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ORIGINAL RESEARCH

A Nomogram for Predicting the Risk of Twin and Preterm Births After Two Cleavage-Stage Embryo Transfer

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Objective: To propose a prediction model aimed at increasing the live birth rate of patients undergoing two-cell embryo transfer while eliminating multiple pregnancy complications.

Methods: Women who underwent their first In Vitro Fertilization (IVF) cycle with transferred cleavage-stage embryos at the Affiliated Hospital of Southwest Medical University between January 2016 and December 2024 were included. This study recruited women who received the GnRH agonist long protocol (GnRH-a) or antagonist protocol (GnRH-ant) for ovarian stimulation. In addition, the clinical parameters related to the basic protocol and embryo characteristics were recorded.

Result(s): In the multivariate analysis, we found that female age, endometrial thickness on the Human Chorionic Gonadotropin (HCG) days, patients with Body Mass Index (BMI) >30, high-quality embryos with 4 cells between 1 and 4 pieces, high-quality embryos with 8 cells, available embryos between 5 and 15 pieces and high-quality embryos between 1 and 4 pieces were crucial factors in the twin pregnancies prediction model ($p < 0.05$). On the basis of these characteristics, we constructed a nomogram for twin pregnancies. Additionally, we compared the difference between premature and normal births and found that endometrial thickness on HCG days positively correlated with the model score. In the premature group, the endometrial thickness on HCG days was lower than that found in patients without premature births.

Conclusion: Endometrial thickness on the HCG day may influence the outcome of twin pregnancies. The high-quality embryos showed a higher positive correlation with the model score. Patients with preterm birth had less endometrial thickness on the HCG day than patients without premature birth. For patients with high-quality embryos, it is advisable to use single embryo transfer to reduce the rate of twin pregnancies. This nomogram will enhance the counseling of patients with infertility challenges.

Plain Language Summary:

A. Multiple pregnancy complications, including preterm births and birth defects associated with assisted reproductive technologies, have shown an increasing trend, mainly due to ovarian stimulation. Therefore, research on the formulation of pregnancy outcome prediction models is necessary to serve as a potential personalized protocol for controlled ovarian stimulation.

B. The study outcome suggests that endometrial thickness on the HCG day is a clinical factor that may increase the rate of multiple pregnancy complications; thus, the lower the endometrial thickness on the HCG day, the higher the risk of preterm birth.

C. We sought to propose a prediction model to achieve a high live birth rate in patients undergoing two cleavage-stage embryos transfer, while eliminating twin pregnancies. This study reports, for the first time, a nomogram that can be used to predict pregnancy outcomes with the clinical characteristics of patients whose first IVF cycles were performed with two cleavage-stage embryos.

Keywords: endometrial thickness, twin pregnancies, cleavage embryos, nomogram model, preterm birth

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Introduction

In the past 40 years of assisted reproductive technology (ART), multiple pregnancy complications have become a challenge worldwide. In 2014, the Society for Assisted Reproductive Technology (SART) reported that the rate of twin delivery was 23% after a successful in vitro fertilization (IVF) cycle,[1] while the twin delivery rate after IVF or intracytoplasmic sperm injection (ICSI) was 17% in Europe.[2] In line with data from the Chinese Society of Reproductive Medicine (CSRM) in 2016, the rate of twin deliveries was more than 30% in China.[3] Although the American Society for Reproductive Medicine has proposed a limited number of embryos to be transferred to reduce the rate of multiple pregnancies, twin gestations are still prevalent with an increased risk of complications.[4] Recently, markers of successful infertility treatment have gradually shifted to live birth rates, including peripartum outcomes; however, preterm births have been reported as a perinatal complication.[5,6] Additionally, it has been reported that infants with preterm delivery have higher rates of morbidities, including a higher risk of infection, impaired respiration, and neurologic and nephrological disabilities than full-term infants.[7]

Concerning the decision on the placement of more than one embryo, it needs to be acknowledged that the pregnancy rate of single embryo transfer (SET) may be lower than that of double embryo transfer (DET),[8] possibly due to the failure of pre-implantation embryos to reach the blastocyst stage. Notably, insurance coverage for IVF procedures is provided for the transfer of fewer embryos. Financial pressure may be a coercive tipping point in favor of multiple-embryo transfer insurance coverage and outcomes.[9,10]

Although many patients believe that twins are the optimum outcome of ART, DET will still be a part of clinical practice in future treatment.[11] Currently, clinical practice faces challenges in terms of personalized ART protocols. Therefore, more supporting evidence is required to improve ART programs. In addition, there are few prediction models for successful DET in fresh cleavage-stage embryo transfer, so we were committed to proposing a model for patients undergoing cleavage-stage transfers to predict a high live birth rate as a DET strategy while significantly eliminating twin pregnancies.

Materials and Methods

Patient Cohorts

Patients who underwent the first IVF cycle of two cleavage-stage embryo transfers at the Affiliated Hospital of Southwest Medical University between January 2016 and December 2024 were included in this retrospective cohort study. The primary outcome was twin delivery, and the secondary outcome was preterm birth. We retrospectively analyzed data from 4328 women without pregnancy, single pregnancy, or biochemical pregnancy (group A), and 1351 women with twin pregnancies (group B) (see Figure 1). Regardless of infertility diagnosis, reproductive history, or insemination method. The exclusion criteria are as follows: [1] women with a history of ovarian surgery, genital tumors, endometriosis, or uterine malformations; [2] women with a history of recurrent spontaneous abortion (≥3 times); [3] women with metabolic abnormalities or endocrine diseases (eg, polycystic ovary syndrome, diabetes, abnormal thyroid function, and Cushing syndrome); [4] chromosomal abnormalities in either the female patient or the male partner; [5] women undergoing complete ICSI or rescue ICSI (RICSI); [6] using donor oocytes and [7] no family history of twins.

