

# Novel Clinically Validated Machine Learning Model for Early Pregnancy Loss in Recurrent Spontaneous Abortion: Integrating Serum Autoantibodies and Ultrasonic Parameters

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**Objective:** To explore the correlation between autoantibodies, ultrasonic endometrial receptivity parameters and early miscarriage in recurrent spontaneous abortion (RSA) patients during subsequent pregnancies, and to establish and validate a predictive model for early miscarriage.

**Methods:** A retrospective analysis was conducted on RSA patients who visited Xi'an People's Hospital from January 2019 to December 2024. Patients were randomly divided into a training set (70%, n=412) and a validation set (30%, n=177). Baseline data, serum autoantibodies (anti- $\beta$ 2-glycoprotein 1 antibody [ $\alpha\beta$ 2-GP1], thyroglobulin antibody [TgAb], anti-sperm antibody [AsAb], anti-cardiolipin antibody [ACA]) and ultrasonic parameters (resistance index [RI], endometrial thickness, endometrial volume, vascularization index [VI], vascularization flow index [VFI]) were collected. Multiple machine learning models (logistic regression [LR], XGBoost, random forest, etc.) were developed. Model performance was evaluated using area under the receiver operating characteristic curve (AUC), accuracy, and other metrics. A nomogram was constructed based on the optimal model.

**Results:** The abortion subgroup had significantly higher positive rates of  $\alpha\beta$ 2-GP1, TgAb, ACA and RI, but lower endometrial thickness, endometrial volume, VI and VFI than the normal subgroup (all  $P < 0.05$ ). Eight variables ( $\alpha\beta$ 2-GP1, TgAb, AsAb, RI, endometrial thickness, endometrial volume, VI, VFI) were identified as candidate predictors. The LR model was optimal, with AUC=0.94 and accuracy=0.93 in the training set, and AUC=0.92 and accuracy=0.90 in the validation set. The nomogram based on this model showed good alignment between predicted probabilities and actual outcomes.

**Conclusion:** A practical and accurate LR model for predicting early miscarriage in RSA patients was established using autoantibodies and ultrasonic parameters. It can assist in clinical risk stratification and individualized intervention. Future multicenter prospective studies with larger samples and more variables are needed to optimize the model.

**Keywords:** recurrent spontaneous abortion (RSA), early miscarriage, autoantibody, ultrasonic parameter, machine learning, prediction model

## Introduction

Recurrent spontaneous abortion (RSA) refers to women who experience multiple ( $\geq 2$ ) miscarriages during pregnancy.<sup>1</sup> Previous studies have reported that the incidence of RSA ranges from 1% to 5%.<sup>2,3</sup> Many women develop psychological issues such as anxiety and depression after experiencing RSA, which not only affects their physical health but also imposes a significant burden on their mental state, exerting negative impacts on both families and society.

The etiologies of RSA mainly include chromosomal or genetic abnormalities, anatomical structural abnormalities (including congenital and acquired types), autoimmune diseases, prethrombotic states (including hereditary and acquired types), endocrine factors, infectious factors, male factors, environmental factors, and psychological factors.<sup>4-9</sup>

A considerable proportion of RSA patients have unclear specific causes and pathogenesis of the disease, thus being classified as “unexplained recurrent spontaneous abortion (URA)”<sup>10</sup> Despite the availability of extensive screening and diagnostic tests with current technologies, more than 40% of patients still have unknown causes of miscarriage.<sup>10,11</sup> These patients face a risk of miscarriage in subsequent pregnancies, especially those with a history of 3 consecutive miscarriages, for whom the recurrence risk is as high as 33%.<sup>12</sup>

In recent years, studies have established models for predicting pregnancy outcomes based on RSA-related factors, but different international guidelines recommend different prediction models. For instance, the 2012 guidelines of the American Society for Reproductive Medicine (ASRM) recommend the Lund model.<sup>13</sup> The latest ESHRE guidelines propose the Kolte & Westergaard model.<sup>14</sup> However, these models have limitations such as incomplete risk factors, single modeling methods, and lack of internal or external validation, leading to room for improvement in their predictive performance.

Furthermore, with the evolution of RSA definitions, changes in demographic characteristics, advances in etiological research, and improvements in treatment methods, there is an urgent clinical need to develop more accurate and practical risk prediction models. These models aim to achieve risk stratification for patients, identify high-risk populations, and provide early intervention and individualized treatment for these groups, thereby effectively preventing recurrent miscarriages. An ideal model should include a small number of easily accessible variables, with clinical practicality and excellent predictive performance.

Establishing a live birth prediction model for RSA patients requires comprehensive consideration of multiple factors, including women’s age, history of previous miscarriages, genetic factors, lifestyle, environmental factors, and male factors.<sup>15,16</sup> In recent years, with the further development of big data and artificial intelligence, technologies such as machine learning and neural networks have demonstrated significant advantages in establishing precision medicine modeling methods.<sup>17,18</sup> It is expected that through continuous iterative training of machine learning algorithms and neural networks, the risk of early miscarriage in RSA patients during subsequent pregnancies can be accurately predicted to guide clinical practice.

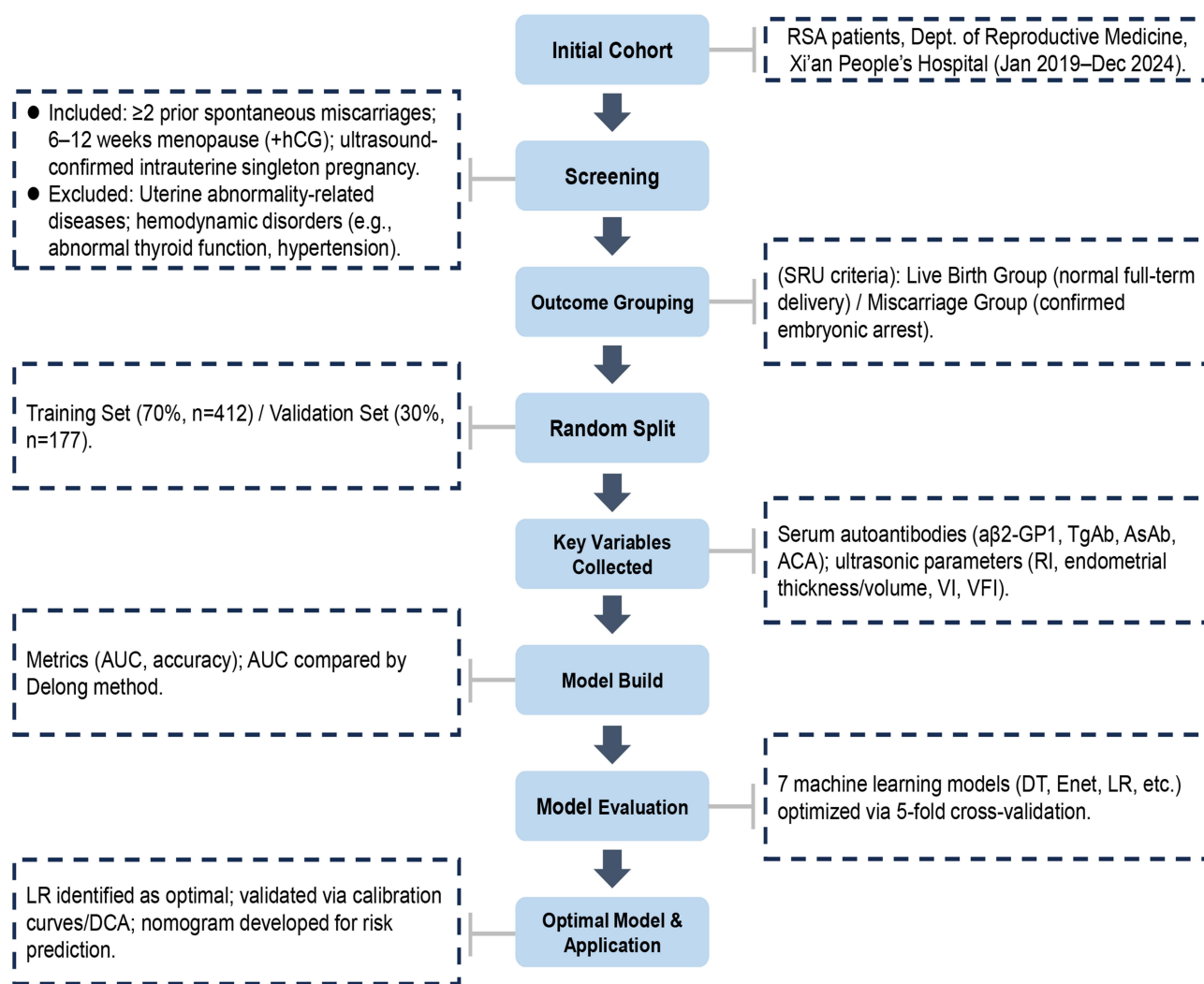
Recent studies in reproductive immunology have shown that more than half of RSA cases are associated with immune dysfunction.<sup>4</sup> The immunopathological changes leading to miscarriage vary among different factors, and immune miscarriage can be divided into autoimmune RSA (AI-RSA) and alloimmune RSA (Allo-RSA).<sup>19,20</sup> Among these, AI-RSA includes the production of tissue-nonspecific autoantibodies (such as anti-phospholipid antibodies [aPL], anti-nuclear antibodies [ANA], and anti-DNA antibodies) and tissue-specific autoantibodies (such as anti-sperm antibodies [AsAb] and anti-thyroid antibodies [ATA]).

