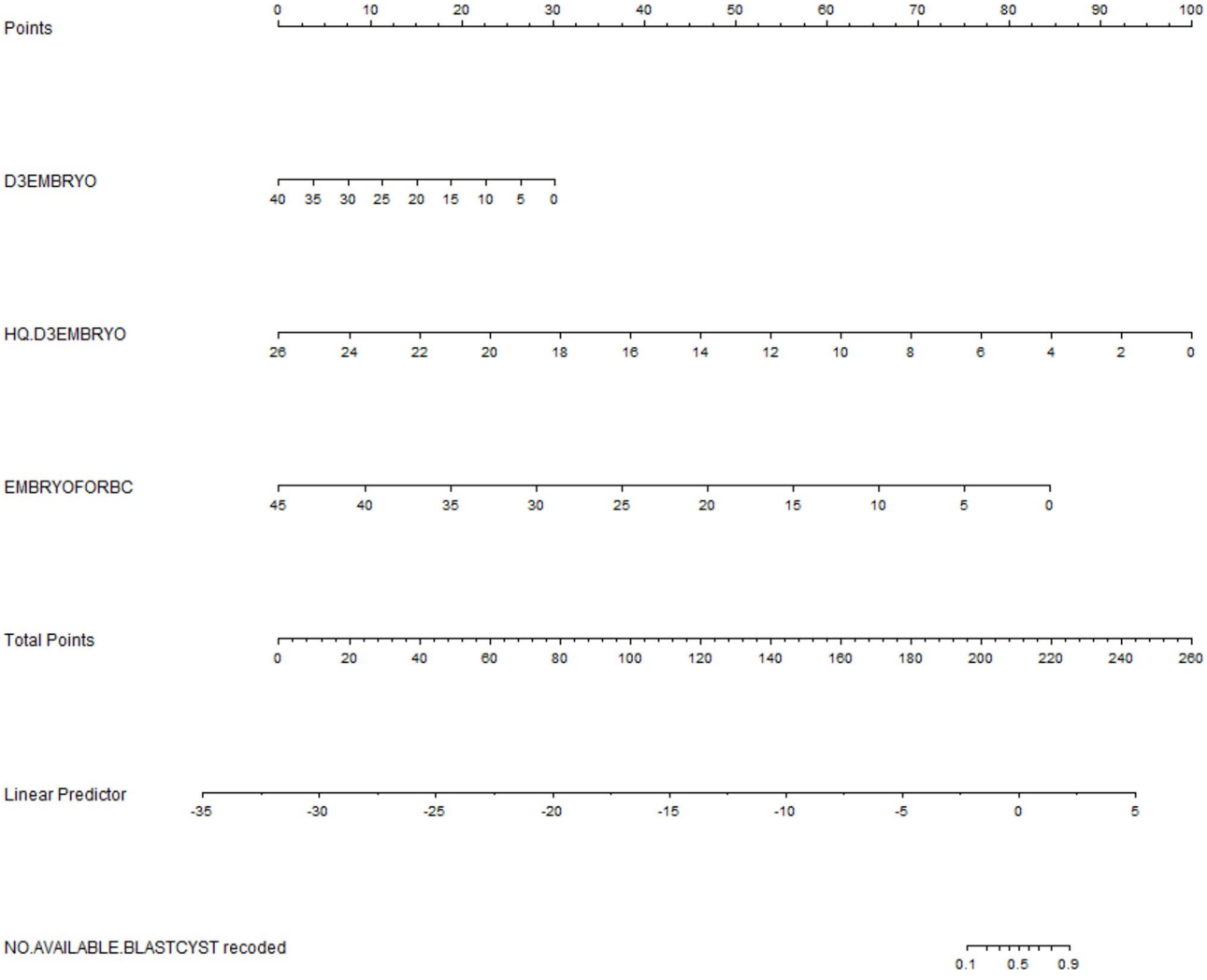

Fig. 3 Nomogram to predict the risk of having no available blastocysts for transfer. The points for each variable were determined by drawing a vertical line from the value of the variable to the point scale. The total points were calculated by summing the individual points, and a downward line was drawn from the "Total Points" axis to intersect with the "Probability of No Available Blastocyst" axis, which provided the estimated probability

