Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Prediction of live birth failure following the first fresh embryo transfer with GnRH antagonist protocol: development and validation of a nomogram

Haiying Zhou
Shandong University of Traditional Chinese Medicine
Zhicheng Jia
Chinese Academy of Chinese Medical Sciences
Mengyu Shi
Shandong University of Traditional Chinese Medicine
Xinxin Yang
Shandong University of Traditional Chinese Medicine
Xinwei Sun
Shandong University of Traditional Chinese Medicine
Wenlong Qi
Shandong University of Traditional Chinese Medicine
Xinyu Li
Shandong University of Traditional Chinese Medicine
Lei Huang
Shandong University of Traditional Chinese Medicine
Junjie Wang
Shandong University of Traditional Chinese Medicine
Ying Guo
71000916@sdutcm.edu.cn

Affiliated Hospital of Shandong University of Traditional Chinese Medicine

Research Article

Keywords: Live birth, Nomogram, GnRH antagonist protocol, Fresh embryo transfer, Assisted reproductive technology, Prediction model, Risk factors

Posted Date: November 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11698551/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Prediction of live birth failure following the first fresh embryo transfer with GnRH antagonist protocol: development and validation of a nomogram

Haiying Zhou[1], Zhicheng Jia[2], Mengyu Shi [1], Xinxin Yang[1], Xinwei Sun [1], Wenlong Qi [1], Xinyu Li [1], Lei Huang [1], junjie Wang [1], Ying Guo[3]

[1]The First Clinical College, Shandong University of Traditional Chinese Medicine, Jinan 250014, China; [2]Department of Gynecology, Guang'anmen Hospital,Chinese Academy of Chinese Medical Sciences, Beijing 100053, China;[3]Department of Reproductive and Genetics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan 250011, China

Corresponding author: Ying Guo, Email: 71000916@sdutcm.edu.cn

Abstract

Objective: To develop and validate a predictive nomogram based on routine clinical indicators for assessing the risk of live birth failure in patients undergoing their first fresh embryo transfer following controlled ovarian stimulation with the GnRH antagonist（GnRH-ant）protocol.

Methods: A total of 1,351 patients receiving the GnRH-ant protocol for their first fresh embryo transfer were retrospectively enrolled. After excluding 293 ineligible cases, 1,058 patients were included and stratified into a live birth success group (n = 385) and a live birth failure group (n = 673) according to reproductive outcomes. The dataset was randomly divided into a training set (n = 741) and a test set (n = 317) at a 7:3 ratio. Univariate and multivariate logistic regression analyses were performed on the training set to identify independent predictors of live birth failure, which were then incorporated into a nomogram prediction model. Model performance was evaluated using receiver operating characteristic (ROC) curves, calibration curves, the

Hosmer-Lemeshow test, and decision curve analysis (DCA).

Results: Multivariate logistic regression identified age, endometrial thickness on trigger day, trigger type, number of viable blastocysts on day 5, number of viable embryos, and number of top-quality embryos as independent predictors of live birth failure (all $P < 0.05$). The nomogram incorporating these six variables demonstrated favorable predictive performance, with a C-index of 0.802 and an area under the curve（AUC） of 0.802 in the training set, and corresponding values of 0.798 in the test set. The Hosmer-Lemeshow test indicated satisfactory model calibration ($P = 0.572$). Calibration curves showed good agreement between predicted and observed probabilities, and decision curve analysis confirmed that the model provided clinical net benefit across a wide range of threshold probabilities.

Conclusions: The proposed nomogram, based on six readily available clinical predictors, is an effective tool for predicting live birth failure in patients undergoing their first fresh embryo transfer with the GnRH-ant protocol. It can assist clinicians in early identification of high-risk patients and support individualized decision-making regarding embryo transfer strategies. Further multicenter prospective studies are warranted to validate its generalizability.

Keywords: Live birth; Nomogram; GnRH antagonist protocol; Fresh embryo transfer; Assisted reproductive technology; Prediction model;Risk factors

1Introduction

With the rapid development and wide application of assisted reproductive technology (ART), the GnRH-ant protocol has become one of the mainstream regimens for controlled ovarian stimulation, owing to its merits including lower total gonadotropin (Gn) dosage, shorter ovarian stimulation period, lower risk of ovarian hyperstimulation syndrome (OHSS), and superior patient

adherence[1][2]. Moreover, the antagonist protocol has been proven more cost-effective than the agonist protocol in fresh embryo transfer cycles [3].

The ultimate goal of ART is to achieve a healthy singleton live birth[4][5]. However, specific to patients receiving their first fresh embryo transfer with the GnRH-ant protocol, there is still a lack of efficient tools to precisely identify individuals at high risk of live birth failure [6][7]. Even for patients receiving uniform antagonist protocol for their first fresh transfer, clinical pregnancy outcomes vary substantially. Some patients still suffer live birth failure caused by implantation failure or clinical pregnancy loss even after embryo transfer. This not only reduces the cumulative clinical efficiency of ART but also imposes heavy psychological and economic burdens on patients.

Ovarian response and ovarian stimulation-related parameters are core indicators reflecting ovarian reserve, follicular developmental potential and the suitability of stimulation regimens, which are closely correlated with embryo quality, endometrial receptivity and subsequent pregnancy outcomes [8]. Therefore, clinicians need to comprehensively evaluate multiple indicators such as oocyte retrieval yield and embryo quality to determine the optimal timing and scheme of fresh embryo transfer. At present, clinical evaluation of transfer prognosis mainly depends on single indicators, and few individualized risk prediction tools integrate multiple clinical characteristics and ovarian stimulation parameters, which hinders the early screening and targeted intervention of high-risk patients.

In view of the above clinical dilemma, this study constructed a prediction model for live birth failure among patients undergoing the first fresh embryo transfer with GnRH-ant protocol based on ovarian response markers, stimulation-related parameters and baseline clinical features. This

model aims to provide an objective reference for early screening of high-risk patients, personalized embryo transfer scheme optimization and individualized clinical management, so as to improve the efficacy and safety of ART.

2Materials and methods

2.1Participants

This retrospective study enrolled 1,351 patients who received their first fresh embryo transfer with the GnRH-ant protocol at the Department of Reproductive and Genetics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, from January 2018 to December 2024. We excluded 175 patients with unavailable clinical data and 118 patients with incomplete clinical data (Figure 1).

Inclusion criteria:

(1) First controlled ovarian stimulation with the GnRH-ant protocol;

(2) First fresh embryo transfer cycle;

(3) Serum anti-Müllerian hormone (AMH) < 7 ng/mL;

(4) Maternal age < 42 years[9].

Exclusion criteria:

(1) Endocrine disorders, including hyperprolactinemia and thyroid dysfunction;

(2) Chromosomal abnormalities;

(3) Organic genital tract abnormalities: uterine malformations, endometrial polyps, submucous fibroids, untreated hydrosalpinges, stage III – IV endometriosis, adenomyosis, or uterine fibroids with a diameter > 3 cm;

(4) Absolute contraindications to IVF/ICSI treatment.

Ultimately, 1,058 eligible patients were retained and randomly assigned to a training set (n = 741) and a test set (n = 317) at a 7:3 ratio. This study was approved by the Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (Approval No. 2026-126-01-YJS). Written informed consent was waived given the retrospective design of the study.