Existing studies have indicated that 5% to 20% of RSA patients exhibit abnormal expression of tissue-nonspecific autoantibodies (eg, aPL) in clinical practice, and the live birth rate of untreated patients in subsequent pregnancies decreases to 10%.<sup>21–23</sup> In view of this, the present study intends to explore the correlation between autoantibodies and early miscarriage in RSA patients during subsequent pregnancies. It also attempts to establish and validate a prediction model for early miscarriage in pregnant RSA patients based on autoantibodies and ultrasonic endometrial receptivity parameters, aiming to better assist in guiding clinical decision-making and fertility guidance for RSA patients.

## Materials and Methods

### Study Subjects

A retrospective analysis was performed on RSA patients who visited the Department of Reproductive Medicine of Xi’an People’s Hospital (Xi’an Fourth Hospital) from January 2019 to December 2024. Inclusion criteria for RSA patients was as follows: (1) History of  $\geq 2$  previous spontaneous miscarriages; (2) Current menopause duration of 6–12 weeks, with positive results for urinary human chorionic gonadotropin (hCG) and serum hCG; (3) Ultrasound examination indicating intrauterine pregnancy and singleton gestation. Exclusion criteria: (1) Complications with other diseases that may cause uterine abnormalities, such as adenomyosis, intrauterine adhesions, inflammation, and tumors; (2) Diseases affecting hemodynamics, including abnormal thyroid function, hypertension, diabetes mellitus, and arrhythmia. This study was approved by the Medical Ethics Committee of Xi’an People’s Hospital, and all patients signed the informed consent form. The process of patient enrollment and predictive model construction is detailed in [Figure 1](#).



**Figure 1** Flowchart of Patient Enrollment and Predictive Model Construction & Validation.

Patients were divided into the live birth group and the miscarriage group based on pregnancy outcomes. Patients with no obvious fetal malformations and normal full-term delivery were assigned to the live birth group, while those with a clear diagnosis of embryonic arrest were included in the miscarriage group. According to the standards of the Society of Radiologists in Ultrasound (SRU), embryonic arrest can only be diagnosed by ultrasound if any of the following criteria are met: (1) Crown-rump length (CRL)  $\geq 7$  mm without cardiac activity; (2) Mean gestational sac diameter (MGSD)  $\geq 25$  mm without detectable fetal bud; (3) Gestational sac with no yolk sac detected by ultrasound, and no embryo with cardiac activity observed in the re-examination after  $\geq 2$  weeks; (4) Gestational sac with a detectable yolk sac by ultrasound, and no embryo with cardiac activity observed in the re-examination after  $\geq 11$  days.

In accordance with the routine clinical diagnosis and treatment protocols of the department, RSA patients underwent three transvaginal ultrasound examinations for monitoring uterine artery hemodynamics at 6–8 weeks, 8–10 weeks, and 10–12 weeks of gestation. Patients who did not undergo transvaginal ultrasound for uterine artery blood flow measurement during pregnancy were excluded.

## Collection and Detection of Clinical Baseline Data and Serum-Related Indicators

Baseline data of all included patients, such as age, Body Mass Index (BMI), and comorbidities (hypertension, diabetes mellitus, dyslipidemia), were collected through the electronic medical record system. A 4 mL sample of fasting venous blood was collected from each subject in the morning (on the day after diagnosis confirmation). The serum was separated

by centrifugation for 10 minutes and stored in a  $-80^{\circ}\text{C}$  refrigerator. The concentrations of various indicators were determined using the enzyme-linked immunosorbent assay (ELISA) method, with strict adherence to the kit instructions. The instruments and kits used were as follows: Centrifuge (Model: 5757, Shanghai Yuanyao Biotechnology Co., Ltd.); Microplate reader (Model: 20220204588, Shanghai Yuanyao Biotechnology Co., Ltd.); Anti- $\beta 2$ -glycoprotein 1 antibody ( $\alpha\beta 2$ -GP1) kit (Catalogue No.: FY-B021407, Shanghai Fuyu Biotechnology Co., Ltd.); Thyroglobulin antibody (TgAb) kit (Catalogue No.: ZK-E1024, Shanghai Zhenke Biotechnology Co., Ltd.); Anti-sperm antibody (AsAb) kit (Catalogue No.: RE52001, Shenzhen Kerunda Biotechnology Co., Ltd., acting as an agent for products of IBL GmbH, Germany); Anti-cardiolipin antibody (ACA) kit (Catalogue No.: FS-E1106, Shanghai Fusheng Industrial Co., Ltd).

## Monitoring and Acquisition of Ultrasonic Parameters

An HD-15 color Doppler ultrasound diagnostic instrument manufactured by Philips (USA) was used, with a transvaginal ultrasound probe frequency of 4–8 MHz. The specific examination procedures were as follows: Examination of the uterus, uterine cavity morphology, and the size, location, and morphology of the gestational sac; observation of the presence of yolk sac, fetal bud, and primitive heart tube pulsation; and assessment of fetal bud development and the morphology and size of bilateral ovaries.

Monitoring of hemodynamic parameters of the left and right uterine arteries separately. After the uterine artery branches off from the internal iliac artery, a transverse section was obtained at the level of the internal cervical os. Multiple tortuous arterial branches were generally detectable, and the ascending branch was the arterial blood flow supplying the uterus. The branch vessel with blood flow away from the probe and abundant blood flow signals was selected as far as possible. The color Doppler was adjusted appropriately to ensure good display of blood flow signals without overflow or loss, and the sampling gate width was set to 2 mm. The sampling line was aligned with the direction of blood flow, with an angle of  $<30^{\circ}$  between the sampling line and the blood flow direction, and the blood flow velocity was greater than 50 m/s. Six consecutive stable cardiac cycle spectra were recorded. The automatic spectrum envelope and calculation function of the system were activated to measure and record the resistance index (RI), pulsatility index (PI), and peak uterine artery systolic/end-diastolic velocity (S/D) of the left and right uterine arteries separately, and the average values were calculated. The relationship between the uterine artery blood flow resistance parameters in early pregnancy and different pregnancy outcomes of RSA patients was analyzed. All parameters were measured three times by a professional technician for each patient.

## Establishment of the Prediction Model and Evaluation of Predictive Efficacy

All included patients were randomly divided into a training set and a validation set at a ratio of 7:3. The occurrence of recurrent miscarriage in RSA patients was used as the outcome variable to establish the prediction model.

In this study, R software (Version 4.2.3) was used, with R packages including “autoReg”, “XGBoost”, “e1071”, “randomForest”, and “caret” to develop and test multiple models. These models included decision tree (DT), elastic net (Enet), logistic regression(LR), multi-layer perceptron (MLP), random forest (RF), regularized support vector machine (Rsvm), and extreme gradient boosting (Xgboost). When constructing the classic machine learning prediction models, univariate analysis was first performed in the training cohort to screen factors related to the “outcome variable”. Subsequently, factors with  $P<0.10$  in the univariate analysis were included in the stepwise binary logistic regression analysis. Due to the small number of original independent variables in this study and the potential positive effect of variables with low importance on model training, all feature variables were included in the training process of the prediction model.