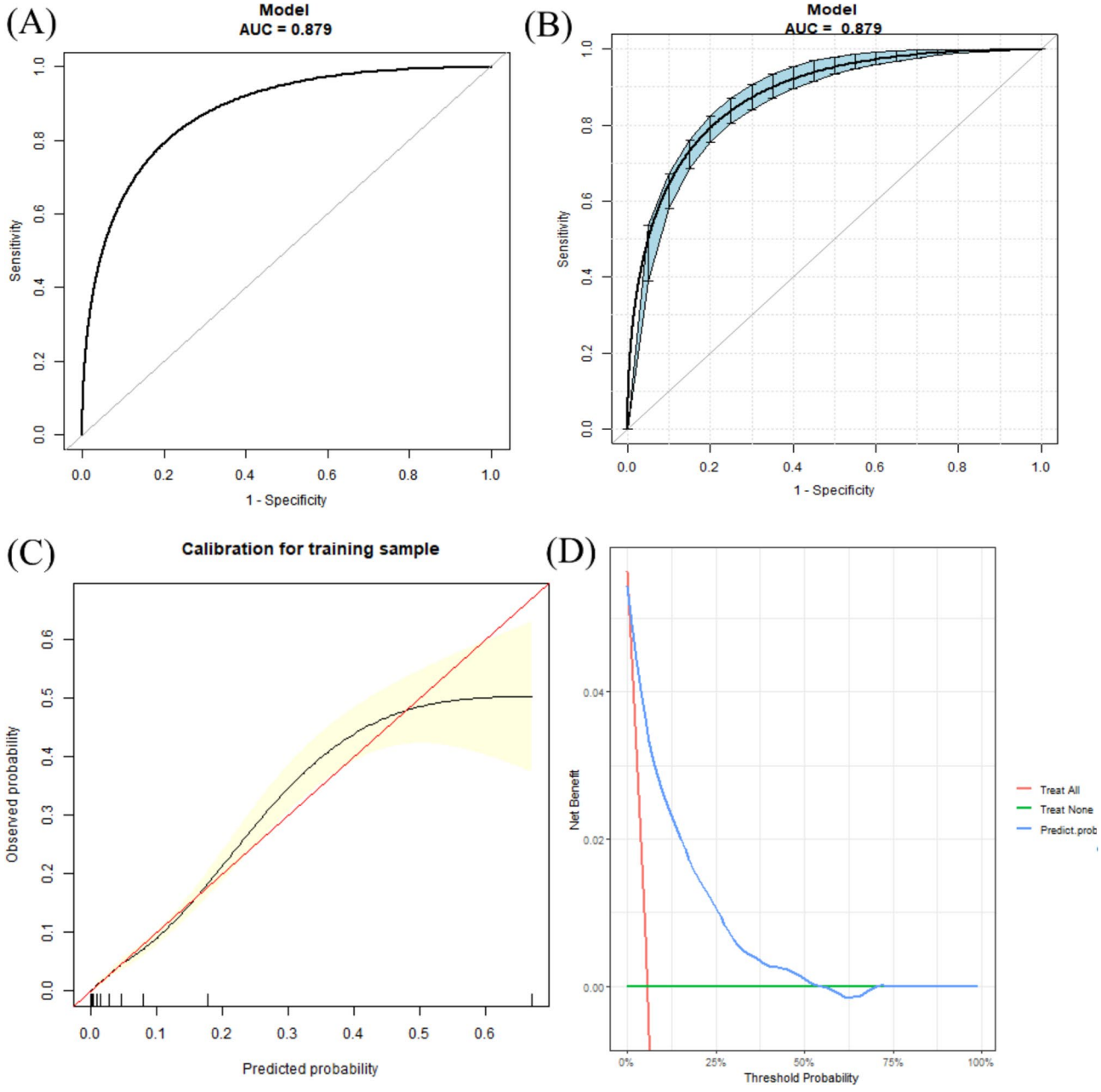

Fig. 4 Model evaluation and validation results. The AUC confidence interval and significance test were obtained using the bootstrap method (bootstrap resampling times = 500) (Fig. **4A** and **B**). The calibration plots of the nomogram model are plotted in Fig. **4C**. The Fig. **4D** displays the Decision Curve Analysis (DCA) curves related to the nomogram

major flaws while utilizing the nomogram for diagnostic and decision-making purposes. This research shows that the nomogram offers substantial net benefits for clinical application through its DCA curve. In addition, we compared the predictive potential of the single variables and the predictive model using the ROC curves. The AUC of the nomogram was significantly. (Table 4)

The nomogram is used to calculate the probability of no blastocyst development. The predictor score that corresponds to each variable (black arrow) is read on the upper scale. The total points (196 points) were calculated by summing the individual points and a downward line was drawn from the "Total Points" axis to intersect with the "Probability of No Available Blastocyst" axis, which provided the estimated probability of no available blastocyst (15.65%). The equation to use linear predictor to predict no available blastocyst is as follows: P (NO. AVAILABLE.BLASTCYST) = 1/ (1 + e− (linear Predictor) (Fig. 5).