Participants were included in this study after signed informed consent were obtained. The study design and arrangements made to address the issue of informed consent were approved by the Ethical Committee of the Affiliated Hospital of Southwest Medical University. All included women received GnRH agonist long-protocol (GnRH-a) or the antagonist protocol (GnRH-ant) for ovarian stimulation, and the initiation times of GnRH-a and GnRH-ant were based on the standard operating procedure (SOP) in our center. We also recorded the BMI and Anti-Mullerian Hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), free 3,5,3 triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) levels.

Group A includes cases of no pregnancy, biochemical pregnancies, and instances of two gestational sacs with only one fetal heartbeat. Also, group B constitutes the presence of two gestational sacs only, each containing an embryo and exhibiting a heartbeat, at 7 weeks of gestation on ultrasound (this was considered a twin pregnancy). Group C includes

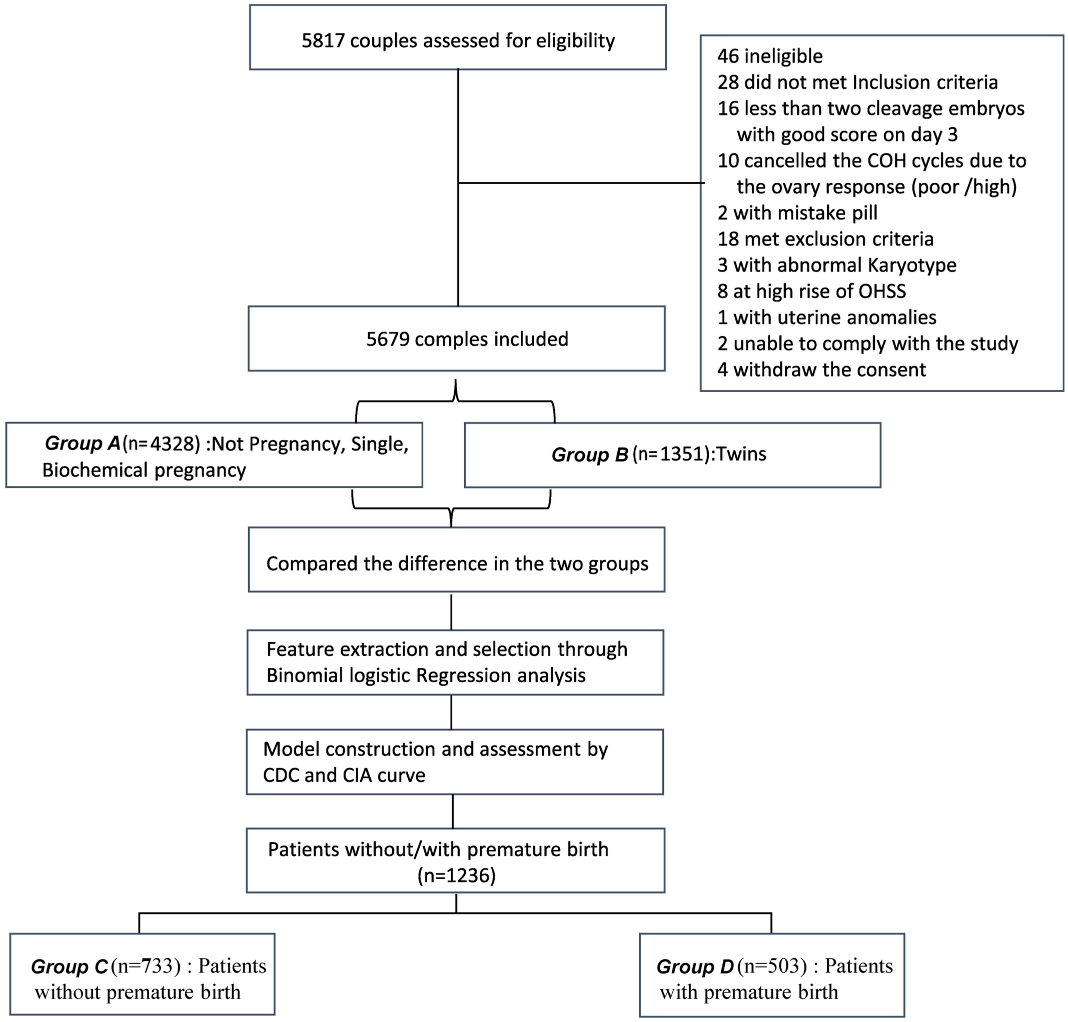

Figure 1 Flowchart describing the study population.

women who underwent the GnRH-agonist long protocol or GnRH-antagonist protocol transferred with two cleavage-stage embryos patients without premature birth, and finally, women with premature birth constituted group D.