All machine learning prediction models were optimized through 5-fold cross-validation or hyperparameter tuning. The “pROC” package was used to plot the receiver operating characteristic (ROC) curve and calculate the area under the curve (AUC). The AUC was used as the comprehensive evaluation index of the model, and the Delong method was adopted to compare the AUC values of different models. In addition, the predictive performance of each model was further evaluated from multiple dimensions, including accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and F1-score. A two-tailed  $P$ -value  $<0.05$  was considered statistically significant.

## Statistical Analysis

GraphPad Prism software was used for statistical analysis. Baseline characteristics were expressed as median and interquartile range. The Mann–Whitney *U*-test was used for nonparametric testing to analyze the differences in baseline characteristics, age, and BMI between RSA patients and the control group. The Kruskal–Wallis test was used in this study. Dunn’s correction for multiple comparisons was adopted to compare two variables among multiple variables. MedCalc software (Version 15.2.2) was used to plot the ROC curve and calculate the AUC. Logistic regression was used to calculate the AUC for the combined use of all parameters of interest under the default settings of MedCalc software. A P-value <0.05 was considered statistically significant.

## Result

### Comparison of Baseline Characteristics Between RSA Patients with and without Early Pregnancy Loss

To compare the baseline characteristics between RSA patients who experienced early pregnancy loss and those who did not, relevant data were summarized in [Table 1](#). In both the training cohort (N=412) and testing cohort (n=177), no significant differences were observed between the abortion and normal subgroups in terms of age, BMI, infertility duration, gravidity, parity, hormone levels (FSH, LH, estradiol, progesterone, TSH, FT4, prolactin), metabolic indicators (fasting glucose, total cholesterol, triglycerides, LDL, HDL), blood pressure (systolic and diastolic), platelet count, or coagulation parameters (PT, APTT) (all  $P>0.05$ ). However, the positive rates of  $\alpha\beta 2$ -GP1, TgAb, and ACA were significantly higher in the abortion subgroup than in the normal subgroup across both cohorts (all  $P<0.05$ ), while AsAb showed a significant difference only in the training cohort ( $P=0.001$ ). Additionally, the abortion subgroup had higher uterine artery RI and lower endometrial thickness, endometrial volume, VI, and VFI in both cohorts (all  $P<0.05$ ). These findings indicate that autoantibody positivity ( $\alpha\beta 2$ -GP1, TgAb, ACA), increased uterine artery resistance, and poor endometrial morphology and vascularity are associated with early pregnancy loss in RSA patients.

### Screening of Candidate Variables for Machine Learning-Based Prediction of Early Pregnancy Loss in RSA Patients

To screen candidate variables for machine learning-based prediction of early pregnancy loss in RSA patients, univariate and multivariate logistic regression analyses were conducted, with findings summarized in [Table 2](#). Univariate analysis identified nine variables significantly associated with early pregnancy loss (all  $P<0.001$ ), including positivity for  $\alpha\beta 2$ -GP1, TgAb, AsAb and ACA, increased RI, as well as decreased endometrial thickness, endometrial volume, VI and VFI. In multivariate analysis, ACA positivity no longer showed significance ( $P=0.072$ ), while the other eight variables remained significantly correlated with the outcome (all  $P<0.001$ ). [Figure 2](#) provided multi-method validation for these variables, covering Pearson correlation analysis heatmap, Lasso regression plots (binomial deviance vs number of trees, variable coefficients vs log lambda) and SHAP value-based candidate variable weight ranking. These results confirm that the eight variables with persistent significance are reliable candidates for constructing the machine learning prediction model for early pregnancy loss in RSA patients.

### Evaluation and Comparison of Predictive Efficacy of Machine Learning Models

To assess the predictive efficacy of different machine learning models for early pregnancy loss in RSA patients, performance metrics were analyzed using [Table 3](#) and [Figure 3](#) (heatmaps for train and test datasets), with multi-dimensional validation via [Figure 4](#). In the training dataset, the logistic regression model showed the highest ROC-AUC (0.94) and high accuracy (0.93), outperforming other models such as decision tree (DT, ROC-AUC 0.78) and Xgboost (ROC-AUC 0.88). In the test dataset, logistic regression remained superior with ROC-AUC 0.92 and accuracy 0.90, exceeding random forest (RF, ROC-AUC 0.86) and multi-layer perceptron (Mlp, ROC-AUC 0.81). [Figures 4A–B](#) decision curve analysis (DCA, Panels A, C) and ROC curve analysis (Panels B, D) further confirmed its favorable clinical utility and discriminative ability in both cohorts. These findings demonstrate that the logistic regression model

**Table 1** Baseline Characteristics for Predicting Early Pregnancy Loss in Patients with Recurrent Spontaneous Abortion

Variables	Training Cohort			P-value	Testing Cohort			P-value
	Overall (N=412)	Abortion (N=58)	Normal (N=354)		Overall (n=177)	Abortion (N=23)	Normal (N=154)	
Age, years (median [IQR])	30.00 [28.00, 32.00]	29.50 [28.00, 33.00]	30.00 [28.00, 32.00]	0.739	31.00 [29.00, 33.00]	31.00 [29.00, 32.00]	30.50 [29.00, 33.00]	0.633
BMI, kg/m <sup>2</sup> (median [IQR])	22.64 [20.39, 24.33]	22.34 [20.46, 24.49]	22.72 [20.36, 24.32]	0.986	22.43 [20.52, 24.11]	23.12 [20.91, 23.84]	22.39 [20.50, 24.11]	0.868
Infertility duration, years (median [IQR])	2.11 [1.49, 2.72]	1.95 [1.34, 2.59]	2.12 [1.53, 2.75]	0.222	2.06 [1.43, 2.80]	1.58 [1.25, 2.40]	2.12 [1.53, 2.82]	0.054
Gravidity (%)								
1	113 (27.4)	20 (34.5)	93 (26.3)	0.568	52 (29.4)	10 (43.5)	42 (27.3)	0.199
2	198 (48.1)	25 (43.1)	173 (48.9)		87 (49.2)	7 (30.4)	80 (51.9)	
3	88 (21.4)	12 (20.7)	76 (21.5)		35 (19.8)	6 (26.1)	29 (18.8)	
4	13 (3.2)	1 (1.7)	12 (3.4)		3 (1.7)	0 (0.0)	3 (1.9)	
Parity (%)								
0	264 (64.1)	35 (60.3)	229 (64.7)	0.813	110 (62.1)	17 (73.9)	93 (60.4)	0.348
1	142 (34.5)	22 (37.9)	120 (33.9)		60 (33.9)	6 (26.1)	54 (35.1)	
2	6 (1.5)	1 (1.7)	5 (1.4)		7 (4.0)	0 (0.0)	7 (4.5)	
Aβ2 GPI_pos								
Negative	352 (85.4)	38 (65.5)	314 (88.7)	<0.001	157 (88.7)	13 (56.5)	144 (93.5)	<0.001
Positive	60 (14.6)	20 (34.5)	40 (11.3)		20 (11.3)	10 (43.5)	10 (6.5)	
TGAb pos (%)								
Negative	370 (89.8)	41 (70.7)	329 (92.9)	<0.001	163 (92.1)	18 (78.3)	145 (94.2)	0.026
Positive	42 (10.2)	17 (29.3)	25 (7.1)		14 (7.9)	5 (21.7)	9 (5.8)	
AsAb pos (%)								
Negative	378 (91.7)	46 (79.3)	332 (93.8)	0.001	166 (93.8)	20 (87.0)	146 (94.8)	0.322
Positive	34 (8.3)	12 (20.7)	22 (6.2)		11 (6.2)	3 (13.0)	8 (5.2)	
ACA pos (%)								
Negative	374 (90.8)	41 (70.7)	333 (94.1)	<0.001	145 (81.9)	14 (60.9)	131 (85.1)	0.012
Positive	38 (9.2)	17 (29.3)	21 (5.9)		32 (18.1)	9 (39.1)	23 (14.9)	
FSH, mIU/mL (median [IQR])	6.70 [5.94, 7.78]	6.61 [6.00, 7.42]	6.71 [5.88, 7.83]	0.422	6.69 [5.70, 7.52]	6.61 [5.58, 7.47]	6.77 [5.85, 7.53]	0.534
LH, mIU/mL (median [IQR])	4.78 [4.11, 5.52]	5.07 [4.07, 5.61]	4.74 [4.11, 5.49]	0.52	4.75 [4.29, 5.26]	4.75 [3.98, 5.06]	4.77 [4.34, 5.27]	0.383
Estradiol, pg/mL (median [IQR])	28.98 [23.89, 34.06]	28.32 [25.05, 31.51]	29.26 [23.83, 34.54]	0.142	29.43 [24.16, 35.06]	26.70 [21.78, 33.93]	29.61 [24.62, 35.15]	0.144
Progesterone, ng/mL (median [IQR])	11.39 [9.70, 13.46]	10.86 [9.56, 13.08]	11.43 [9.71, 13.47]	0.389	12.06 [10.08, 13.90]	11.81 [10.40, 14.34]	12.27 [9.90, 13.86]	0.963
TSH, μIU/mL (median [IQR])	1.88 [1.64, 2.21]	1.84 [1.64, 2.17]	1.89 [1.63, 2.21]	0.491	1.87 [1.60, 2.19]	1.98 [1.67, 2.10]	1.84 [1.58, 2.21]	0.861
FT4, ng/dL (median [IQR])	1.04 [0.97, 1.13]	1.06 [0.98, 1.13]	1.03 [0.97, 1.12]	0.342	1.03 [0.95, 1.12]	1.05 [0.94, 1.09]	1.02 [0.95, 1.12]	0.922
Fasting glucose, mmol/L (median [IQR])	4.95 [4.69, 5.26]	5.02 [4.72, 5.34]	4.94 [4.69, 5.24]	0.167	5.01 [4.74, 5.30]	5.18 [4.81, 5.39]	5.00 [4.74, 5.26]	0.151
Total_cholesterol, mmol/L (median [IQR])	5.04 [4.62, 5.46]	5.03 [4.65, 5.49]	5.04 [4.60, 5.43]	0.868	5.05 [4.57, 5.51]	5.05 [4.58, 5.48]	5.04 [4.58, 5.50]	0.913
Triglycerides, mmol/L (median [IQR])	1.17 [0.95, 1.41]	1.19 [0.98, 1.39]	1.17 [0.94, 1.42]	0.955	1.26 [1.03, 1.44]	1.26 [1.04, 1.48]	1.25 [1.02, 1.44]	0.74
LDL, mmol/L (median [IQR])	3.09 [2.68, 3.51]	3.00 [2.56, 3.37]	3.10 [2.69, 3.55]	0.132	3.04 [2.69, 3.42]	3.20 [2.76, 3.46]	3.01 [2.69, 3.42]	0.497
HDL, mmol/L (median [IQR])	1.38 [1.17, 1.58]	1.45 [1.30, 1.59]	1.38 [1.17, 1.57]	0.19	1.41 [1.21, 1.59]	1.49 [1.28, 1.60]	1.40 [1.21, 1.59]	0.664
Systolic BP, mmHg (median [IQR])	118.56 [114.03, 122.93]	119.82 [114.00, 124.40]	118.11 [114.09, 122.54]	0.073	118.27 [114.23, 123.06]	121.67 [115.06, 124.90]	118.13 [114.16, 122.69]	0.191