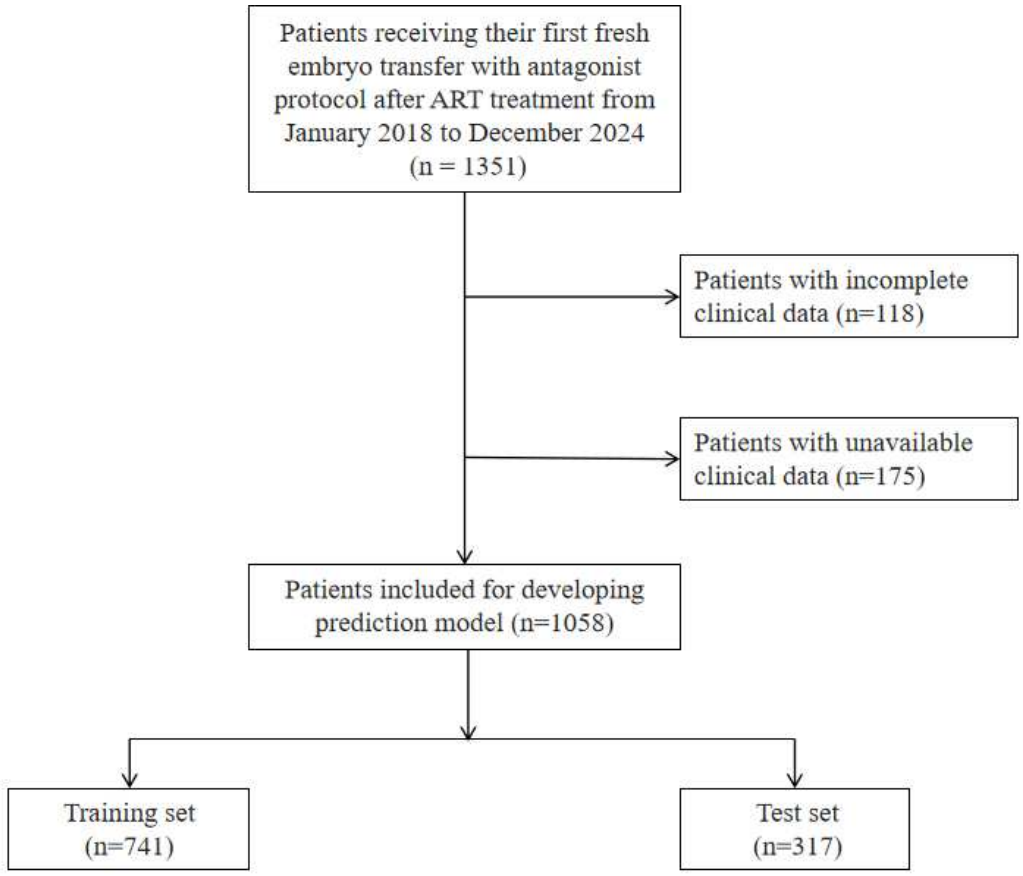


Fig1. Flowchart of this study.

2.2 Ovarian stimulation and embryo transfer protocol

Daily subcutaneous cetrorelix acetate (Merck Serono, Switzerland) was administered from days 5–6 after Gn initiation until the day of human chorionic gonadotropin (hCG) trigger. Triggering was performed when at least two dominant follicles had reached a diameter of ≥18

mm. Patients received either a single trigger of 250 μg recombinant hCG (Ovidrel; Merck Serono, Switzerland) or a dual trigger consisting of 0.2 mg triptorelin acetate (Decapeptyl; Ferring Pharmaceuticals, Switzerland) plus 250 μg recombinant hCG on the same evening. Transvaginal ultrasound-guided oocyte retrieval was conducted 36 hours after trigger administration.

Fertilization was performed via standard IVF or intracytoplasmic sperm injection (ICSI) according to clinical history and semen analysis results of male partners. Embryo transfer was carried out on day 3 (cleavage-stage embryos) or day 5 (blastocyst-stage embryos) following oocyte retrieval. Luteal phase support was initiated on the day of oocyte retrieval and maintained until serum β-hCG testing. For patients with positive β-hCG results, luteal support was continued for approximately 50 days after embryo transfer, followed by a gradual tapering regimen before complete discontinuation.

2.3 Embryo culture and morphological evaluation

Embryo morphological evaluation was performed according to our center's laboratory standards and the Gardner blastocyst grading system[10][11].

Number of viable embryos: Defined as the total number of cleavage-stage embryos on day 3 (D3) after oocyte retrieval that met the criteria for transfer or cryopreservation based on morphological assessment. Inclusion criteria were: ≥ 6 blastomeres, < 20% fragmentation, relatively uniform blastomere size, and no obvious multinucleation.

Number of top-quality embryos: Defined as the total number of embryos that met our center's top-quality morphological criteria among the viable embryos. Inclusion criteria were: 7–9 blastomeres, < 10% fragmentation, uniform blastomere size, and no multinucleation.

Number of viable blastocysts on day 5: Defined as the total number of embryos that

developed to the blastocyst stage on day 5 after oocyte retrieval and met the criteria for transfer or cryopreservation. Blastocyst grading was performed according to the Gardner grading system. Inclusion criteria were: blastocyst expansion grade $\geqslant$ 3, and both inner cell mass (ICM) and trophectoderm (TE) grades $\geqslant$ C (i.e., $\geqslant$ 3CC).

Number of top-quality blastocysts: Defined as the total number of blastocysts with expansion grade $\geqslant$ 4, ICM grade $\geqslant$ B, and TE grade $\geqslant$ B (i.e., $\geqslant$ 4BB).

2.4 Outcome definition

Live birth was defined as the primary outcome of this study. Live birth was defined as the complete expulsion or extraction from the mother of a newborn weighing ≥ 1000 g or with a gestational age of ≥ 28 weeks, which, after separation from the mother, exhibited any of the following signs of life: heartbeat, spontaneous respiration, umbilical cord pulsation, or voluntary muscle contraction.

2.5 Data collection

Baseline characteristics included age, body mass index (BMI), antral follicle count (AFC), endometrial thickness, anti-Müllerian hormone (AMH), baseline luteinizing hormone (LH), estradiol (E_2), progesterone (P), and follicle-stimulating hormone (FSH), as well as infertility type, duration of infertility, gravidity, and infertility factors. Ovarian stimulation-related parameters included duration of Gn stimulation, total Gn dose, trigger type, serum E_2, LH, and P levels on the day of hCG trigger, and endometrial thickness on the day of hCG trigger. Laboratory data included number of oocytes retrieved, fertilization method, number of normally fertilized oocytes (2PN), number of viable blastocysts on day 5, number of viable embryos, and number of top-quality embryos.

2.6 Prediction model development

The dataset was randomly allocated to a training set (n = 741) and a test set (n = 317) at a 7:3 ratio. Univariate logistic regression analysis was performed on the training set to preliminarily screen potential predictors, with $P < 0.05$ as the threshold for inclusion. Variables with $P < 0.05$ in the univariate analysis were then entered into multivariate logistic regression analysis to identify independent predictors of live birth failure. Prior to multivariate logistic regression, multicollinearity was assessed using linear regression analysis, with tolerance > 0.1 and variance inflation factor (VIF) < 10 considered as indicating no significant multicollinearity. A nomogram prediction model was subsequently constructed based on the final identified predictors.

2.7 Statistical analysis

Statistical analyses were performed using SPSS 26.0, and risk prediction model construction and internal validation were conducted using R 4.5.2. The Shapiro-Wilk test was used to assess the normality of data distribution. Normally distributed continuous variables were presented as mean ± standard deviation (SD), while non-normally distributed variables were expressed as median with interquartile range [M (P25, P75)]. Categorical variables were summarized as frequencies and percentages (%). For between-group comparisons, the independent samples t-test was used for normally distributed continuous variables, the Mann-Whitney U test for non-normally distributed continuous variables, and the chi-square test for categorical variables (with Fisher's exact test applied when expected frequencies were < 5). A P value < 0.05 was considered statistically significant. Model discrimination was evaluated using the area under the receiver operating characteristic (ROC) curve (AUC). Model calibration was assessed using calibration curves and the Hosmer-Lemeshow test ($P > 0.05$ indicated good fit). Clinical utility was evaluated using

DCA. Internal validation was performed using the Bootstrap method with 1,000 resamples, and the concordance index (C-index) was calculated.

2.8 Variable definitions and assignments

Variable definitions and assignments are presented in Table 1.

Table 1.Variable definitions and assignments.

Variable	Definition	Assignment
Trigger type	Type of trigger drug	0 = single trigger; 1 = dual trigger
Infertility type	Type of infertility	0 = primary infertility; 1 = secondary infertility
Live birth outcome	Outcome variable	0 = live birth; 1 = live birth failure
Fertilization method	Method of fertilization	0 = IVF; 1 = ICSI
Infertility factor	Cause of infertility	0 = female factor; 1 = male factor; 2 = combined factors

3 Result

3.1 Baseline characteristics of the training set

A total of 741 patients were included in the training set, comprising 285 patients (38.5%) in the live birth group and 456 patients (61.5%) in the live birth failure group. Baseline characteristics of the training set stratified by live birth outcome are presented in Table 2.

Patients in the live birth group were younger (31.68 ± 4.21 vs. 34.58 ± 4.13 years) and had a lower BMI (23.42 ± 2.98 vs. 24.14 ± 3.84 kg/m²). No significant differences were observed in duration of infertility (3.18 ± 2.15 vs. 3.46 ± 2.75 years) or gravidity [0.00 (0.00, 1.00) vs. 1.00 (0.00, 2.00)] between the two groups. Primary infertility was more common in the

live birth group than in the live birth failure group (55.4% vs. 38.2%), whereas secondary infertility was more common in the live birth failure group than in the live birth group (61.8% vs. 44.6%). In both groups, female factor was the predominant cause of infertility (78.2% vs. 80.9%), with no significant differences in the distribution of male factor or combined factors.