Table 4 Predictive accuracy of the model and various variables for no available blastocyst

Variable	AUC	95%CI	Best threshold	Specificity(%)	Sensitivity(%)	PPV(%)	NPV(%)
No. of D3 embryo	0.8232	0.796–0.857	5.5	0.6943	0.8408	0.1413	0.9865
No. of high quality D3 embyo	0.8431	0.822–0.8609	2.5	0.719	0.8251	0.1494	0.9857
No. of embryo used for blastocyst culture	0.827	0.807–0.848	4.5	0.7657	0.7332	0.1577	0.9796
Model	0.8794	0.8612-0.890	-2.5961	0.7902	0.8251	0.1905	0.9869

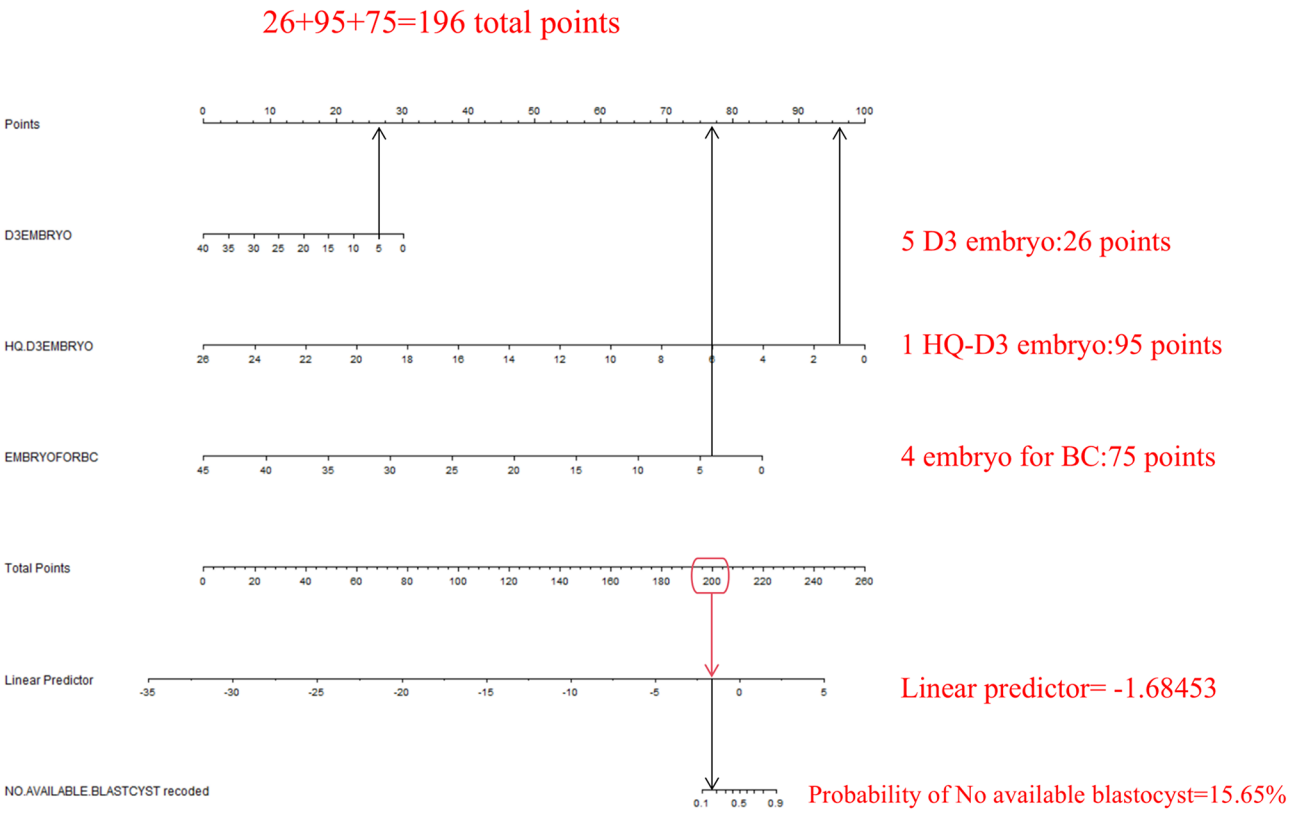

Fig. 5 A worked example for how to use the nomogram—A patient obtained 5 Day 3 embryos, among which 1 was high quality, 4 embryos were used for blastocyst culture