Diastolic BP, mmHg (median [IQR])	78.28 [75.24, 81.49]	79.44 [75.84, 82.39]	78.25 [75.13, 81.42]	0.183	78.29 [75.02, 80.77]	79.39 [76.73, 81.13]	78.25 [74.68, 80.76]	0.311
Platelet count, $\times 10^9/L$ (median [IQR])	244.64 [225.20, 264.10]	246.14 [227.16, 261.12]	244.36 [224.96, 264.40]	0.99	244.58 [227.45, 262.73]	229.13 [221.82, 253.30]	246.48 [229.02, 263.49]	0.101
Prolactin, ng/mL (median [IQR])	14.75 [12.56, 17.38]	15.25 [12.28, 17.59]	14.66 [12.58, 17.29]	0.726	14.84 [12.06, 17.91]	14.19 [13.04, 17.09]	14.88 [11.83, 18.04]	0.775
PT, sec (median [IQR])	12.59 [12.08, 13.07]	12.58 [12.22, 13.17]	12.59 [12.06, 13.04]	0.612	12.42 [11.87, 12.91]	12.52 [11.83, 13.04]	12.41 [11.92, 12.90]	0.619
APTT, sec (median [IQR])	30.14 [28.15, 32.14]	30.67 [28.71, 32.61]	30.01 [27.88, 31.94]	0.08	30.41 [28.71, 32.40]	31.03 [29.80, 32.17]	30.35 [28.57, 32.43]	0.42
RI (median [IQR])	0.64 [0.61, 0.67]	0.74 [0.70, 0.78]	0.63 [0.61, 0.66]	<0.001	0.64 [0.60, 0.67]	0.76 [0.70, 0.79]	0.63 [0.60, 0.65]	<0.001
Endometrial thickness, mm (median [IQR])	8.84 [8.25, 9.43]	6.89 [6.44, 7.66]	9.02 [8.47, 9.50]	<0.001	8.89 [8.22, 9.39]	7.23 [6.45, 7.62]	9.06 [8.48, 9.45]	<0.001
Endometrial volume, mL (median [IQR])	3.71 [3.30, 4.02]	2.42 [2.20, 2.62]	3.79 [3.53, 4.10]	<0.001	3.72 [3.42, 3.99]	2.47 [2.32, 2.69]	3.80 [3.53, 4.03]	<0.001
VI, % (median [IQR])	11.82 [10.06, 13.15]	7.13 [5.76, 8.96]	12.11 [10.89, 13.52]	<0.001	11.73 [10.17, 13.50]	7.87 [6.04, 8.35]	12.30 [10.96, 13.69]	<0.001
VFI (median [IQR])	6.46 [5.50, 7.14]	3.76 [3.11, 4.41]	6.61 [6.00, 7.24]	<0.001	6.30 [5.40, 7.05]	3.87 [3.26, 4.34]	6.46 [5.90, 7.16]	<0.001

**Abbreviations:** BMI, Body mass index; A $\beta$ 2\_GPI, Anti- $\beta$ 2-glycoprotein I antibody; TGAb, Thyroglobulin antibody; AsAb, Anti-sperm antibody; ACA, Anticardiolipin antibody; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; TSH, Thyroid-stimulating hormone; FT4, Free thyroxine; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; BP, Blood pressure; PT, Prothrombin time; APTT, Activated partial thromboplastin time; RI, Resistance index; VI, Vascularization index; VFI, Vascularization flow index.

**Table 2** Univariate and Multivariate Logistic Regression Analyses for Predicting Early Pregnancy Loss in Patients with Recurrent Spontaneous Abortion

Variables	Univariate Logistic Regression		Multivariate Logistic Regression	
	OR (95% CI)	P-value	OR (95% CI)	P-value
A $\beta$ 2 GPI (Positive vs Negative)	4.82 (2.91–7.98)	<0.001	5.13 (3.01–8.75)	<0.001
TGAb (Positive vs Negative)	4.95 (2.76–8.86)	<0.001	5.02 (2.78–9.07)	<0.001
AsAb (Positive vs Negative)	3.68 (1.92–7.06)	<0.001	3.75 (1.95–7.22)	<0.001
ACA (Positive vs Negative)	4.99 (2.77–8.98)	<0.001	1.82 (0.95–3.49)	0.072
RI (per 0.1 increase)	8.25 (5.83–11.68)	<0.001	8.53 (6.01–12.11)	<0.001
Endometrial thickness (per 1 mm decrease)	2.38 (1.95–2.90)	<0.001	2.45 (2.00–2.99)	<0.001
Endometrial volume (per 1 mL decrease)	3.12 (2.53–3.85)	<0.001	3.20 (2.58–3.96)	<0.001
VI (per 1% decrease)	1.56 (1.35–1.81)	<0.001	1.60 (1.38–1.86)	<0.001
VFI (per 1 unit decrease)	1.89 (1.62–2.21)	<0.001	1.95 (1.66–2.29)	<0.001

**Notes:** 1. For binary variables (A $\beta$ 2 GPI, TGAb, AsAb, ACA), “Negative” is set as the reference group, and OR represents the risk ratio of early pregnancy loss when the variable is “Positive” compared with “Negative”; 2. For continuous variables (RI, Endometrial thickness, Endometrial volume, VI, VFI), OR is presented per specified unit change to reflect the association with increased risk of early pregnancy loss (per 0.1 increase for RI, per 1 mm decrease for Endometrial thickness, per 1 mL decrease for Endometrial volume, per 1% decrease for VI, per 1 unit decrease for VFI).