Ovarian Stimulation

All the included women received GnRH-a or GnRH-ant for ovarian stimulation. Women who received GnRH-a, had serum progesterone levels measured at Days 19–23 of menstruation, after progesterone >2 ng/mL and short-acting GnRH-a (Triptorelin Acetate Injection, Changchun Jinsai Pharmaceutical Co., Ltd.) 0.1 mg were subcutaneously injected during the midluteal phase of the last menstrual cycle and antral follicle, and the endometrial thickness was measured with B-ultrasound. Serum LH, serum FSH, and serum estradiol (E_2) were recorded, when the women had endometrial thickness ≤5 mm, follicular size <5 mm: LH, FSH ≤5 mIU/mL, E_2 ≤20 pg/mL, and start the Gn protocol with gonadotropin. Women who underwent GnRH-ant therapy had Gn stimulation initiated on day two or three of the menstrual cycle until ovulation induction. When the primary follicle diameter reached 12–14 mm, according to serum estradiol (E_2) and luteinizing hormone (LH), gonadotropin-releasing hormone antagonist (GnRH-ant, 0.25 mg/d, Cetrorelix, Merck, Germany) was administered at 0.25–0.5 mg/d. Gn drugs (Gonna-f, Urofolotropin, Merck. Jinsaiheng, Lizhu Group Lizhu Pharmaceutical Factory) were selected based on the patient's economic status. During the ovulation induction process, the dosage of medication was adjusted based on the size of the follicles observed through ultrasound monitoring and the level of serum E_2 when at least three follicles reached ≥17 mm, two follicles reached ≥18 mm, or one follicle reached ≥19 mm in diameter, and patients were considered ready for ovum pick-up (OPU). OPU was performed 36–38 hours later, after which oocytes were retrieved under transvaginal B-ultrasound by qualified surgeons.

All transvaginal ultrasound (TVU) assessments were performed at our center by specialist clinicians using the same standardized protocol on the same ultrasound instrument (Manufactured for Ultrasound HD9, Philips Ultrasound, USA). We measured the endometrial thickness (EMT) and endometrial patterns in the medial sagittal plane of the uterus on the day of HCG administration and the maximum thickness from one interface to the other at the junction of the endometrial muscle.

Embryo Culture and Transfer

Conventional IVF is chosen for fertilization when the male's sperm exhibits a normal concentration (≥15 million/mL), with ≥32% of sperm demonstrating forward motility and ≥4% having normal morphology. Embryonic pronuclei (PN) were observed at 18+1h post-insemination. An Embryo quality was assessed based on its blastomeres, fragmentation, and symmetry; cells were scored based on cell number, fragmentation classification (fragmentation < 5% score 4, 5% <fragmentation <10% score 3, 10% <fragmentation <30% score 2, fragmentation >30% score l), and homogeneity (symmetry score 1; asymmetry score 0). Available Embryos: Embryos that are deemed suitable for transfer or cryopreservation based on their morphological quality and developmental potential. These embryos meet specific criteria for further use in assisted reproductive treatments: those derived from 2PN, with a cell count exceeding 5, and assigned a grade of 2 to 4. High-quality embryos: Embryos that exhibit optimal morphological characteristics, such as appropriate cell number, symmetry, and fragmentation rate, and are considered to have the highest potential for successful implantation and development: High-quality embryos were defined as stage embryos with 7–9 blastomeres of 2PN origin, with fragments <10% at day 3.[12] Discarded Embryos: Embryos that do not meet the criteria for transfer or cryopreservation due to poor morphological quality, arrested development, or other abnormalities, and are therefore not used in further treatment cycles.

For fresh embryo transfer cycles, two cleavage-stage embryos were transferred into the uterus under ultrasound guidance. Endocrine support was administered through exogenous estradiol and vaginal progesterone and was continued until week 11 of gestation. Pregnancy was tested by measuring serum HCG levels two weeks after embryo transfer.

Statistical Analysis

The results of the normality test showed that all the measurement data in the present study were non-normally distributed. Baseline clinical characteristics are presented as median and inter-quartile range (IQR). For non-normally distributed data, the Mann–Whitney *U*-test for variables with a skewed distribution was used to calculate the relative risk and 95% CI of numerical parameters. Univariate and multivariate logistic regression analyses were conducted to identify factors associated with twin pregnancies. The C-index and AUC were used to evaluate the discriminative ability, and the optimal cutoff values for the Receiver Operating Characteristic (ROC) curves were determined using the Youden index. Statistical analysis was performed using the SPSS software (version 22.0; Statistical Package for the Social Sciences, Chicago, IL, USA). GraphPad Prism software version 8.02 (GraphPad Software Inc.) was used for forest plots. The nomogram, ROC curves, calibration curves, Decision Curve Analysis (DCA), and Clinical Impact Curve (CIC) were constructed using the R software version 4.3.0 (R Project for Statistical Computing, Vienna, Austria).

Results

Baseline Characteristics

A total of 5679 patients who fulfilled the inclusion criteria were retrospectively analyzed. Baseline patient characteristics are summarized in Table 1. We described the clinical parameters of the basic, protocol, and embryo characteristics using the median and inter-quartile range (IQR).

Table 1 Patients' Baseline Characteristics

	Group A (n=4328)	Group B (n=1351)
Female age(years)	32(29–36)	31(28–33)
BMI (kg/m^2)	22.00(20.30–24.00)	21.83(20.10–23.80)
AMH (ng/mL)	1.94(1.10–3.33)	2.18(1.33–3.71)
FSH/LH	1.96(1.44–6.25)	1.86(1.40–2.51)
Antral Follicle	7(5–10)	8(6–11)
Endometrial thickness on the HCG day (mm)	10(8–11)	10(9–11)
Total Gn day	10(9–11)	10(9–11)
Total Gn dose (bottles)	2400(1800–2850)	2175(1600–2700)
Number of retrieved oocytes (pieces)	9(6–12)	10(7–13)
Total number of oocytes	10(7–13)	11(8–13)
MII	8(5–11)	9(6–11)
2PN	5(2–8)	6(3–9)
Embryos with ≥30% fragments	1(0–3)	1(0–3)
Vacuoles	0(0–1)	0(0–1)
High-quality embryo with 4 cells	1(1–3)	2(0–4)
High-quality embryo with 8-cells	1(0–3)	2(1–3)
Available embryo	3(2–5)	4(3–6)
High-quality embryo	0(0–1)	1(0–2)