Regarding baseline endocrine and ovarian reserve parameters, the live birth group had higher baseline AMH levels (2.87 ± 1.67 vs. 2.34 ± 1.54 ng/mL) and antral follicle count (8.88 ± 3.74 vs. 7.96 ± 3.89), and lower baseline FSH levels compared to the failure group (6.99 ± 2.87 vs. 7.65 ± 3.45 U/L). No significant differences were observed in baseline LH, E_2, or P levels between the two groups.

In terms of ovarian stimulation parameters, the two groups were similar in duration of Gn stimulation, total Gn dose, and LH, E_2, and P levels on the trigger day. Notably, the live birth group had significantly greater endometrial thickness on the trigger day compared to the failure group (12.07 ± 5.33 vs. 9.84 ± 3.02 mm). The proportion of patients receiving dual trigger was higher in the live birth group (49.8% vs. 33.1%), whereas single trigger was more frequently used in the failure group (66.9% vs. 50.2%).

Regarding embryological parameters, the live birth group outperformed the failure group across all indicators, including number of oocytes retrieved [8 (6, 11) vs. 7 (5, 10)], number of normally fertilized oocytes [6 (4, 8) vs. 5 (3, 7)], number of viable embryos [2 (2, 3) vs. 2 (2, 2)], number of top-quality embryos [1 (0, 2) vs. 1 (0, 1)], number of viable blastocysts on day 5 [2 (0, 3) vs. 1 (0, 2)], and number of top-quality blastocysts [0 (0, 1) vs. 0 (0, 0)].

The distribution of fertilization methods was similar between the two groups, with IVF being the predominant method in both the live birth group (84.9%) and the failure group (80.5%), while

ICSI accounted for 15.1% and 19.5%, respectively.

Table 2. Baseline characteristics of the training set.

Variable	Live birth group (n = 285)	Live birth failure group (n = 456)
Baseline characteristics		
Age (years)	31.68 ± 4.21	34.58 ± 4.13
BMI (kg/m²)	23.42 ± 2.98	24.14 ± 3.84
Duration of infertility (years)	3.18 ± 2.15	3.46 ± 2.75
Gravidity (times)	0.00 (0.00, 1.00)	1.00 (0.00, 2.00)
Infertility type		
Primary infertility	158（55.4%）	174（38.2%）
Secondary infertility	127（44.6%）	281（61.8%）
Infertility factor		
Female factor	223 (78.2%)	369 (80.9%)
Male factor	13 (4.6%)	17 (3.7%)
Combined factors	49 (17.2%)	70 (15.4%)

Variable	Live birth group (n = 285)	Live birth failure group (n = 456)
Baseline endocrine and ovarian reserve		
Baseline AMH (ng/mL)	2.87 ± 1.67	2.34 ± 1.54
Baseline FSH (U/L)	6.99 ± 2.87	7.65 ± 3.45
Baseline LH (U/L)	4.24 (3.17, 6.03)	4.13 (3.12, 5.48)
Baseline E_2 (pg/mL)	37.56 (29.20, 49.67)	38.00 (29.18, 53.10)
Baseline P (ng/mL)	0.40 (0.24, 0.65)	0.44 (0.25, 0.70)
Antral follicle count (n)	8.88 ± 3.74	7.96 ± 3.89
Ovarian stimulation and trigger day parameters		
Duration of Gn stimulation (days)	8.89 ± 1.44	8.98 ± 1.73
Total Gn dose (U)	2025.00 (1668.75, 2475.00)	2100.00 (1800.00, 2625.00)
LH on trigger day (U/L)	2.80 ± 2.18	3.02 ± 2.87
E_2 on trigger day (pg/mL)	1809.81 ± 915.00	1846.98 ± 1022.88

Variable	Live birth group (n = 285)	Live birth failure group (n = 456)
P on trigger day (ng/mL)	0.89 ± 0.40	0.91 ± 0.47
Endometrial thickness on trigger day (mm)	12.07 ± 5.33	9.84 ± 3.02
Trigger type		
Dual trigger	142 (49.8%)	151 (33.1%)
Single trigger	143 (50.2%)	305 (66.9%)
Embryological parameters		
Number of oocytes retrieved	8 (6, 11)	7 (5, 10)
Number of normally fertilized oocytes (2PN)	6 (4, 8)	5 (3, 7)
Number of viable embryos	2 (2, 3)	2 (2, 2)
Number of top-quality embryos	1 (0, 2)	1 (0, 1)
Number of viable blastocysts on day 5	2 (0, 3)	1 (0, 2)
Number of top-quality blastocysts	0 (0, 1)	0 (0, 0)
Technical interventions		
Fertilization method		

Variable	Live birth group (n = 285)	Live birth failure group (n = 456)
IVF	242 (84.9%)	367 (80.5%)
ICSI	43 (15.1%)	89 (19.5%)

205

206 **3.2 Univariate logistic regression analysis**

207 Univariate logistic regression analysis was performed to identify factors associated with live
208 birth failure in the training set. As shown in Table 3, age (OR = 1.177, 95% CI: 1.131 – 1.224, *P* <
209 0.001), BMI (OR = 1.064, 95% CI: 1.018 – 1.112, *P* = 0.006), gravidity (OR = 1.389, 95% CI:
210 1.221 – 1.581, *P* < 0.001), secondary infertility (OR = 2.009, 95% CI 1.49 – 2.71, P < 0.001) , and
211 baseline FSH (OR = 1.079, 95% CI: 1.020 – 1.141, *P* = 0.008) were identified as risk factors for
212 live birth failure. In contrast , baseline AMH (OR = 0.818, 95% CI: 0.746 – 0.897, *P* < 0.001),
213 antral follicle count (OR = 0.940, 95% CI: 0.904 – 0.978, *P* = 0.002), endometrial thickness on
214 trigger day (OR = 0.697, 95% CI: 0.642 – 0.756, *P* < 0.001), dual trigger (OR = 0.500, 95% CI:
215 0.369 – 0.678, *P* < 0.001), and all embryological parameters including number of oocytes
216 retrieved, number of normally fertilized oocytes (2PN), number of viable embryos, number of
217 top-quality embryos, number of viable blastocysts on day 5, and number of top-quality blastocysts
218 were protective factors (all *P* < 0.05). No significant associations with live birth failure were
219 observed for duration of infertility, baseline LH, duration of Gn stimulation, hormone levels on
220 trigger day, fertilization method, or infertility factors (all *P* > 0.05).

221 **Table 3.Univariate logistic regression analysis of factors associated with live birth failure in the**

222 **training set.**

Variable	β (B)	se (S.E.)	OR（95% CI）	Wald χ^2	*P* value
Baseline characteristics					
Age (years)	0.163	0.020	1.177 (1.131–1.224)	68.697	<0.001
BMI (kg/m²)	0.062	0.022	1.064 (1.018–1.112)	7.489	0.006
Duration of infertility (years)	0.046	0.031	1.047 (0.985–1.112)	2.263	0.132
Gravidity (times)	0.329	0.066	1.389 (1.221–1.581)	24.868	<0.001
Infertility type (Secondary vs. Primary)	0.698	0.153	2.009 (1.49–2.71)	20.707	<0.001
Infertility factor (overall)				0.801	0.670
Male factor vs. Female factor	0.144	0.205	1.155 (0.773–1.727)	0.497	0.481
Combined factors vs. Female factor	-0.088	0.413	0.915 (0.408–	0.046	0.830

Variable	β (B)	se (S.E.)	OR（95% CI）	Wald χ^2	*P* value
			2.056)		
Baseline endocrine and ovarian reserve					
Baseline AMH (ng/mL)	-0.201	0.047	0.818 (0.746–0.897)	18.045	<0.001
Baseline FSH (U/L)	0.076	0.029	1.079 (1.020–1.141)	6.974	0.008
Baseline LH (U/L)	-0.052	0.029	0.950 (0.898–1.005)	3.200	0.074
Baseline E_2 (pg/mL)	0.000	0.001	1.000 (0.998–1.002)	0.001	0.970
Baseline P (ng/mL)	-0.030	0.090	0.970 (0.813–1.158)	0.114	0.736
Antral follicle count (n)	-0.062	0.020	0.940 (0.904–0.978)	9.827	0.002
Ovarian stimulation and trigger day					