**Abbreviations:** A $\beta$ 2 GPI, Anti- $\beta$ 2-glycoprotein I antibody; TGAb, Thyroglobulin antibody; AsAb, Anti-sperm antibody; ACA, Anticardiolipin antibody; RI, Resistance index; VI, Vascularization index; VFI, Vascularization flow index; OR, Odds ratio; CI, Confidence interval.

has the most robust and stable predictive efficacy for early pregnancy loss in RSA patients among the seven tested models.

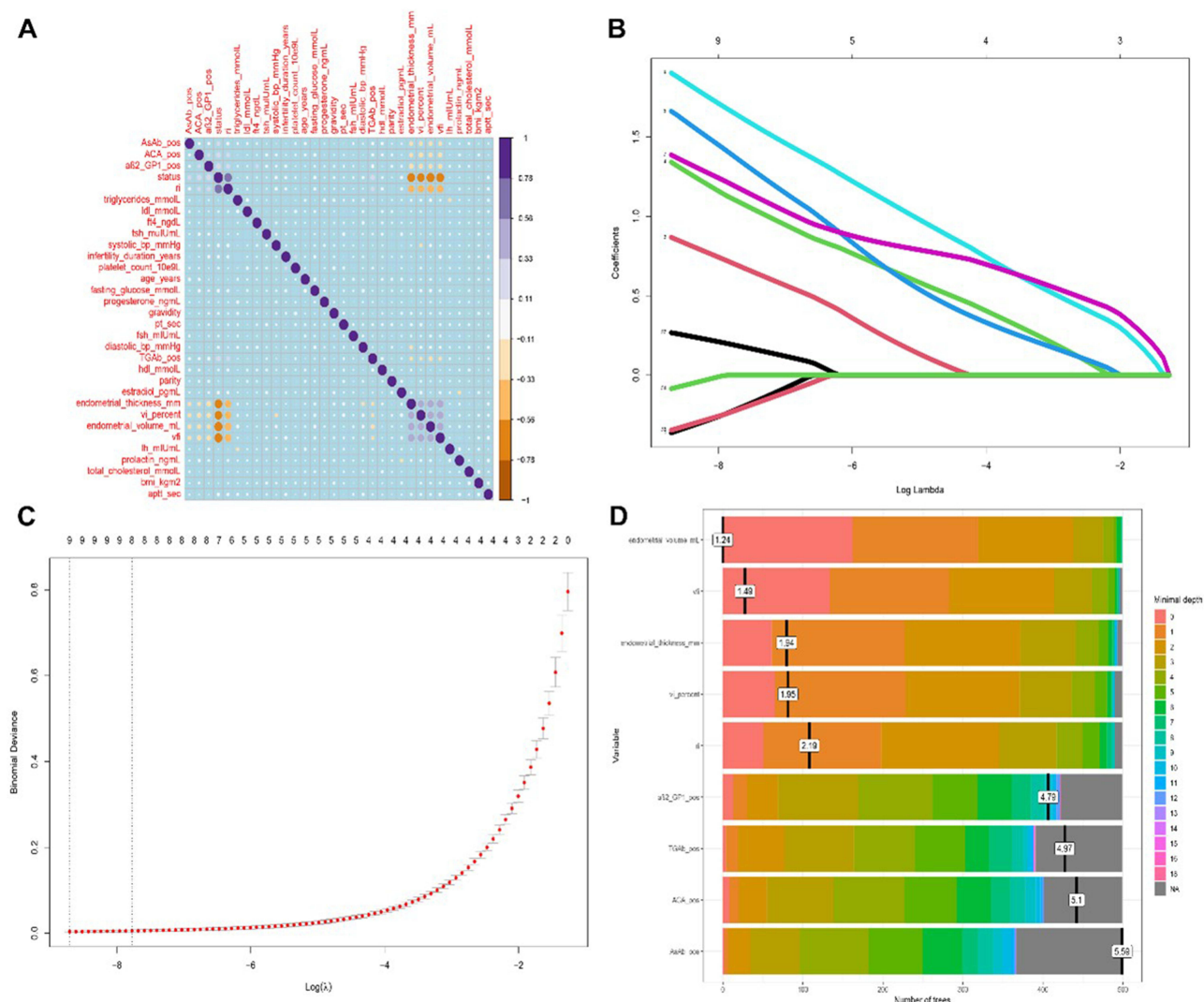
## Clinical Application of the Optimal Predictive Model in Machine Learning

Since the logistic regression model was confirmed as the optimal one for predicting early pregnancy loss in RSA patients, a clinical prediction tool was developed and displayed in Figure 5. Figure 5A shows the online prediction nomogram, which integrates the eight validated candidate variables (a $\beta$ 2-GPI, TgAb, AsAb, RI, endometrial thickness, endometrial volume, VI, VFI). Each variable is assigned corresponding points; clinicians can sum the points of individual variables to get a patient’s total points, thereby estimating the probability of early pregnancy loss. Additionally, calibration curves were used to evaluate the model’s prediction consistency. Figure 5B corresponds to the training set and Figure 5C to the test set, and both curves demonstrate good alignment between predicted probabilities and actual outcomes. This indicates that the nomogram’s predictive results are highly consistent with real clinical situations. Overall, the nomogram derived from the optimal logistic regression model provides a practical and accurate tool for clinicians to assess the risk of early pregnancy loss in RSA patients, which is conducive to formulating individualized clinical intervention strategies.

## Discussion

Globally, RSA remains a major clinical challenge in the field of reproductive medicine. Even after completing comprehensive examinations covering chromosomes, anatomy, immunity, and other aspects, more than half of RSA patients still have unclear etiologies and are thus diagnosed with URA.<sup>24</sup> These patients often experience intense anxiety during subsequent pregnancies, creating an urgent need for reliable pregnancy monitoring methods. Currently, serum  $\beta$ -hCG, estradiol, and progesterone have been used in early pregnancy assessment, but the predictive value of their dynamic changes for pregnancy outcomes remains unclear.<sup>25–27</sup> The machine learning prediction model established in this study based on positive a $\beta$ 2-GPI, positive TgAb, endometrial thickness, endometrial volume, and VFI can effectively evaluate the risk of early miscarriage in RSA patients during subsequent pregnancies.

To date, the specific mechanism by which prethrombotic states (PTS) cause spontaneous miscarriage has not been fully clarified. It is generally recognized that the hypercoagulable state during pregnancy alters the blood flow status at the uteroplacental interface, easily leading to the formation of local microthrombi or even placental infarction.<sup>28,29</sup> This reduces blood supply to placental tissue, causing ischemia and hypoxia in the embryo or fetus, and ultimately resulting in poor embryonic or fetal development and miscarriage. Known acquired PTS mainly include antiphospholipid syndrome



**Figure 2** Multi-Method Validation for Candidate Variable Selection in Machine Learning. (A) Pearson Correlation Analysis Heatmap; (B) Lasso Regression Plot (Binomial Deviance vs Number of Trees); (C) Lasso Regression Plot (Variable Coefficients vs Log Lambda); (D) Candidate Variable Weight Ranking Based on SHapley Additive exPlanations Value Analysis.

(APS), acquired hyperhomocysteinemia, and other diseases that cause hypercoagulable states.<sup>30</sup> Currently, indicators commonly used to detect PTS include coagulation-related tests and relevant autoantibodies, such as aPL, aβ2-GP1, lupus anticoagulant (LA), and homocysteine (Hcy). Among them, the aβ2-GP1 is widely present in human plasma, and the placenta participates in the production of aβ2-GP1 during pregnancy.<sup>31</sup> aβ2-GP1 plays an important role in the occurrence of autoimmune diseases, adverse pregnancy histories, and other conditions. Previous studies have shown that positive expression of aβ2-GP1 antibodies in pregnant women may lead to habitual abortion, stillbirth, and pregnancy-induced hypertension, and has predictive value for adverse pregnancy outcomes.<sup>32</sup>