Logistic Regression Analysis

The purpose of this study was to establish a twin live birth prediction model using 15 variables in 5679 fresh double cleavage embryo transfer patients. References and logistic regression usually include female age, AMH, ratio of FSH to LH, antral follicle, endometrial thickness on the HCG day, total Gn day, and total Gn dose. Besides, we adjusted the confounders for the model: BMI, MII, embryos with ≥30% fragments, vacuoles, high-quality embryos with 4 cells, high-quality embryos with 8 cells, available embryos, and the high-quality embryos. In the multivariate analysis, we found that female age (0.919 (0.903–0.936), $p < 0.05$), endometrial thickness on the HCG day (1.132 (1.09–1.176), $p < 0.05$), patients with BMI >30(0.48 (0.249–0.925), $p < 0.05$), high-quality embryos with 4 cells between 1 and 4 pieces (1.212 (1.04–1.412), $p < 0.05$), high-quality embryos with 8 cells (1.653 (1.349–2.024), $p < 0.05$), available embryos between 5 and 15 pieces (9.109 (1.233–67.292), $p < 0.05$ and 10.828 (1.422–82.44), $p < 0.05$), and high-quality embryos between 1 and 4 pieces (1.217 (1.04–1.423), $p < 0.05$) were crucial factors in the twin pregnancies predictive model (Table 2).

Table 2 Univariate and Multivariate Analysis to Identify the Influential Factors of Twin Pregnancies

	Univariate analysis OR (95% CI)	*P* value	Multivariate analysis OR (95% CI)	*P* value
Female age(years)	0.903(0.889–0.917)	0.000	0.919(0.903–0.936)	0.000*
AMH (ng/mL)	1.047(1.023–1.071)	0.000	0.983(0.953–1.014)	0.276
FSH/LH	0.988(0.964–1.013)	0.023	0.999(0.98–1.018)	0.909
Antral Follicle (pieces)	1.053(1.037–1.069)	0.000	0.996(0.971–1.021)	0.734
Endometrial thickness on the HCG day (mm)	1.156(1.115–1.198)	0.000	1.132(1.09–1.176)	0.000*
Total Gn day (bottles)(days)	1.046(1.006–1.088)	0.023	1.016(0.946–1.091)	0.663
Total Gn dose (bottles)	1(1–1)	0.000	1(1–1)	0.144
BMI (kg/m^2)				
<18.5	1.109(0.873–1.409)	0.396	1.019(0.794–1.309)	0.880
25–29.9	0.899(0.756–1.069)	0.227	0.936(0.781–1.123)	0.478
>30	0.495(0.261–0.938)	0.031	0.48(0.249–0.925)	0.028*

(*Continued*)

Table 2 (Continued).

	Univariate analysis OR (95% CI)	*P* value	Multivariate analysis OR (95% CI)	*P* value
MII				
5–9	1.666(1.365–2.034)	0.000	1.268(0.879–1.831)	0.204
10–15	2.105(1.709–2.593)	0.000	0.995(0.639–1.548)	0.983
>15	2.472(1.847–3.309)	0.000	0.959(0.534–1.722)	0.889
Embryos with ≥30% fragments				
1–4	0.963(0.845–1.098)	0.577	0.982(0.848–1.138)	0.811
>5	1.1(0.889–1.361)	0.381	1.262(0.97–1.643)	0.083
Vacuoles				
1–4	1.302(1.143–1.482)	0.577	1.09(0.947–1.255)	0.228
>5	1.527(0.86–2.713)	0.381	0.975(0.534–1.781)	0.935
High-quality embryo with 4 cells				
1–4	1.368(1.196–1.565)	0.000	1.212(1.04–1.412)	0.014*
>5	2.004(1.658–2.422)	0.000	1.198(0.947–1.515)	0.133
High-quality embryo with 8-cells				
1–4	2.153(1.818–2.548)	0.000	1.653(1.349–2.024)	0.000*
>5	2.832(2.242–3.576)	0.000	1.498(1.078–2.08)	0.016*
Available embryo		0.000		
1–4	11.891(1.639–86.262)	0.014	6.873(0.937–50.41)	0.058
5–9	21.951 (3.024–159.351)	0.002	9.109(1.233–67.292)	0.030*
10–15	26.699 (3.623–196.769)	0.001	10.828(1.422–82.44)	0.021*
>15	12.25(0.639–234.81)	0.096		0.999
High-quality embryo				
1–4	1.678(1.479–1.904)	0.000	1.217(1.04–1.423)	0.014*
5–9	1.9(1.33–2.714)	0.000	1.166(0.753–1.806)	0.491
10–15		0.999		0.999

Note: The asterisk (*) denotes a statistically significant difference ($p < 0.05$).

Nomogram Construction and Validation

Based on the data from the multivariate analyses, we constructed a nomogram for twin pregnancies that underwent the GnRH-agonist long-protocol and GnRH-antagonist protocol with two cleavage-stage embryo transfers. A total score was calculated with the use of the style of female age, endometrial thickness on the HCG day, the high-quality embryo with 8-cells (0: no embryo, 1: between 1 and 4 pieces, 2: more than 5 pieces), the high-quality embryo with 4 cells (0: no embryo, 1: between 1 and 4 pieces, 2: more than 5 pieces), the available embryo (0: no embryo, 1: between 1 and 4 pieces, 2: between 5 and 10 pieces, 3: between 10 and 15 pieces, 4: more than 15 pieces), the high-quality embryo (0: no embryo, 1: between 1 and 4 pieces, 2: between 5 and 10 pieces, 3: between 10 and 15 pieces, 4: more than 15 pieces) and BMI (0: between 18.5 and 24.9 kg/m^2, 1: below 18.5 kg/m^2, 2: between 25 and 29.9 kg/m^2, 3: more than 30 kg/m^2). The total score was determined based on the individual scores calculated using the nomogram; the higher the value, the higher the probability of women with twin pregnancies of transferred two-cleavage stage embryos who underwent the GnRH-agonist long protocol or GnRH-antagonist protocol (Figure 2).