Variable	β (B)	se (S.E.)	OR（95% CI）	Wald χ^2	*P* value
parameters					
Duration of Gn stimulation (days)	0.035	0.047	1.036 (0.945–1.136)	0.568	0.451
Total Gn dose (U)	0.000	0.000	1.000 (1.000–1.000)*	4.052	0.044
LH on trigger day (U/L)	0.033	0.031	1.034 (0.973–1.098)	1.175	0.278
E_2 on trigger day (pg/mL)	0.000	0.000	1.000 (1.000–1.000)#	0.194	0.660
P on trigger day (ng/mL)	0.082	0.174	1.086 (0.773–1.525)	0.224	0.636
Endometrial thickness on trigger day (mm)	-0.362	0.042	0.697 (0.642–0.756)	73.892	<0.001
Trigger type (Dual vs.Single)	-0.693	0.155	0.500(0.369–0.678)	20.039	<0.001
Embryological parameters					

Variable	β (B)	se (S.E.)	OR（95% CI）	Wald χ^2	*P* value
Number of oocytes retrieved	-0.088	0.022	0.916 (0.877–0.957)	16.571	<0.001
Number of normally fertilized oocytes (2PN)	-0.098	0.026	0.906 (0.861–0.954)	14.010	<0.001
Number of viable embryos	-0.129	0.063	0.879 (0.777–0.995)	4.137	0.042
Number of top-quality embryos	-0.291	0.066	0.748 (0.657–0.851)	19.247	<0.001
Number of viable blastocysts on day 5	-0.182	0.036	0.833 (0.777–0.894)	25.959	<0.001
Number of top-quality blastocysts	-0.444	0.116	0.641 (0.511–0.805)	14.610	<0.001
Technical interventions					
Fertilization method (ICSI vs. IVF)	-0.314	0.203	0.731 (0.491–1.089)	2.380	0.123

223

224 **3.3 Multivariate logistic regression analysis**

Multivariate logistic regression analysis results are presented in Table 4. Age (OR = 1.171, 95% CI: 1.117–1.228, $P < 0.001$) was identified as an independent risk factor for live birth failure. Endometrial thickness on trigger day (OR = 0.664, 95% CI: 0.602–0.732, $P < 0.001$), trigger type (dual vs. single trigger, OR = 0.431, 95% CI: 0.298–0.623, $P < 0.001$), number of viable embryos (OR = 0.835, 95% CI: 0.702–0.994, $P = 0.042$), number of top-quality embryos (OR = 0.761, 95% CI: 0.640–0.904, $P = 0.002$), and number of viable blastocysts on day 5 (OR = 0.883, 95% CI: 0.793–0.982, $P = 0.022$) were independent protective factors for live birth failure. Multicollinearity diagnostics revealed that the variance inflation factor (VIF) of the included variables ranged from 1.014 to 1.222, and tolerance values ranged from 0.818 to 0.986, indicating no significant multicollinearity.

BMI, antral follicle count, baseline AMH, baseline FSH, gravidity, infertility type, number of oocytes retrieved, number of normally fertilized oocytes (2PN), and number of top-quality blastocysts were not independently associated with live birth (all $P > 0.05$).

Table 4. Multivariate logistic regression analysis of factors associated with live birth failure in the training set.

Variable	β (B)	se (S.E.)	Wald χ^2	*P* value	OR (95% CI)
Age (years)	0.157	0.024	41.686	<0.001	1.171 (1.117–1.228)
Endometrial thickness on trigger day (mm)	-0.409	0.050	68.060	<0.001	0.664 (0.602–0.732)

Variable	β (B)	se (S.E.)	Wald χ^2	*P* value	OR (95% CI)
Trigger type (Dual vs.Single)	-0.846	0.188	20.142	<0.001	0.431 (0.298–0.623)
Number of viable embryos	-0.180	0.089	4.140	0.042	0.835 (0.702–0.994)
Number of top-quality embryos	-0.274	0.088	9.733	0.002	0.761 (0.640–0.904)
Number of viable blastocysts on day 5	-0.125	0.055	5.221	0.022	0.883 (0.793–0.982)

3.4 Nomogram construction

Based on the independent predictors identified by multivariate logistic regression analysis, a nomogram for predicting the risk of live birth failure was constructed using R 4.5.2 software (Figure 2). In the nomogram, each variable was assigned a corresponding point value. The sum of the points across all variables corresponded to a predicted probability, which represented the estimated risk of live birth failure for an individual patient.

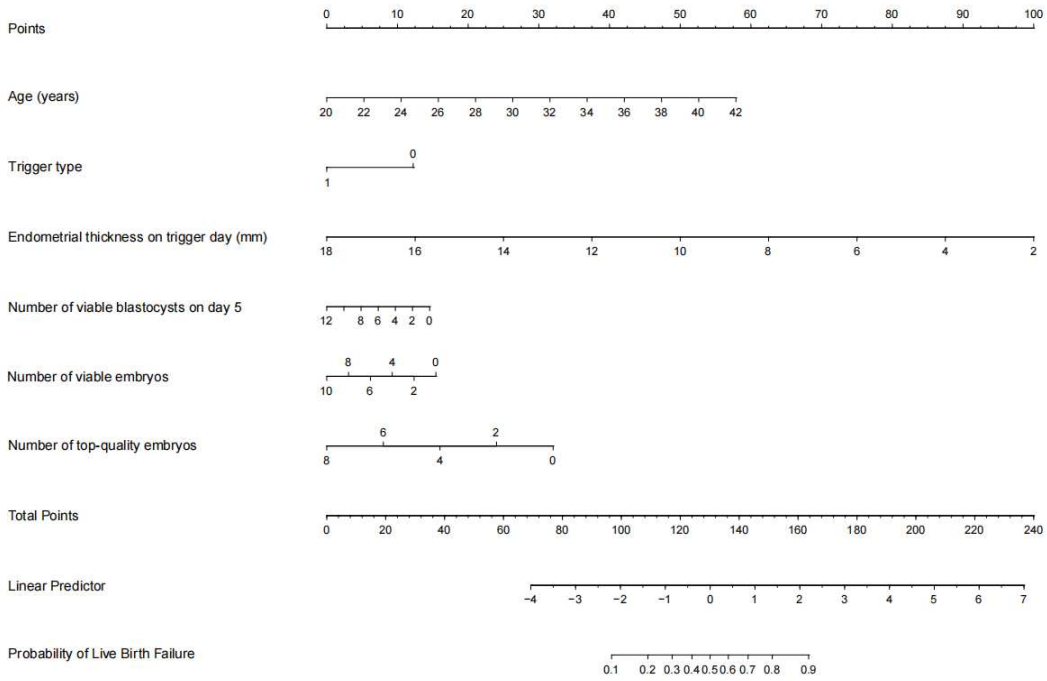


Fig2. Nomogram for predicting live birth failure.

3.5 Validation of the nomogram model

The discriminative ability of the nomogram was assessed using ROC curve analysis in both the training and test sets. As presented in Figure 3, the AUC for the training set was 0.802 (95% CI: 0.770 – 0.802), while the AUC for the test set was 0.798 (95% CI: 0.750 – 0.798). These findings suggest that the nomogram model demonstrated favorable discriminative ability and satisfactory generalizability.