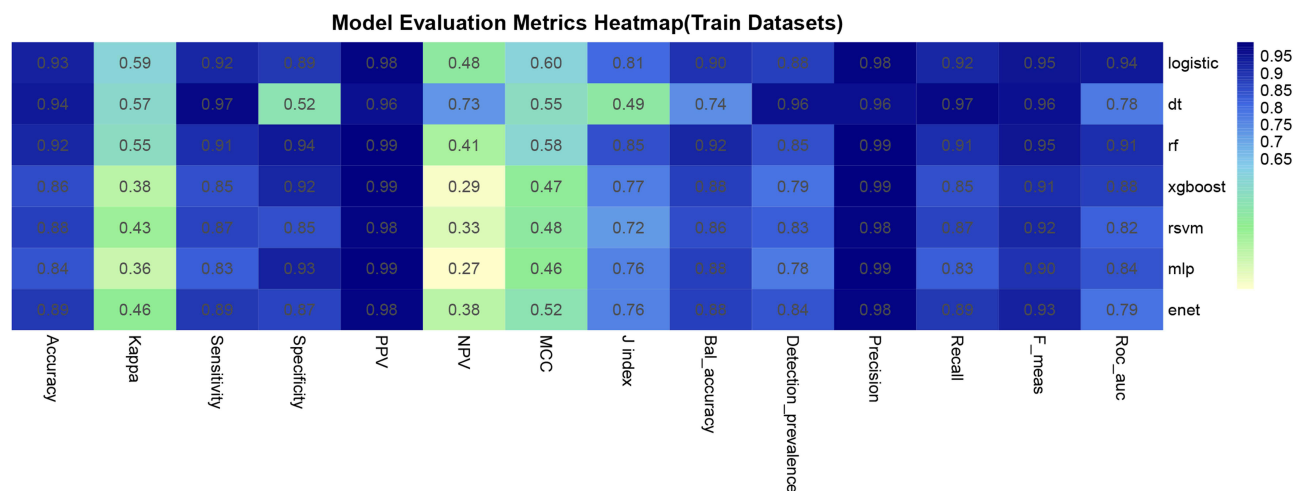
In this study, serum aβ2-GP1 antibodies were detected in all subjects, and the results showed that the positive rate of serum aβ2-GP1 antibodies in RSA patients was significantly higher than that in the control group, which was consistent with the above findings. Moreover, the positive rate of serum aβ2-GP1 antibodies in RSA patients was significantly positively correlated with the risk of recurrent miscarriage in subsequent pregnancies, indicating that an increased positive rate of serum aβ2-GP1 antibodies is associated with an elevated risk of miscarriage in RSA patients during subsequent pregnancies. It is speculated that serum aβ2-GP1 antibodies may play a role in the assessment of RSA severity.

**Table 3** Performance Metrics of Various Models on Training and Testing Datasets

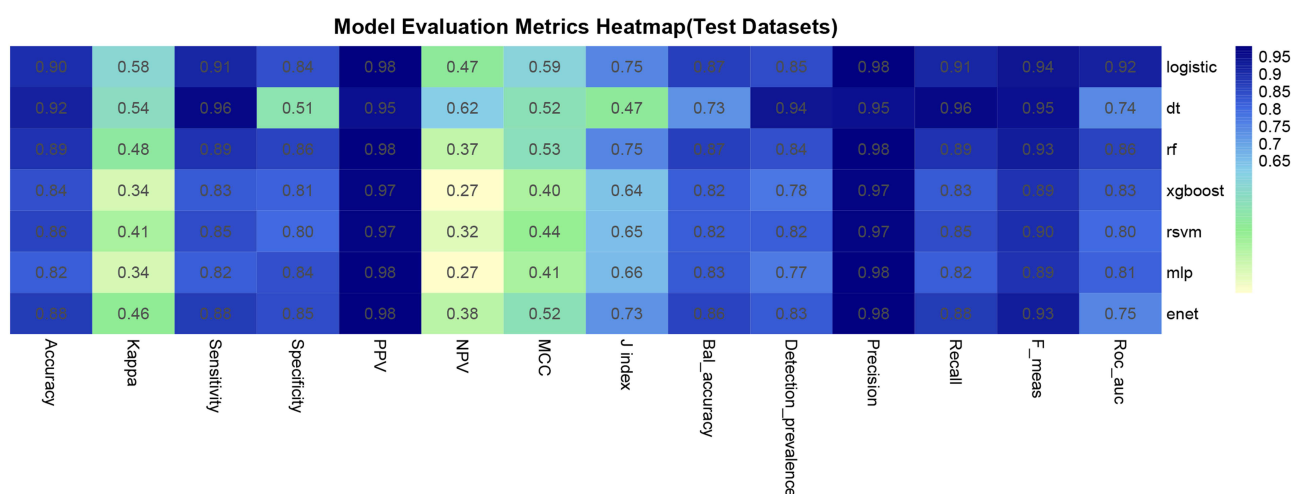
Dataset	Model	Accuracy	Kappa	Sensitivity	Specificity	PPV	NPV	MCC	J index	Bal_accuracy	Detection_prevalence	Precision	Recall	F_meas	Roc_auc
Train	Logistic	0.93	0.59	0.92	0.89	0.98	0.48	0.6	0.81	0.90	0.88	0.98	0.92	0.95	0.94
	Dt	0.94	0.57	0.97	0.52	0.96	0.73	0.55	0.49	0.74	0.96	0.96	0.97	0.96	0.78
	Rf	0.92	0.55	0.91	0.94	0.99	0.41	0.58	0.85	0.92	0.85	0.99	0.91	0.95	0.91
	Xgboost	0.86	0.38	0.85	0.92	0.99	0.29	0.47	0.77	0.88	0.79	0.99	0.85	0.91	0.88
	Rsvm	0.88	0.43	0.87	0.85	0.98	0.33	0.48	0.72	0.86	0.83	0.98	0.87	0.92	0.82
	Mlp	0.84	0.36	0.83	0.93	0.99	0.27	0.46	0.76	0.88	0.78	0.99	0.83	0.9	0.84
	Enet	0.89	0.46	0.89	0.87	0.98	0.38	0.52	0.76	0.88	0.84	0.98	0.89	0.93	0.79
Test	Logistic	0.9	0.58	0.91	0.84	0.98	0.47	0.59	0.75	0.87	0.85	0.98	0.91	0.94	0.92
	Dt	0.92	0.54	0.96	0.51	0.95	0.62	0.52	0.47	0.73	0.94	0.95	0.96	0.95	0.74
	Rf	0.89	0.48	0.89	0.86	0.98	0.37	0.53	0.75	0.87	0.84	0.98	0.89	0.93	0.86
	Xgboost	0.84	0.34	0.83	0.81	0.97	0.27	0.4	0.64	0.82	0.78	0.97	0.83	0.89	0.83
	Rsvm	0.86	0.41	0.85	0.8	0.97	0.32	0.44	0.65	0.82	0.82	0.97	0.85	0.9	0.8
	Mlp	0.82	0.34	0.82	0.84	0.98	0.27	0.41	0.66	0.83	0.77	0.98	0.82	0.89	0.81
	Enet	0.88	0.46	0.88	0.85	0.98	0.38	0.52	0.73	0.86	0.83	0.98	0.88	0.93	0.75

**Abbreviations:** PPV, Positive Predictive Value; NPV, Negative Predictive Value; MCC, Matthews Correlation Coefficient; J index, Youden's Index; Bal\_accuracy, Balanced Accuracy; Detection\_prevalence, Detection Prevalence; F\_meas, F-measure; Roc\_auc, Receiver Operating Characteristic Area Under Curve.