Clinical Value of the Nomogram and Risk Stratification

Figure 3 shows the concordance index (C-index = 0.665) and receiver operating characteristic curve (AUC) (0.665 (0.649–0.681)) (see Figure 3a and c); the threshold was 0.221 (specificity, 0.555; sensitivity, 0.702; Youden's index, 0.257). Calibration curves were constructed for the nomograms. The x-axis represents the nomogram-predicted probability and the y-axis represents the actual probability of a high-quality embryo. The perfect prediction corresponds to the 45° red line, the black dotted line represents the entire cohort (n = 5679), and the orange line is bias-corrected by bootstrapping (1000 repetitions), indicating a good predictive accuracy between the actual and predicted probabilities

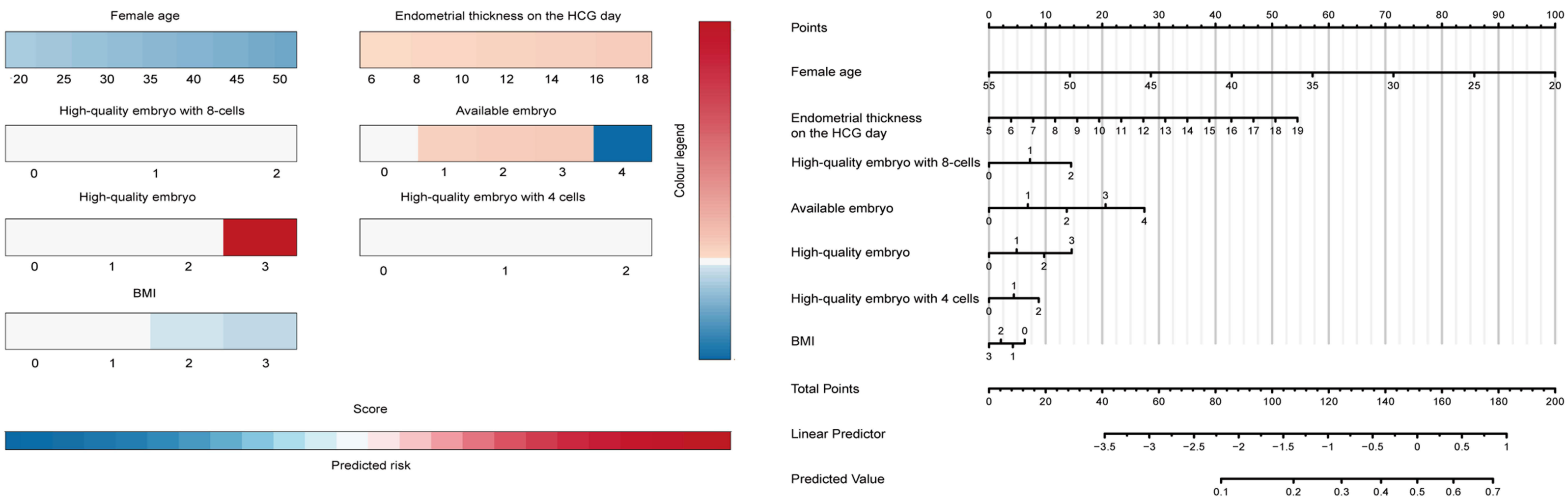

Figure 2 Nomogram model for patients with twin pregnancies.

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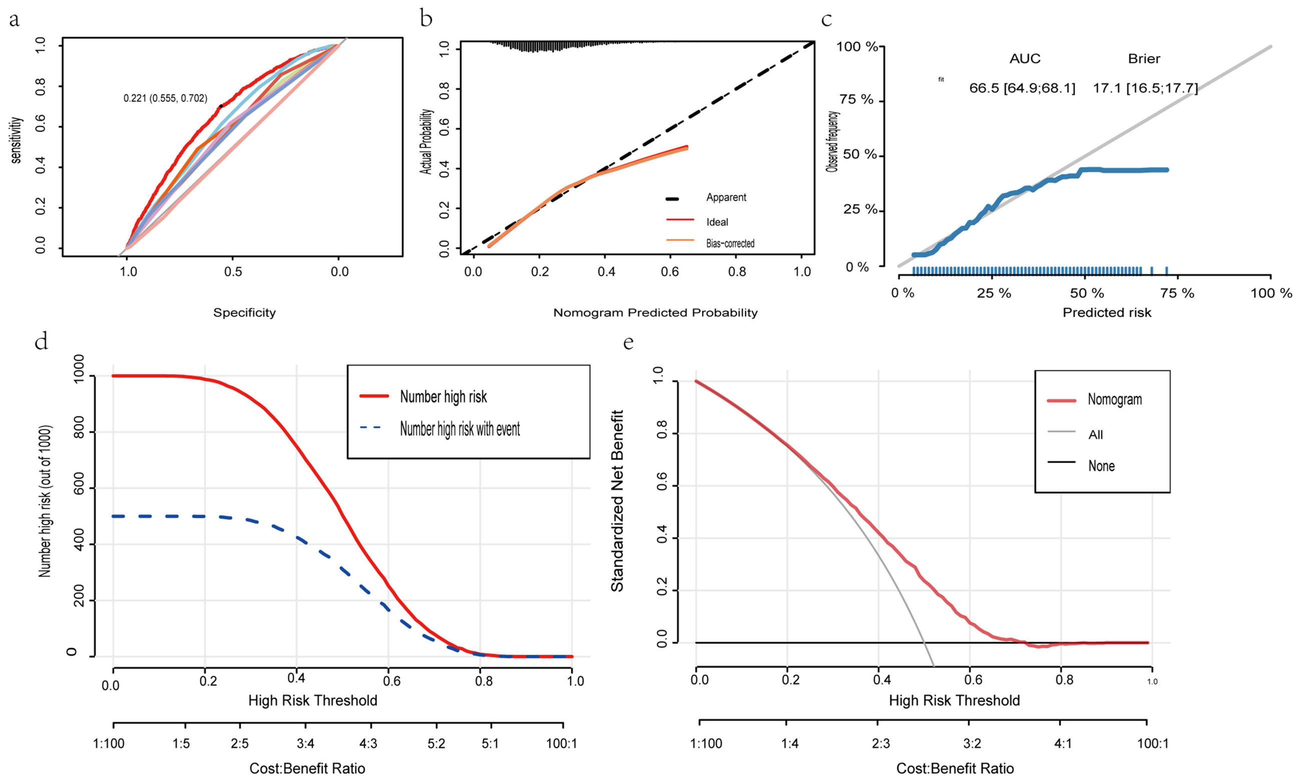