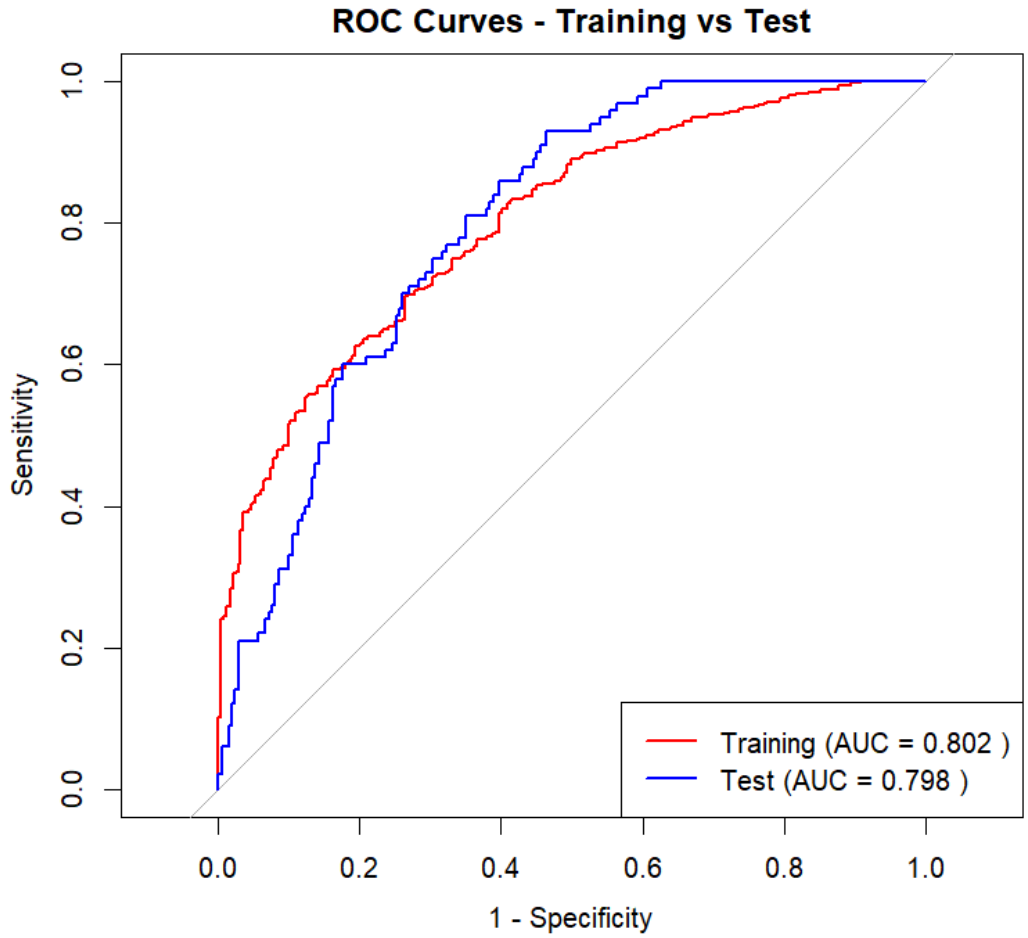
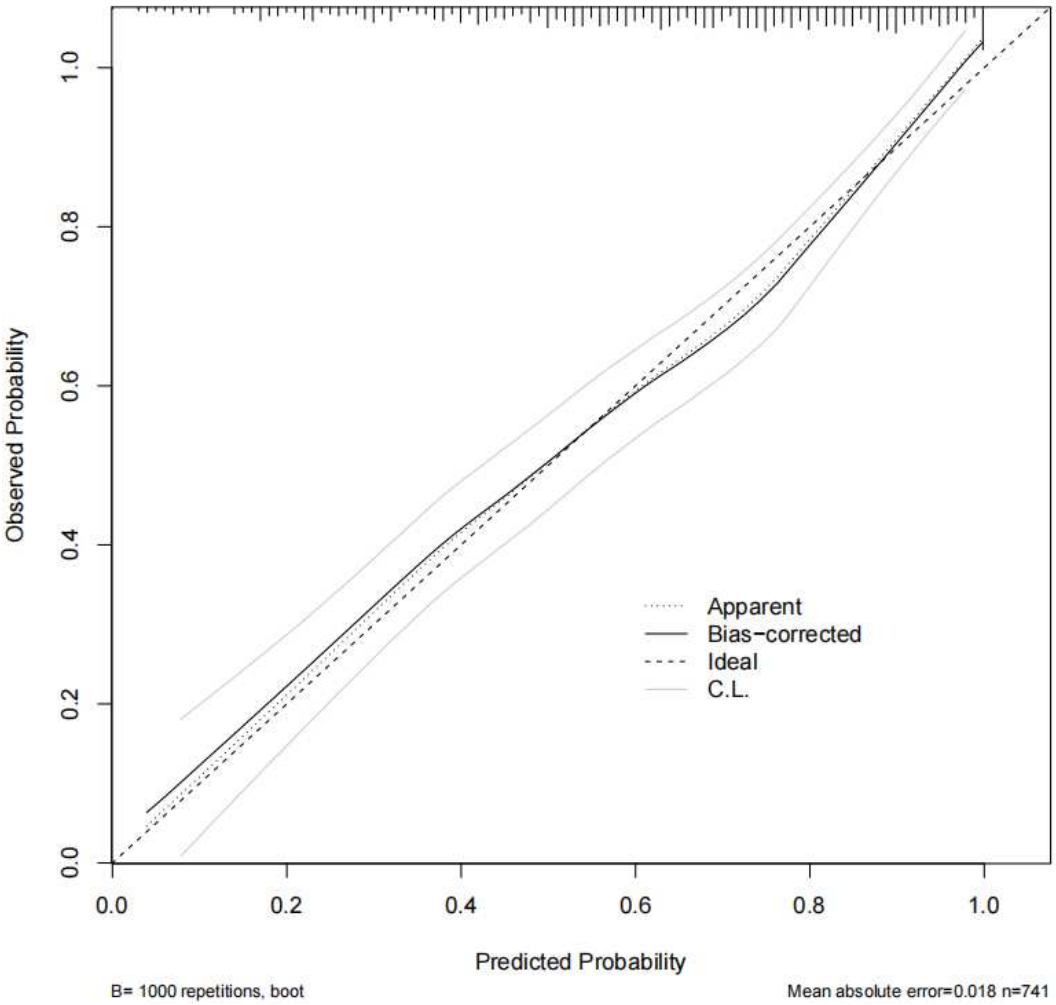


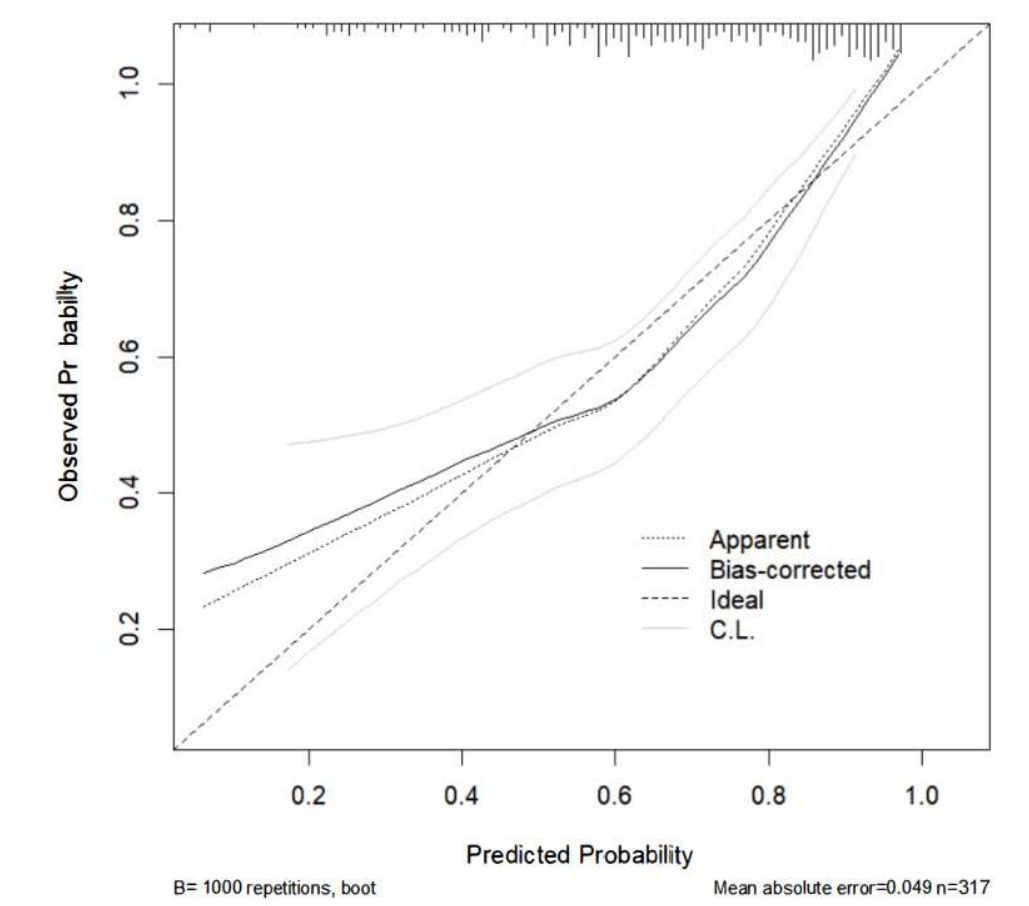
Fig3. ROC curve graph for model with training set and test set.