A



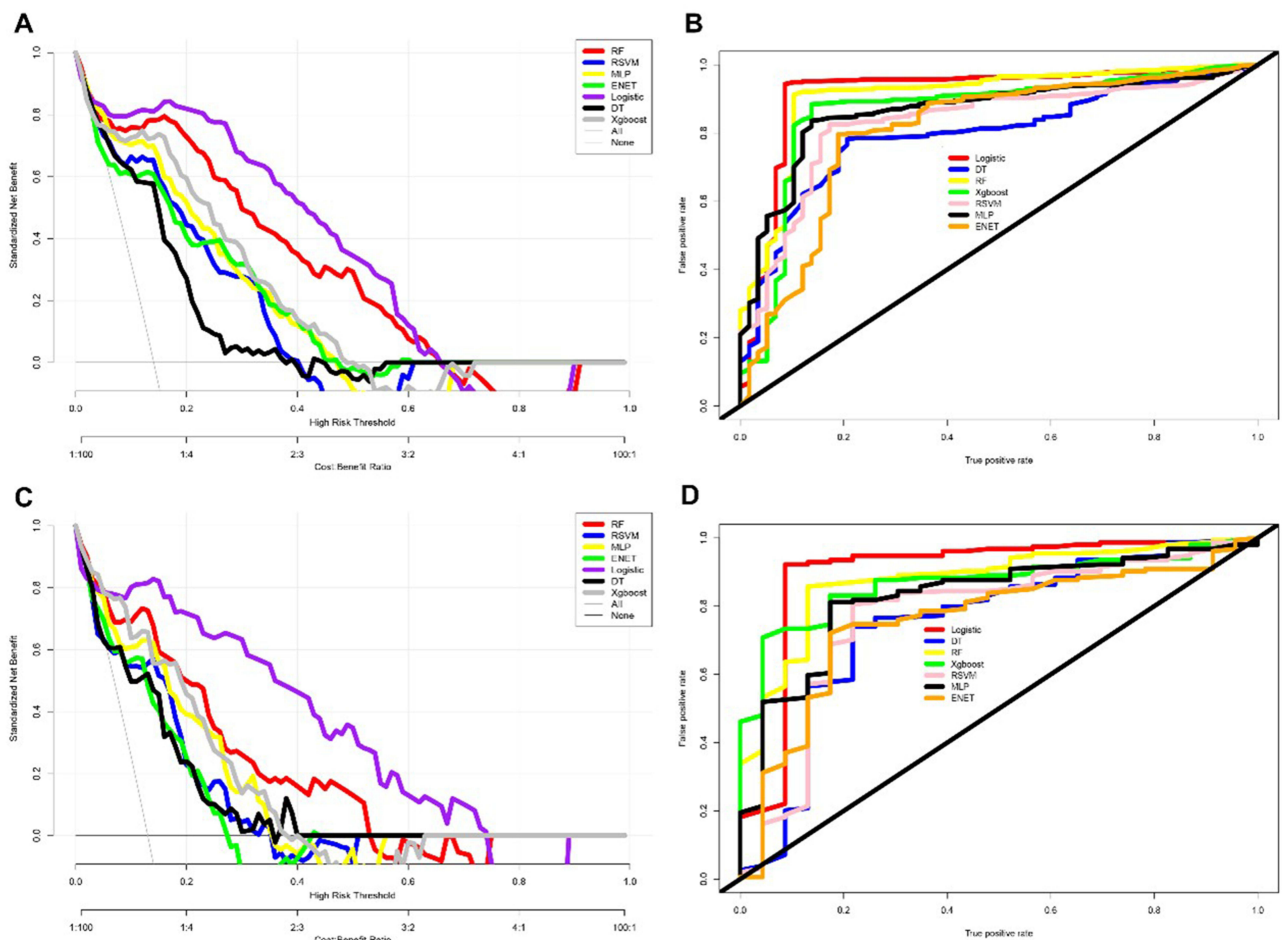
B



**Figure 3** Heatmaps of Model Evaluation Metrics for Train (A) and Test (B) Datasets.

Previous studies have indicated that pregnancy is regulated by multiple factors, including the uterine environment, hormones, and immunity.<sup>33–35</sup> Dysfunction of any of these factors may lead to adverse pregnancy outcomes.  $\alpha\beta$ 2-GP1 can inhibit the activity of prothrombin on the platelet surface, promote thrombosis, and damage trophoblast cells, ultimately resulting in adverse pregnancy outcomes. Consistent with the results of this study, the positive rate of serum  $\alpha\beta$ 2-GP1 antibodies in patients with adverse pregnancy outcomes was significantly higher than that in patients with favorable pregnancy outcomes, and  $\alpha\beta$ 2-GP1 antibodies had high accuracy in predicting adverse pregnancy outcomes in RSA patients. These findings suggest that  $\alpha\beta$ 2-GP1 antibodies have certain value in predicting adverse pregnancy outcomes in patients with RSA.

Previous studies have shown that thyroid autoimmunity (TAI) is a common autoimmune disease in women of childbearing age, with a prevalence of approximately 18%.<sup>36</sup> TAI is associated with a variety of anti-thyroid antibodies (ATA), mainly including anti-thyroid peroxidase antibody (TPOAb), anti-thyroglobulin antibody (TgAb), and thyroid-stimulating hormone receptor antibody (TSHRab). Epidemiological data show that ATA can be detected in approximately 5% to 15% of women of childbearing age.<sup>37–39</sup> A large-scale epidemiological survey in China showed that 23.3% of pregnant women tested positive for TPOAb and 9.9% tested positive for TgAb.<sup>40</sup> Consistent with the results of



**Figure 4** Multidimensional Evaluation of the Predictive Performance of Various Predictive Models.

**Notes:** (A) (Training cohort) and (C) (Testing cohort) refer to the analysis results for evaluating model performance from one dimension, corresponding to Decision Curve Analysis (DCA); (B) (Training cohort) and (D) (Testing cohort) refer to the analysis results for evaluating model performance from another dimension, corresponding to Receiver Operating Characteristic (ROC) Curve Analysis.

previous studies, this study found that TgAb was significantly associated with adverse outcomes of recurrent miscarriage in RSA patients during subsequent pregnancies.

It is speculated that the regularity of the female menstrual cycle and normal ovulation depend on the precise regulation of the hypothalamic-pituitary-ovarian axis (HPO axis), and the presence of ATA may disrupt this balance mechanism, thereby causing ovulation disorders and menstrual irregularities. In addition, previous studies have found that the presence of ATA is associated with increased levels of proinflammatory cytokines (interferon-gamma [IFN- $\gamma$ ] and interleukin-17A [IL-17A]) and decreased levels of transforming growth factor-beta (TGF- $\beta$ ). These changes can affect embryo implantation and pregnancy maintenance.

This study also found that AsAb is an independent risk factor for early miscarriage in RSA patients during subsequent pregnancies. In fact, there is no consistent clinical consensus in the academic community regarding studies on recurrent spontaneous abortion caused by AsAb. However, it has long been recognized that sperm act as antigens in the female body. When sperm enter the female bloodstream under the influence of factors such as infection or trauma, the body produces immune antibodies, and AsAb is the immune complex generated by the body. Previous studies have shown that AsAb can inhibit the tight binding between sperm and the zona pellucida (ZP) through two mechanisms: one is direct binding to the ZP ligand in the sperm membrane, and the other is binding to the corresponding antigen near the ZP ligand in the sperm membrane.<sup>41–43</sup> Both mechanisms interfere with the binding between the ZP ligand in the sperm membrane and the sperm receptor in the ZP, ultimately leading to miscarriage and infertility. However, sperm antigens are quite