Figure 3 ROC, DCA and CIC curves show the predictive model's impact on clinical practice.
Notes: (**a**) and (**c**) show the concordance index, receiver operating characteristic curve and the threshold. (**b**) The predictive accuracy between the actual probability and predicted probability. (**d**) The clinical impact curve. (**e**) The decision curve.

(Figure 3b). In addition, we constructed a CIC to assess the impact of predictive models on actual clinical practice, where the x-axis is the threshold, and the y-axis is the number of high-risk patients; the red line represents the number of people who were adjudged to be of high risk by the model, and the blue line represents the number of people who were adjudged by the model as high risk at different probability thresholds and the outcome event occurred. It has been suggested that if the threshold is greater than 0.6, the model prediction is highly matched with the actual occurrence, and the clinical prediction is highly effective (Figure 3d). We used the DCA to calculate a clinical "net benefit" for this prediction model. The result of the prediction model is the red line; the other two lines are for "intervention for all" (gray line) and "intervention for none" (black line). The y-axis represents the benefit and the x-axis represents the threshold. It is obvious to identify the red line as the highest benefit across a wide range of values of preference (Figure 3e).

The Clinical Characters of Patients With/Without Premature Birth

To gain insight into the underlying clinical characteristics of IVF in women who underwent the GnRH-agonist long protocol or GnRH-antagonist protocol transferred with two cleavage-stage embryos, we compared whether there were significant differences between premature and non-premature births (Table 3). D-dimer (0.19 (0.19–0.27) VS 0.2

Table 3 Mann–Whitney *U*-Test About Clinical Characters of Patients Without/With Premature Birth

	C group (n=733)	D group (n=503)	Z	*P* value
Female age(years)	30(28–32)	30(28–33)	−0.03	0.98
BMI (kg/m^2)	21.6(19.9775–23.8325)	21.9(20.2–23.7)	−0.56	0.58
AMH (ng/mL)	2.24(1.38–3.715)	2.2(1.28–3.79)	−0.80	0.43
FSH/LH	1.85(1.37–2.4025)	1.88(1.47–2.64)	−1.35	0.18
E_2	31(22.83–42)	29.81(21.97–40)	−1.54	0.12

(*Continued*)

Table 3 (Continued).

	C group (n=733)	D group (n=503)	Z	*P* value
PRL	16.725(12.11–22.9)	15.98(12–22.7)	−0.51	0.61
FT_3	4.505(4.23–4.8425)	4.52(4.18–4.88)	−0.79	0.43
FT_4	13.17(12.32–14.2225)	13.22(12.14–14.27)	−0.31	0.76
TSH	1.905(1.28–2.5525)	1.86(1.37–2.47)	−0.02	0.98
International normalized ratio	0.9(0.86–0.95)	0.91(0.86–0.95)	−0.67	0.50
Thrombin time	17(16–18)	17(16–18)	−0.41	0.68
Fibrinogen	2.75(2.5–3.2)	2.8(2.5–3.2)	−0.97	0.33
D-dimer	0.19(0.19–0.27)	0.2(0.19–0.29)	−2.10	0.04*
Antral Follicle	8(6–11)	8(6–11)	−1.24	0.22
Endometrial thickness on the HCG day (mm)	10(9–11)	10(9–11)	−2.32	0.02*
Total Gn day (bottles)(days)	10(9–11)	10(9–11)	−0.26	0.80
Total Gn dose (bottles)	2175(1575–2700)	2150(1600–2700)	−0.31	0.76
Number of retrieved oocytes (pieces)	10(7–13)	10(7–13)	−0.59	0.56
Total number of oocytes (pieces)	9(6–11)	9(6–11)	−0.53	0.59
Embryos with ≥30% fragments	1(0–3)	1(0–2)	−1.27	0.21
Vacuoles	0(0–1)	0(0–1)	−1.17	0.24
Available embryo	5(3–7)	4(3–7)	−0.01	0.99
High-quality embryo	1(0–2)	1(0–2)	−0.33	0.74
Serum β-hCG levels (IU/L)	901.8(679.525–1152.825)	911(697.4–1160.4)	−2.43	0.02*
Weight of the Newborn 1	2600(2400–2850)	2300(1920–2500)	−19.17	0.00*
Length of the Newborn 1	47(46–49)	46(45–48)	−17.23	0.00*
Weight of the Newborn 2	2600(2400–2800)	2250(1970–2550)	−13.62	0.00*
Length of the Newborn 2	48(46–49)	46(44–48)	−10.94	0.00*

Note: The asterisk (*) denotes a statistically significant difference (p < 0.05).