The calibration of the nomogram model was further evaluated to assess the agreement between predicted risks and actual outcomes (Figure 4A, 4B). In the training set, the calibration curve demonstrated good agreement, as confirmed by the Hosmer-Lemeshow test ($\chi^2 = 6.674$, $P = 0.572$). Similarly, in the test set, the model also showed good consistency between predicted and observed outcomes, supported by the Hosmer-Lemeshow test ($\chi^2 = 13.257$, $P = 0.103$). These findings indicate that the nomogram model possesses favorable calibration and reliability in predicting the risk of live birth failure. DCA further confirmed that the nomogram provided clinical net benefit across a wide range of threshold probabilities (Figure 4C, 4D).

266 A.

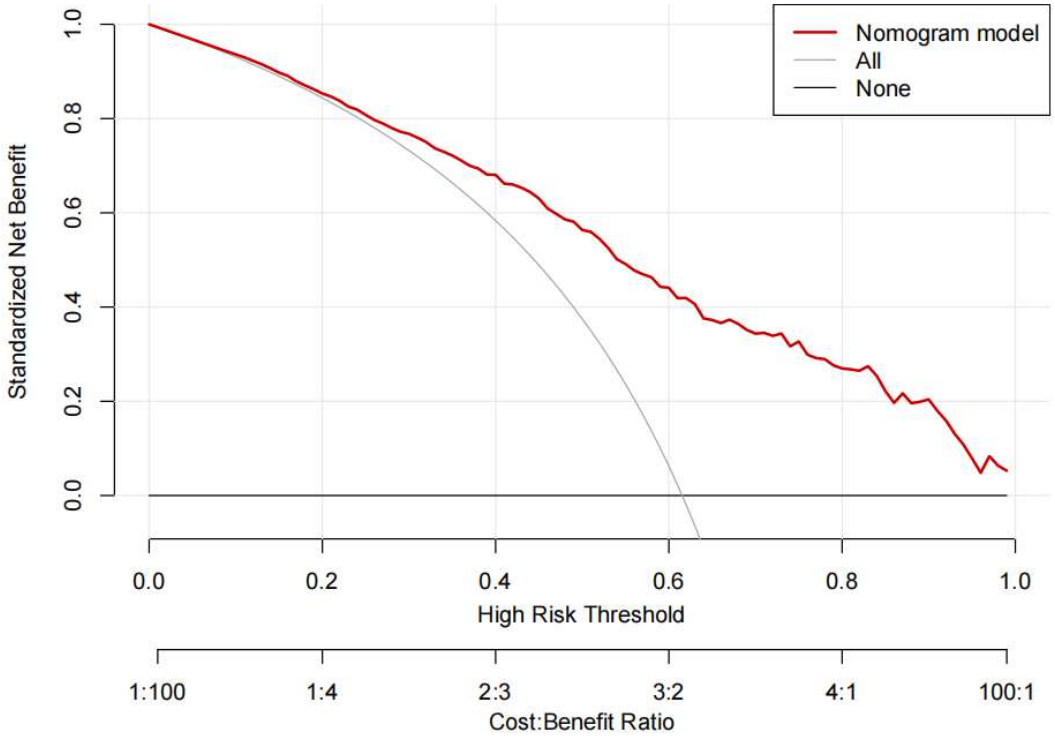

Observed Probability
0.0
0.2
0.4
0.6
0.8
1.0
Predicted Probability
Apparent
Bias−corrected
Ideal
C.L.
B= 1000 repetitions, boot
Mean absolute error=0.018 n=741

267


Observed Pr babilty
0.2
0.4
0.6
0.8
1.0
Apparent
Bias-corrected
Ideal
C.L.
0.2
0.4
0.6
0.8
1.0
Predicted Probability
B= 1000 repetitions, boot
Mean absolute error=0.049 n=317

268 B.

C.


Nomogram model
All
None
Standardized Net Benefit
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
High Risk Threshold
1:100
1:4
2:3
3:2
4:1
100:1
Cost:Benefit Ratio

D.

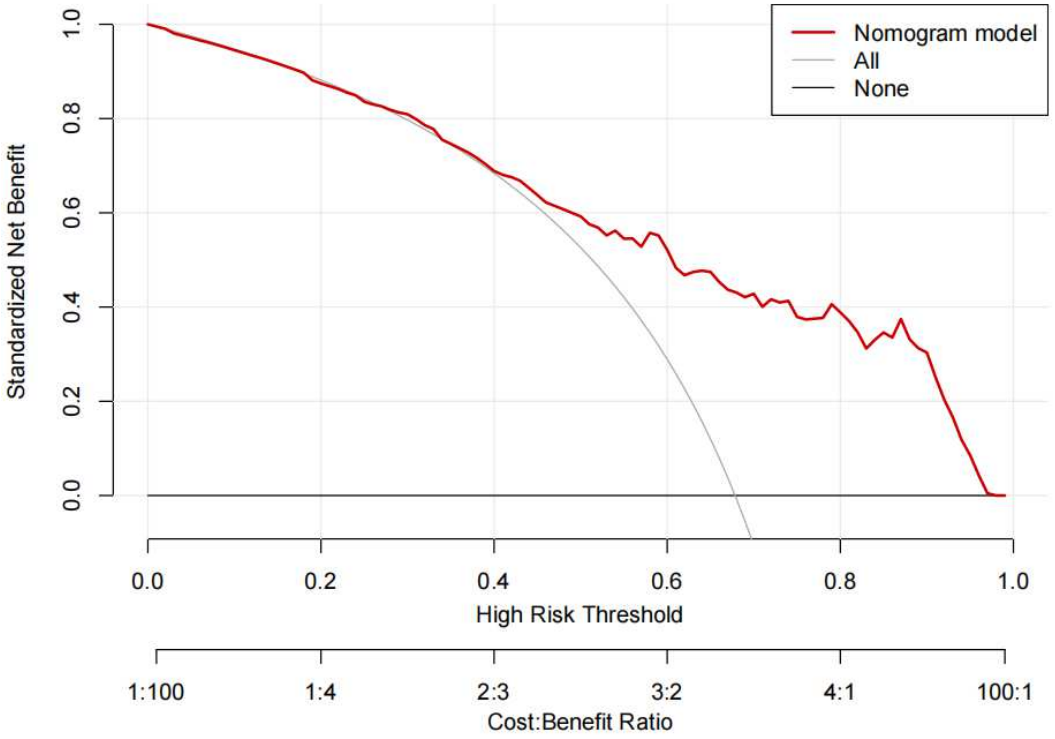

Nomogram model
All
None
Standardized Net Benefit
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
High Risk Threshold
1:100
1:4
2:3
3:2
4:1
100:1
Cost:Benefit Ratio

Fig4. Calibration curves and DCA of the nomogram model for predicting live birth failure in the training and test sets. (A) Calibration curve of the nomogram model in the training set; (B) Calibration curve of the nomogram model in the test set; (C) DCA curve of the nomogram model in the training set; (D) DCA curve of the nomogram model in the test set.

4 Discussion

Live birth is the ultimate goal of ART. Accurately identifying factors that affect live birth outcomes and optimizing embryo transfer strategies remain central concerns in reproductive medicine. In patients undergoing their first fresh embryo transfer with the GnRH-ant protocol, early identification and risk stratification of live birth failure are essential for improving cumulative live birth rates. Several studies have investigated potential predictors of IVF/ICSI live birth outcomes[12][13][14][15], and factors such as age, endometrial thickness, embryo quality, and the number of available embryos have been associated with live birth in fresh embryo transfer cycles. However, few studies have integrated multiple clinical and ovarian stimulation parameters into individualized prediction tools to guide transfer decisions[16][17] .

In this study, six independent predictors were identified using logistic regression analysis: age, endometrial thickness on trigger day, trigger type, number of viable blastocysts on day 5, number of viable embryos, and number of top-quality embryos. A nomogram incorporating these variables was constructed and demonstrated favorable discrimination, calibration, and clinical net benefit upon internal validation, indicating its potential utility for individualized risk assessment prior to embryo transfer.

Age is a well-established predictor of reproductive outcomes [18]. Advanced maternal age is associated with diminished ovarian reserve and reduced oocyte quality, primarily due to mitochondrial dysfunction and increased aneuploidy rates, which compromise embryonic developmental competence and lead to live birth failure[19]. Notably, even with the use of preimplantation genetic testing for aneuploidy (PGT-A) to select euploid embryos, maternal age remains an independent determinant of pregnancy success [20].

Trigger type represents a modifiable factor in controlled ovarian stimulation. The dual trigger strategy, combining a GnRH agonist with hCG, mimics the physiological LH surge to promote oocyte nuclear maturation and cumulus expansion, while hCG provides luteal phase support [21]. Accumulating evidence suggests that the dual trigger may offer clinical benefits in patients with poor ovarian response, low fertilization rates, or those undergoing fertility preservation[22][23][24][25]. Our findings support the preferential use of the dual trigger in appropriately selected patients.