Discussion

The lack of available embryos imposes not only a financial burden on patients undergoing IVF but also induces substantial psychological stress and can lead to strained doctor-patient relationships. In this study, we identified key clinical and laboratory factors influencing blastocyst formation through robust data analysis, and developed a predictive model for patients under 40 with normal ovarian reserve post-oocyte retrieval (Fig. 5). Our model primarily incorporates predictors such as the number of day 3 embryos, top-quality day 3 embryos, and the number of embryos used for blastocyst culture, with these variables proving statistically significant in multivariate logistic regression analysis.

Detailed methodological approach

Embryo quality assessment and blastocyst culture are key laboratory routines. Current methods include traditional morphological assessment [8, 13], non-invasive oocyte and embryo viability assessment via metabolite measurement [14, 15], and time-lapse imaging [16, 17]. These focus on individual embryos, not entire retrieval cycles and they incur additional costs. Predictive models based on clinical data could advise on blastocyst culture necessity, reducing IVF costs and aiding clinician decisions.

We utilized LASSO regression for effectively managing variable selection and interaction testing without overfitting. This ensured the inclusion of clinically relevant variables, aligning well with established literature. Specifically, factors like the quantity and quality of day 3 embryos were emphasized due to their documented impact on blastocyst formation, supported by studies [18, 19]. This methodological advancement results in a more streamlined and concise model that maintains high predictive accuracy evidenced by an AUC of 0.88. (Researchs before reported an AUC of 0.80) [20]. The resulting nomogram provides a graphical synthesis of the predictive model, facilitating intuitive calculations for clinicians

to estimate specific probabilities of outcomes. This is especially beneficial given the increased accessibility and application of nomograms in reproductive medicine [21, 22].

Our research also reflects real-world scenarios by allowing patients the option to select some or all embryos for blastocyst culture, which is more consistent with actual clinical practices compared to the more rigid criteria in Dessolle et al. Their models predict day 5 blastocyst formation [20], assuming least five zygotes on Day 1 after fertilization and all were used for blastocyst culture. Embryonic transfer decisions are complex, and our model can support the implementation of individualized transplantation strategies in clinical practice.

Our model demonstrated robust performance upon testing through repeated sampling, evidenced by strong calibration and discrimination metrics. However, calibration plots suggested a slight overestimation of results, which we acknowledge as a potential area for refinement. Furthermore, our study evaluates the model's clinical utility through internal validation and the incorporation of decision curve analysis (DCA). This analysis helps clinicians determine the most beneficial threshold for treatment decisions, balancing the benefits of true positives against the costs of false positives.

Limitations and future directions

While retrospective analysis naturally carries inherent biases, we meticulously controlled selection bias by setting stringent inclusion criteria related to ovarian reserves and COS protocols. These measures aimed to ensure population homogeneity and enhance the clinical applicability of our findings. Acknowledging that our model's validation was restricted to internal data, we recognize the need for external validation across multiple centers. Such validation is crucial before clinical adoption, given the common performance variances seen in models tested outside their original population [23, 24]. Future studies should thus focus on broader data collection to validate and generalize our model's utility.

Conclusion

We have successfully devised a cycle-specific predictive model for assessing the risk of unsuccessful blastocyst formation in women undergoing IVF. Despite our model's promising calibration and performance metrics, further validation is essential. Once validated externally, our model could substantially aid clinical decision-making, potentially recommending day 3 embryo transfer for patients at elevated risk of blastocyst formation failure. This approach promises to enhance the outcomes of assisted reproductive technologies by personalizing patient care.

Author contributions

S-DZ supervised the whole research, including the processes, conception, design, and completion. She also contributed to the analysis of the study results and made amendments to the publication. XW made significant contributions to the analysis of the data and also wrote the first version of the paper. C-YD conducted data collecting and authored portions of the text. C-LZ made substantial contributions to design of the work and revised it. Each author made contributions to the paper and endorsed the submitted version. The authors affirm that they have no conflicts of interest.

Funding

NA.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Reproductive Medicine Ethics Committee of Henan Provincial People's Hospital committees (No: SYSZ-LL-2021091501), China. The procedures followed were in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association.

Consent for publication

The Reproductive Medicine Ethics Committee of Henan Provincial People's Hospital committees waived the need for obtaining informed consent from the participants.

Competing interests

The authors declare no competing interests.

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