(0.19–0.29), $p < 0.05$) and serum beta human chorionic gonadotropin (β-hCG) levels 14 days after ET (901.8 (679.525–1152.825) VS 911 (697.4–1160.4), $p < 0.05$) in patients without premature birth (group C) were lower than those in patients with premature birth (group D). While the weight of newborn 1 (2600 (2400–2850) VS 2300 (1920–2500), $p < 0.05$) and newborn 2 (2600 (2400–2800) VS 2250 (1970–2550), $p < 0.05$) in group C were better than those in group D, the length of newborn 1 (47 (46–49) VS 46 (45–48), $p < 0.05$) and newborn 2 (48 (46–49) VS 46 (44–48), $p < 0.05$) in group C were higher than those in group D. Additionally, in terms of endometrial thickness on the HCG day (10 (9–11) VS 10 (9–11), $p < 0.05$), we used a histogram and ridgeline plot to describe the difference between the two groups (Figure 4).

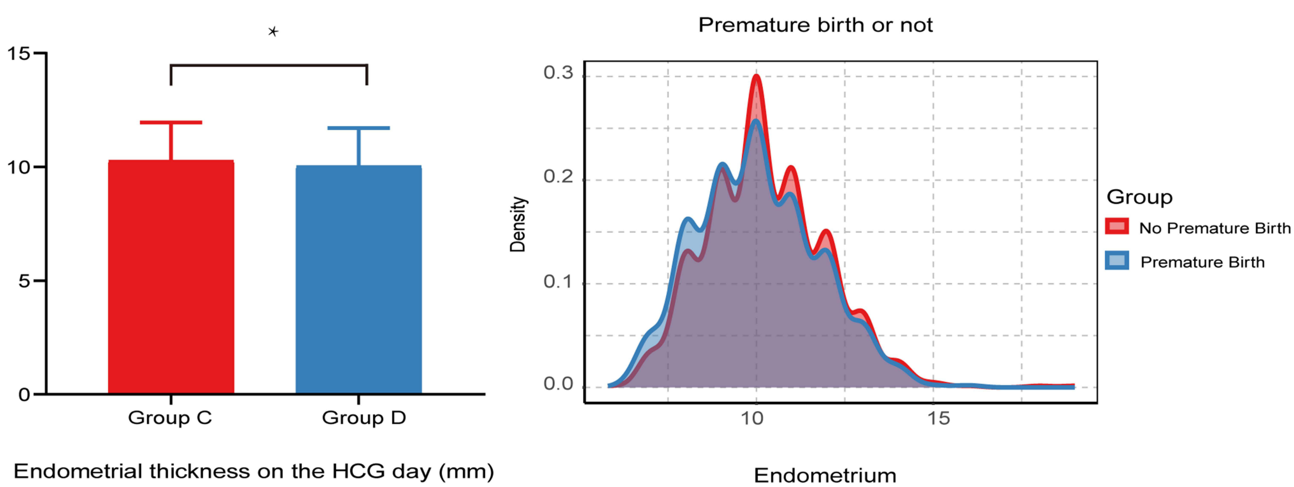

Figure 4 Histogram and Ridgeline Plot.
Note: The asterisk (*) denotes a statistically significant difference (p < 0.05).

Discussion

In this study, we retrospectively analyzed data from 4328 women without pregnancy or single pregnancy or biochemical pregnancy and 1351 women with twin pregnancies. The rate of twin pregnancy was found to be 23.78%, which is similar to the SART in 2014 about the twin birth rate.[1] Based on the multivariate analysis, we found that female age (0.919 (0.903–0.936), $p < 0.05$), endometrial thickness on the HCG day (1.132 (1.09–1.176), $p < 0.05$), patients with BMI >30 (0.48 (0.249–0.925), $p < 0.05$), high-quality embryos with 4 cells between 1 and 4 pieces (1.212 (1.04–1.412), $p < 0.05$), high-quality embryos with 8 cells (1.653 (1.349–2.024), $p < 0.05$), available embryo between 5 and 15 pieces (9.109 (1.233–67.292), $p < 0.05$ and 10.828 (1.422–82.44), $p < 0.05$), and high-quality embryos between 1 and 4 pieces (1.217 (1.04–1.423), $p < 0.05$) were crucial factors in the twin pregnancy predictive model. Simultaneously, the nomogram model showed that female age and BMI negatively correlated with the score of the twin pregnancy predictive model which is well known. In addition, age is reported to affect fertility; thus, younger age is a risk factor for twin pregnancies after IVF with DET,[13] Duy Le Nguyen et al indicated that patient age and high-quality embryos were predictors of twin pregnancies in DET cycles.[14] Apart from a young age, maternal BMI has been reported to be associated with a higher rate of fraternal twins, BMI of 30 or more should be considered an independent risk factor for a poor reproductive outcome,[15] previous study found the rate of pregnancy as 42% in a BMI of 20–22.4 kg/m^2; however, a BMI of overweight and obesity (BMI 25–29.9 kg/m^2, BMI ≥ 25 kg/m^2) was found to be associated with a lower pregnancy rate (23% and 22%, respectively).[16] Given this evidence, it is recommended that overweight or obese women lose weight before implantation to improve fertility.[17] Hence, single-embryo transfer might be advisable for couples with risk factors for twin pregnancy. In agreement with previous studies,[18,19] we found that high-quality embryos positively correlated with the model score. Embryo quality is a crucial factor not only in implantation and pregnancy rates but also in the live birth rate;[20] therefore, for patients with high-quality embryos, it is advisable to use single embryo transfer in order to reduce the rate of twin pregnancies. To better understand the clinical performance and value of this nomogram, we included a case example: A 24-year-old patient underwent the GnRH-agonist long-protocol/GnRH-antagonist protocol and the endometrial thickness on the HCG day was 7.8mm. Also, the high-quality embryo with 8-cells was 2 pieces, the high-quality embryo with 4 cells was 4 pieces, the available embryo was 4 pieces, the high-quality embryo was 2 pieces and BMI was 22.5. Density plot of total points and age shows their distribution. The importance of each variable was ranked according to the standard deviation along nomogram scales. The sum (132) of these points located on the Total Points axis determines the probability of twin pregnancies (34%) that underwent the GnRH-agonist long-protocol and GnRH-antagonist protocol with two cleavage-stage embryo transfers.