Endometrial thickness on trigger day is an important sonographic indicator of endometrial receptivity and is closely associated with implantation and clinical pregnancy outcomes[26]. A thin endometrium may indicate insufficient functional layer development, thereby increasing the risk of adverse pregnancy outcomes. Specifically, an endometrial thickness below 8 mm, and particularly below 6 mm, has been associated with a significant decline in live birth rates[12][27][28].

Morphological assessment remains the primary method for selecting embryos with the highest developmental potential. On day 3 , the degree of cytoplasmic fragmentation reflects metabolic activity and chromosomal stability during early cleavage[29]. These fragments, which

consist of degenerated cytoplasmic components or apoptotic bodies, may disrupt normal blastomere arrangement and functional integrity when excessively accumulated, thereby reducing the number of transferable embryos[30]. At the blastocyst stage (D5), morphological evaluation based on inner cell mass quality, trophectoderm status, and blastocoel expansion provides objective indicators of developmental competence, with higher-quality blastocysts associated with higher live birth rates [31][32][33]. Thus, from D3 to D5, a sequential screening process is formed, and the number of viable embryos, number of top-quality embryos, and number of viable blastocysts on day 5 serve as key quantitative indicators at each critical node of this cascade.

The predictors identified in our study partially overlap with those reported in previous studies [34][35][36]. However, unlike prior prediction models that focused on specific populations such as patients with polycystic ovary syndrome or endometriosis, our study was conducted in a non-selective infertile population, which may enhance the generalizability of our findings. The nomogram provides a visual presentation of risk factors, enabling clinicians to rapidly assess individualized risk prior to embryo transfer. The incorporation of decision curve analysis further supports the clinical utility of the model by demonstrating net benefit across a range of threshold probabilities.

Several limitations should be acknowledged. First, this was a single-center retrospective study with a relatively limited sample size (n = 1,058), which may introduce selection bias and limit the generalizability of our findings. Second, although internal validation was performed using the bootstrap method, external validation in independent cohorts is warranted. Third, certain molecular biomarkers, including embryo chromosomal ploidy status, sperm DNA fragmentation index[19][37][38][39], and genetic polymorphisms related to ovarian response, such as FSHR and

AMHR2[40][41][42], were not included in the analysis and may affect ovarian response and pregnancy outcomes.

Future studies integrating multi-omics approaches—including genomics, proteomics, and metabolomics—may help identify additional molecular markers associated with ovarian reserve, endometrial receptivity, and embryonic developmental potential, thereby further enhancing the predictive accuracy and individualization of the current nomogram model.

5 Conclusion

In this study, we developed a nomogram prediction model for live birth failure in patients undergoing their first fresh embryo transfer with the GnRH-ant protocol, based on six variables: age, endometrial thickness on trigger day, trigger type, number of viable embryos, number of top-quality embryos, and number of viable blastocysts on day 5. The model demonstrated favorable discrimination, calibration, and clinical net benefit in both the training and test sets, suggesting its potential utility as a clinical tool for early identification of high-risk patients and optimization of individualized embryo transfer strategies. Further multicenter prospective studies are warranted to validate its generalizability and stability.

Declarations

Ethics approval and consent to participate

The study was conducted at a public tertiary referral university hospital in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine on May 6, 2026 (approval no. 2026-126-01-YJS).

Clinical trial number: not applicable.

Consent for publication

This manuscript does not contain any individual person's data in any form (including any individual details, images or videos).

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China [Grant No. 82374505] and the Taishan Scholars Youth Expert Program [Grant No. 202211353].

Authors' contributions

All authors have made substantial contributions to this manuscript. H.Y. Zhou collected the data and drafted the manuscript. Z.C. Jia, M.Y. Shi, and X.X. Yang guided the study design and critically reviewed the manuscript. W.L. Qi, X.W. Sun, and X.Y. Li contributed to data collection. L. Huang and J.J. Wang assisted with data analysis. Y. Guo conceived and designed the study, supervised the implementation, and acquired funding. All authors reviewed and approved the final manuscript for submission.

Acknowledgments

The authors acknowledge all the Chinese patients and the Reproductive and Genetic Center for their contributions to this study.

References

[1] ESHRE Guideline Group on Ovarian Stimulation; Ata B, Bosch E, Broer S, Griesinger G, Grynberg M, Kolibianakis E, Kunicki M, La Marca A, Lainas G, Le Clef N, Massin N, Polyzos NP, Sunkara SK, Timeva T, Töyli M, Urbancsek J, Broekmans F. ESHRE guideline: ovarian stimulation for IVF/ICSI: an update in 2025†. Hum Reprod. 2026 Apr 1;41(4):498-514. doi: 10.1093/humrep/deag018.

[2] Venetis CA, Storr A, Chua SJ, Mol BW, Longobardi S, Yin X, D'Hooghe T. What is the optimal GnRH antagonist protocol for ovarian stimulation during ART treatment? A systematic review and network meta-analysis. Hum Reprod Update. 2023 May 2;29(3):307-326. doi: 10.1093/humupd/dmac040.

[3] Jing M, Lin C, Zhu W, Tu X, Chen Q, Wang X, Zheng Y, Zhang R. Cost-effectiveness analysis of GnRH-agonist long-protocol and GnRH-antagonist protocol for in vitro fertilization. Sci Rep. 2020 May 26;10(1):8732. doi: 10.1038/s41598-020-65558-0.

[4] Practice Committee of the American Society for Reproductive Medicine and the Practice Committee for the Society for Assisted Reproductive Technologies. Electronic address: ASRM@asrm.org. Guidance on the limits to the number of embryos to transfer: a committee opinion. Fertil Steril. 2021 Sep;116(3):651-654. doi: 10.1016/j.fertnstert.2021.06.050.

[5] Zhu S, Zhang Q, Hu M, Zhang Y, Sun A, Gao X, Chen H, Yan J. Karyotypic analysis of 62,587 Han Chinese infertile couples undergoing assisted reproductive technology treatments: insights into chromosomal variations. BMC Genomics. 2025 Jul 7;26(1):640. doi: 10.1186/s12864-025-11851-z.

[6] Wang H, Zhu J, Li T. Comparison of perinatal outcomes and pregnancy complications between fresh embryo transfer and frozen embryo transfer in singleton pregnant women. Zhejiang

Da Xue Xue Bao Yi Xue Ban. 2023 Feb 25;52(1):24-32. English, Chinese. doi: 10.3724/zdxbyxb-2022-0510.

[7] Wei D, Sun Y, Zhao H, Yan J, Zhou H, Gong F, Zhang A, Wang Z, Jin L, Bao H, Zhao S, Xiao Z, Qin Y, Geng L, Cui L, Sheng Y, Sun M, Liu P, Ding L, Liu H, Wu K, Li Y, Lu Y, Xu B, Xu B, Zhang L, Zhang H, Legro RS, Chen ZJ. Frozen versus fresh embryo transfer in women with low prognosis for in vitro fertilisation treatment: pragmatic, multicentre, randomised controlled trial. BMJ. 2025 Jan 29;388:e081474. doi: 10.1136/bmj-2024-081474.

[8] Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. J Clin Endocrinol Metab. 2020 Nov 1;105(11):3361–73. doi: 10.1210/clinem/dgaa513.

[9] Nukaga S, Tokoro M, Asano E, Inoue D, Hashiba Y, Fukunaga N, Asada Y. Two-year cumulative live-birth rates and maximum number of transfer cycles in women aged ≥ 40 years. Sci Rep. 2024 Oct 22;14(1):24814. doi: 10.1038/s41598-024-74460-y.

[10] Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000;73(6):1155-1158.doi:10.1016/s0015-0282(00)00518-5.

[11] Gonen Y, Casper R. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). J In Vitro Fert Embryo Transf. 1990;7(3):146-152.doi：10.1007/BF01135678.