**A**

**RSAnomogram**

Building nomogram

aβ2\_GP1  
0.171477079796265

TGAb  
0.127334465195246

AsAb  
0.101867572156197

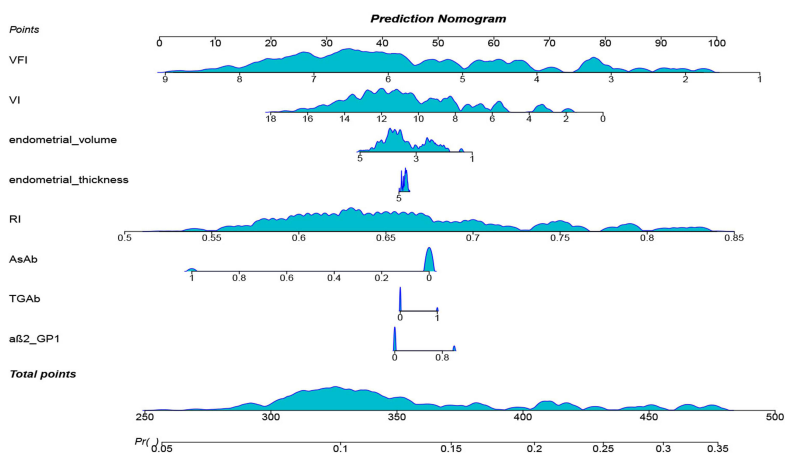
RI  
0.660933786078098

endometrial\_thickness  
8.40655348047538

endometrial\_volume  
3.39908319185059

VI  
10.729337860781

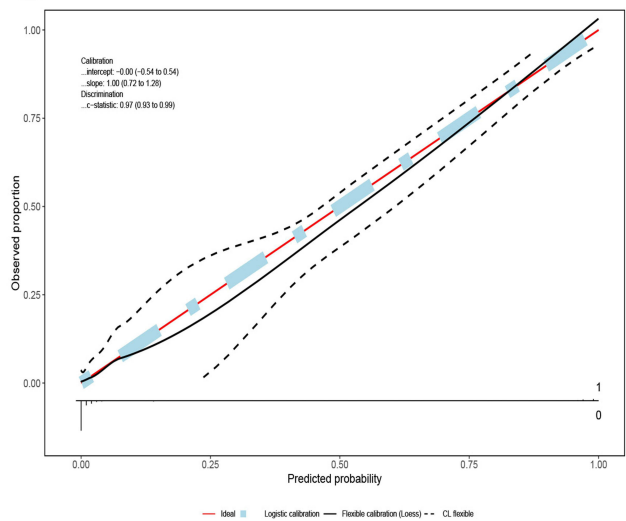
VFI  
5.77528013582343



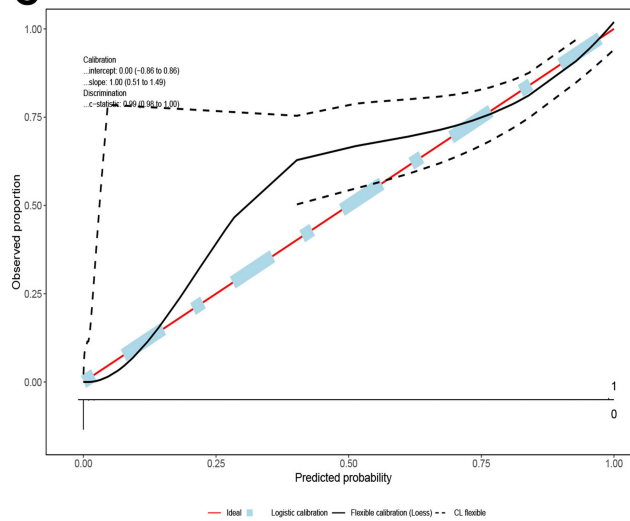
Prediction result:

[1]	-2.0365998	-2.4492266	-1.8048052	-2.1444645	-2.3584922	-2.1431658	-2.1661362	-2.3442641	-2.4184635
[10]	-1.6731682	-1.9904432	-2.0364011	-1.6185334	-1.8320766	-2.1943482	-1.3316539	-1.6023202	-0.9138344
[19]	-1.7266336	-1.2798210	-1.4589537	-0.7465044	-1.1330781	-0.9832921	-1.2382886	-1.6533706	-0.7505916
[28]	-1.3505993	-0.6238042	-1.3365852	-0.8776722	-0.6572272	-1.2366223	-2.5679474	-2.0817828	-2.1292528
[37]	-1.9427951	-1.9963026	-1.9712715	-2.0768923	-2.0576411	-2.2405765	-1.6616880	-2.1150038	-2.2595643
[46]	-2.6394721	-1.9617454	-2.3379716	-1.7720364	-2.0532707	-2.1724202	-2.1571840	-2.3098982	-1.9275791
[55]	-2.0528416	-1.7992575	-2.2459636	-2.3602197	-1.9093752	-2.0474250	-2.2327724	-2.1176468	-2.5674684
[64]	-2.2099854	-2.8209578	-2.6008094	-1.9509523	-2.2599731	-2.2672324	-2.2160296	-2.0971762	-2.3048926
[73]	-2.2984768	-2.5355449	-2.3297700	-2.3131623	-2.4649216	-1.9427439	-2.2381823	-2.3425922	-2.1225759
[82]	-2.0564515	-2.0497728	-2.3125148	-1.6185334	-1.8320766	-2.1943482	-1.3316539	-1.6023202	-0.9138344
[91]	-1.7266336	-1.2798210	-1.4589537	-0.7465044	-1.1330781	-0.9832921	-1.2382886	-1.6533706	-0.7505916
[100]	-1.3505993	-0.6238042	-1.3365852	-0.8776722	-0.6572272	-1.2366223	-2.2439567	-2.3456732	-2.2625222
[109]	-2.1276869	-2.2921328	-2.1963082	-2.3227594	-2.0615743	-2.1264786	-2.3733534	-2.0844871	-2.0911846

**B**



**C**



**Figure 5** Online Nomogram for Predicting Early Pregnancy Loss.

**Notes:** Subfigure (A) shows the online prediction nomogram; Subfigures (B and C) denote the calibration curves of the training set and test set, respectively.

complex, and the known types include sperm-specific antigens, sperm membrane antigens, ABO blood group antigens, and histocompatibility antigens.

Studies have indicated that the presence of antisperm antibodies (AsAb) in the human body may lead to infertility, and this factor is associated with a considerable proportion of infertile cases.<sup>44,45</sup> Additionally, among women who experience spontaneous abortion, the rate of AsAb positivity also constitutes a notable percentage.<sup>46</sup> Therefore, immune infertility caused by AsAb has received widespread clinical attention. Since the production of AsAb is not correlated with age and can cause infertility in all main reproductive age groups, the detection of AsAb is a prerequisite for the effective treatment of infertile patients.

In this study, endometrial thickness, endometrial volume, and Vascular Flow Index (VFI) were found to be independent protective factors against early miscarriage in RSA patients during subsequent pregnancies. The endometrium is a key site for embryo implantation and development, and its morphology, thickness, and microenvironment have important impacts on pregnancy outcomes. From the perspective of the microenvironment, the endometrial microenvironment consists of complex components such as cells, molecules, and flora, which work together to affect the process of embryo implantation and development.<sup>47</sup> Among these, endometrial stromal cells and metabolism influence the decidualization process. Notably, during early pregnancy, significant changes occur in maternal blood circulation to meet the growing needs of the uterus and fetus. Embryo implantation, trophoblast invasion, and placental development are critical for successful pregnancy, and uterine artery blood flow plays an important role in these processes. Domestic and international studies have confirmed that abnormal uterine artery blood flow is associated with adverse pregnancy outcomes.<sup>48</sup> In the monitoring of uterine artery blood flow parameters in this study, the mean systolic/diastolic ratio (mS/D), mean pulsatility index (mPI), and mean resistance index (mRI) in the miscarriage group were higher than those in the live birth group at 8–10 weeks of gestation (ie, the second ultrasound examination), with statistically significant differences. This suggests that patients with recurrent pregnancy loss have increased uterine artery Doppler parameters during the 8–10 weeks period, which is associated with a corresponding increase in the risk of miscarriage.

In addition, some scholars have shown that trophoblast invasion has little effect on uterine artery blood flow at 4–5 weeks of gestation, and the blood flow resistance of the uterine artery decreases very slowly before 8 weeks of gestation.<sup>49–51</sup> Other scholars have also compared the uterine artery PI values between women with no history of miscarriage and those with unexplained recurrent pregnancy loss, and found that the PI values of patients with unexplained recurrent pregnancy loss were significantly higher than those of women without a history of miscarriage.<sup>52,53</sup> Based on these findings, we speculate that the uterine artery may regulate endometrial receptivity, and poor uterine blood perfusion may be one of the causes of unexplained miscarriage and may also contribute to implantation failure. In conclusion, the results of our study indicate that VFI in early pregnancy can be used as a risk factor for miscarriage, and in particular, the rate of decrease in uterine artery blood flow parameters in early pregnancy can predict adverse pregnancy outcomes.

This study also inevitably has the following three key limitations. First, it employed a retrospective single-center design, with all data derived from the electronic medical records of Xi'an People's Hospital. This design may introduce selection bias (eg, the exclusion of patients with incomplete records) and information bias (eg, unrecorded factors like psychological stress), which could compromise the reliability of the established model. Second, the sample size was little and homogeneous, as participants were recruited from a single institution and had similar demographic backgrounds. This restricts the model's external validity when applied to RSA patients in other regions or of different ethnic groups. Third, the set of variables included was incomplete. The model did not account for lifestyle factors (eg, environmental toxin exposure), male-related indicators (eg, sperm quality), and dynamic biochemical markers (eg, serial  $\beta$ -hCG changes), thereby reducing its comprehensiveness.

## Conclusion

This study established a logistic regression model for early pregnancy loss in RSA patients using 8 predictors ( $\alpha\beta$ 2-GP1, TgAb, AsAb, RI, endometrial thickness, endometrial volume, VI, VFI), with an AUC of 0.94 in the training set and 0.92 in the validation set. The model's nomogram enables practical risk stratification, thereby supporting individualized clinical intervention. Limitations include a single-center, retrospective study design; however, future multicenter prospective studies incorporating more variables (eg, male factors, lifestyle) are expected to optimize the model.

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## Disclosure

The authors report no conflicts of interest in this work.

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