We also compared the clinical characteristics of patients with and without premature births with two transferred cleavage-stage embryos from their first IVF cycle. Our data showed that D-dimer (0.19 (0.19–0.27) VS 0.2 (0.19–0.29), $p < 0.05$) and serum β-hCG levels 14 days after ET (901.8 (679.525–1152.825) VS 911 (697.4–1160.4), $p < 0.05$) were lower in patients without premature birth (group C) than in patients with premature birth (group D). D-dimer is a degradation product of a cross-linked blood clot that increases during acute thrombotic processes and is a marker of a hypercoagulable state. Pregnancy itself is a prothrombotic state, especially with ovarian hyperstimulation; therefore, pregnant women are at an even higher risk of complications including immune system dysregulation and recurrent pregnancy loss (RPL). These disorders can have a negative impact on embryo implantation and placentation.[21] Zhu et al confirmed this result using a mouse model.[22] On the other hand, a previous study showed that pregnancy outcomes of the patients with initially low serum β-hCG levels were poor, with only 18.6% of live births,[23] while in our study, serum β-hCG levels 14 days in the patients without premature birth (group C) were lower than those patients with premature birth (group D). In our research, the data showed that serum β-hCG levels in group D were about 10 IU/L higher than those in group C, and the β-hCG fold increase should be analyzed, for these reasons, to accurately and fundamentally assess the role of β-hCG levels 14 days after ET, it is very important to make individualized follow-up plans according to different serum β-hCG intervals. Unsurprisingly, the weights of newborn 1 (2600 (2400–2850) VS 2300 (1920–2500), $p < 0.05$) and newborn 2 (2600 (2400–2800) VS 2250 (1970–2550), $p < 0.05$) in group C were better than those in group D. The lengths of newborns 1 (47 (46–49) VS 46 (45–48), $p < 0.05$) and 2 (48 (46–49) VS 46 (44–48), $p < 0.05$) in group C were higher than those in group D.

Interestingly, in the multivariate analysis, we found that endometrial thickness on the day of HCG administration is a clinical factor that influences the outcome of twin pregnancy. Similarly, it emerged during the comparison of patients without/with premature births, based on the current evidence, we used the nomogram model, the histogram, and ridgeline plot to describe the difference between the two groups, and we found the endometrial thickness on the HCG day to be positively correlated with the score of the model. In the premature group, the endometrial thickness on HCG days was lower than that in patients without premature births. There is ongoing conflict regarding whether there is a correlation between pregnancy rates and acceptable endometrial thickness.[24,25] Previous studies showed no adverse effects of a thickened endometrium during implantation and pregnancy,[26,27] while a systematic review and meta-analysis demonstrated a lower chance of pregnancy if the endometrial thickness was <7 mm in IVF cycles.[28] Additionally, studies on women with poor endometrial development have demonstrated that a thin endometrium or hyperechogenic endometrium on the day of HCG administration may be associated with pathological abnormalities and may eventually reduce the rate of pregnancy.[29] Theoretically, during implantation, the endometrium undergoes anatomical and functional changes to become decidua, which is crucial for successful implantation, pregnancy maintenance, and parturition.[30] In addition to pathological abnormalities, the tumor suppressor protein p53 is also associated with decidual senescence, as demonstrated by reduced mammalian target of rapamycin (mTOR) complex 1 signaling, and administration of rapamycin or progesterone attenuates premature decidual senescence and preterm birth. Owing to the limited number of studies, long-term outcomes need to be tracked and studied further to provide a more complete evaluation and recommendations.

Conclusion

In conclusion, we sought to evaluate the risk factors for twin pregnancies after the transfer of two cleavage embryos in the first IVF/ICSI cycle. To the best of our knowledge, the present study had the largest sample size, with 5679 IVF/ICSI cycles. This is the first predictive model to analyze the clinical characteristics of the twin pregnancies of first IVF cycles with transferred two cleavage-stage embryos. The limitation of our study is that it is retrospective, based on data from a single-center. This model provides clinicians with control over the possibility of twin pregnancies when transferring two cleavage-stage embryos, as well as whether a patient will have a high risk of preterm birth after a twin pregnancy. From our model, we found that high-quality embryos positively correlated with the model score; therefore, for patients with high-quality embryos, it is advisable to use single embryo transfer to reduce the rate of twin pregnancies. We also found that endometrial thickness on HCG days is not only associated with twins but also with preterm birth after fresh cleavage embryo transfers; however, future large prospective cohort studies and multi-centers with a longer follow-up period are required to validate our findings.

Data Sharing Statement

The data are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study was approved by the ethics committee of the Affiliated Hospital of Southwest Medical University (ethical approval number: KY2024317) on August 15, 2024, and promised to comply with the Helsinki Declaration.

Author Contributions

Yunzhu Lan and Shuang Liu are co-first authors. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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