[12] Mahutte N, Hartman M, Meng L, Lanes A, Luo ZC, Liu KE. Optimal endometrial thickness in fresh and frozen-thaw in vitro fertilization cycles: an analysis of live birth rates from 96,000

autologous embryo transfers. Fertil Steril. 2022 Apr;117(4):792-800. doi:
10.1016/j.fertnstert.2021.12.025.
[13] Winter HG, Rolnik DL, Mol BWJ, et al. Can dietary patterns impact fertility outcomes? A systematic review and meta-analysis. Nutrients. 2023;15(11):2589. doi:10.3390/nu15112589.
[14] Ranisavljevic N, Huberlant S, Montagut M, et al. Low luteal serum progesterone levels are associated with lower ongoing pregnancy and live birth rates in ART: systematic review and meta-analyses. Front Endocrinol (Lausanne). 2022;13:892753. doi:10.3389/fendo.2022.892753.
[15] van Loendersloot LL, van Wely M, Limpens J, Bossuyt PM, Repping S, van der Veen F. Predictive factors in in vitro fertilization (IVF): a systematic review and meta-analysis. Hum Reprod Update. 2010;16(6):577-589. doi:10.1093/humupd/dmq015.
[16] Wu S, Wang X, Liu Y, et al. Predictive models for live birth outcomes following fresh embryo transfer in assisted reproductive technologies using machine learning. J Transl Med. 2025;23(1):1004. doi:10.1186/s12967-025-07045-6.
[17] Pérez-Milán F, Caballero-Campo M, Carrera-Roig M, et al. Impact of endometrial thickness on reproductive outcome in fresh and frozen-thawed embryo transfer: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2025;66(3):271-281. doi:10.1002/uog.29270.
[18] Liu Z, Zhang H, Xiong F, et al. Prediction of clinical pregnancy outcome after single fresh blastocyst transfer during in vitro fertilization: an ensemble learning perspective. Hum Fertil (Camb). 2024;27(1):2422918. doi:10.1080/14647273.2024.2422918.
[19] Fu W, Cui Q, Bu Z, Shi H, Yang Q, Hu L. Elevated sperm DNA fragmentation is correlated with an increased chromosomal aneuploidy rate of miscarried conceptus in women of advanced

447 age undergoing fresh embryo transfer cycle. Front Endocrinol (Lausanne). 2024;15:1289763.
448 doi:10.3389/fendo.2024.1289763.
449 [20] Cromack SC, Pavone M. Even with preimplantation genetic testing for aneuploidy, age still
450 matters. Fertil Steril. 2023;120(2):266-267. doi:10.1016/j.fertnstert.2023.05.169.
451 [21] Zhu H, Zhao C, Pan Y, et al. Dual trigger for final follicular maturation improves
452 cumulative live-birth rate in ovarian stimulation for freeze-all in vitro
453 fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne).
454 2021;12:708247. doi:10.3389/fendo.2021.708247.
455 [22] Syam HH, Ritonga MA, Adrianto N. Efficacy of double trigger versus hCG trigger alone in
456 GnRH-antagonist cycles: a systematic review and meta-analysis. Gynecol Obstet Invest. 2025.
457 doi:10.1159/000549461.
458 [23] Hong YH, Kim SK, Lee JR, Jee BC, Suh C. Clinical efficacy of dual trigger with human
459 chorionic gonadotropin and a gonadotropin-releasing hormone agonist for women undergoing
460 fertility preservation. Reprod Med Biol. 2022;21:e12440. doi:10.1002/rmb2.12440.
461 [24] Riobó A, Martínez Acosta A, Martinez-Rocca L, Taboas E, López de Uralde B, Fernandez I,
462 Garrido N, Muñoz E. Dual triggering for final oocyte maturation: a narrative review. Front
463 Endocrinol (Lausanne). 2025;16:1556732. doi:10.3389/fendo.2025.1556732.
464 [25] González VG, Triana AM, García IS, et al. Dual trigger vs. conventional trigger outcomes in
465 in vitro fertilization: systematic review and meta-analysis. JBRA Assist Reprod.
466 2023;27(1):112-119. doi:10.5935/1518-0557.20220035.
467 [26] Ouyang G, Xiao H, Li N, Niu Y, Fang Y, Zou J, Zhao D, Wei D. Association between
468 endometrial thickness and cumulative live birth rate in one oocyte-retrieval cycle: a retrospective

cohort study including 26,127 patients. BMC Pregnancy Childbirth. 2026;26(1):396. doi:10.1186/s12884-026-08897-6.

[27] Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. Hum Reprod Update. 2014;20(4):530-541. doi:10.1093/humupd/dmu011.

[28] Fu K, Li Y, Lv H, Wu W, Song J, Xu J. Development of a model predicting the outcome of in vitro fertilization cycles by a robust decision tree method. Front Endocrinol (Lausanne). 2022;13:877518. doi:10.3389/fendo.2022.877518.

[29] Tesarik J, Mendoza Tesarik R. Sperm-derived dysfunction of human embryos: molecular mechanisms and clinical resolution. Int J Mol Sci. 2025;26(13):6217. doi:10.3390/ijms26136217.

[30] Yazdani A, Halvaei I, Boniface C, Esfandiari N. Effect of cytoplasmic fragmentation on embryo development, quality, and pregnancy outcome: a systematic review of the literature. Reprod Biol Endocrinol. 2024;22(1):55. doi:10.1186/s12958-024-01217-7.

[31] Oh HS, Jang JM, Yoon HJ, Choo CW, Lim KS, Lim JH, Cheon YP. The kinetics of nucleolar precursor bodies clustering at the pronuclei interface: positive correlations with the morphokinetic characteristics of cleaving embryos and euploidy in preimplantation genetic testing programs. Clin Exp Reprod Med. 2025;52(2):150-156. doi:10.5653/cerm.2024.06926.

[32] Zou H, Kemper JM, Hammond ER, et al. Blastocyst quality and reproductive and perinatal outcomes: a multinational multicentre observational study. Hum Reprod. 2023;38(12):2391-2399. doi:10.1093/humrep/dead212.

[33] Zhu S, Huang Z, Chen X, et al. Construction and evaluation of machine learning-based prediction model for live birth following fresh embryo transfer in IVF/ICSI patients with polycystic ovary syndrome. J Ovarian Res. 2025;18(1):70. doi:10.1186/s13048-025-01654-x.

[34] Zhu S, Chen X, Li R, Jiang W, Zheng B, Sun Y. Constructing a predictive model for live birth following fresh embryo transfer in antagonist protocol for polycystic ovary syndrome. J Assist Reprod Genet. 2024;41(10):2709-2719. doi:10.1007/s10815-024-03232-4.

[35] Zhu S, Liao X, Jiang W, Sun Y, Chen X, Zheng B. Development and validation of a nomogram model for predicting clinical pregnancy in endometriosis patients undergoing fresh embryo transfer. BMC Pregnancy Childbirth. 2023;23(1):764. doi:10.1186/s12884-023-06082-7.

[36] Si M, Jiang H, Zhao Y, et al. Nomogram for predicting live birth after the first fresh embryo transfer in patients with PCOS undergoing IVF/ICSI treatment with the GnRH-ant protocol. Diagnostics (Basel). 2023;13(11):1927. doi:10.3390/diagnostics13111927.

[37] Wan B, Fu Y, Ma N, Zhou Z, Lu W. Effect of sperm DNA fragmentation on embryo euploidy rate in assisted reproductive technologies: a systematic review and meta-analysis. Eur J Med Res. 2025;30(1):1138. doi:10.1186/s40001-025-03402-9.

[38] Qu Z, Zhao D, Wang L, Yang S, Zhao S. Effect of sperm DNA fragmentation on the cumulative live birth rate in patients undergoing in vitro fertilization/intracytoplasmic sperm injection treatment. Andrology. 2025;13(4):869-881. doi:10.1111/andr.13754.

[39] Zhu C, Chen F, Zhang S, She H, Ju Y, Wen X, Yang C, Sun Y, Dong N, Xue T, Liu K, Li F, Cui H. Influence of sperm DNA fragmentation on the clinical outcome of in vitro fertilization-embryo transfer (IVF-ET). Front Endocrinol (Lausanne). 2022;13:945242. doi:10.3389/fendo.2022.945242.

[40] Hu S, Jing Y, Fu Y, Ye X. Association of FSHR gene polymorphisms with poor ovarian response in patients undergoing IVF: a systematic review and meta-analysis. Gene. 2024;909:148314. doi:10.1016/j.gene.2024.148314.

[41] Wang Z, Deng Z, Tian Y, Li E, Yang Q. The FSH/FSHR axis in reproductive biology and oncogenesis: mechanisms and emerging targeted therapies. Biol Reprod. 2026;114(4):1168-1185. doi:10.1093/biolre/ioaf287.

[42] Colaco S, Achrekar S, Patil A, et al. Association of AMH and AMHR2 gene polymorphisms with ovarian response and pregnancy outcomes in Indian women. J Assist Reprod Genet. 2022;39(7):1633-1642. doi:10.1007/s10815-022-02541-w.