ORIGINAL RESEARCH

Analysis of Influencing Factors of Embryo Development Arrest During Early Pregnancy and Construction and Validation of its Prediction Model

Yongjun Ji, BM; Hong Xu, MM; Jianing Wang, MM; Ruiheng Zhao, MM

ABSTRACT

Context • The early symptoms of embryo development arrest are not typical. There is currently no model tool available to predict embryo development arrest.

Objective • To explore the influencing factors of embryo development arrest in early pregnancy and build a risk prediction model.

Methods • From May 2019 to March 2023, 277 patients suspected of embryonic development arrest during the first ultrasound examination in the Department of Obstetrics and Gynecology of the Ninth Affiliated Hospital of Soochow University were retrospectively selected as the study subjects. They were divided into diapause group and non-diapause group according to the second ultrasound (review after 1-2 weeks) to diagnose whether embryo development arrest. Collect two sets of data for analysis, Screen out the influencing factors of early pregnancy embryo development arrest. The logistic regression model and random forest model were constructed respectively. Evaluate the predictive performance of two statistical models.

Results • Out of 277 suspected cases of embryonic developmental arrest, 88 were ultimately confirmed. Older age (*OR*: 2.259, *P* = .017), higher ultrasonic blood flow resistance index (RI) (*OR*: 1.728, *P* = .038), higher ultrasonic gestational sac diameter/embryo head hip length ratio (MSD/CRL) (*OR*:1.919, *P* = .007), lower progesterone (*OR*: 0.562, *P* = .011), and lower pregnancy-associated protein A (PAPP-A) (*OR*: 0.495, *P* = .023). The low expression of vascular endothelial growth factor (VEGF) (*OR*: 0.618, *P* = .005) was the influencing factor of embryo development arrest in early pregnancy. Building a prediction model based on the above indicators, it was found through testing that the random forest model is superior to the logistic regression model in predicting the risk of embryo development arrest.

Conclusion • A random forest model based on age, ultrasound RI, progesterone, PAPP-A, ultrasound MSD/CRL ratio, and VEGF index can help clinicians identify the risk of embryonic developmental arrest. (*Altern Ther Health Med.* 2024;30(10):432-437).

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INTRODUCTION

Embryo development arrest in the first trimester of pregnancy means that the development of embryos within 12 weeks of pregnancy stops for some reason, the incidence rate is 10% -15%,[1] The outcome was spontaneous abortion or missed abortion.[2,3] The causes of embryo development arrest are complex, in addition to common genital tract malformations, endocrine abnormalities, chromosome abnormalities, and infection factors.[4,5] There are still about 50% of embryos whose causes are unknown.[6] It leads to difficulties in clinical diagnosis and treatment, And there are currently no specific indicators or model tools for predicting embryo development arrest. Studies have shown that Embryos that have been suspended for more than 4 weeks have not been detected and remain in the mother's body, which can seriously damage the maternal coagulation function,[7] It greatly damages the physiological function of the mother. The placenta serves as a temporary organ for the normal growth and development of the fetus during pregnancy. Numerous studies have shown that placental vascular insufficiency is associated with the occurrence of embryonic arrest.[8,9] It is evident that angiogenesis and remodeling at the maternal-fetal interface are crucial for maintaining early pregnancy. However, the process of placental angiogenesis is extremely complex. It is regulated by various growth factors and cytokines.[10]

In this study, the ultrasound characteristics and blood cytokine indexes of patients with suspected early embryo

pregnancy arrest were comprehensively analyzed. The aim is to identify the influencing factors of embryo suspension and construct a logistic regression model. To make it more user-friendly and efficient, draw a column chart. After that, big data mining technology and the random forest algorithm to create a random forest model. Then, compare it with the logistic regression model to provide a reference for clinical evaluation of the risk of early embryo termination during pregnancy.

MATERIALS AND METHODS

Study population

From May 2019 to March 2023,277 patients with suspected embryo development arrest by the first ultrasound examination in the Department of Obstetrics and Gynecology of Suzhou Ninth Hospital affiliated with Soochow University (Suzhou Ninth People's Hospital) were retrospectively selected as the research objects. According to the final diagnosis of embryonic diapause, the patients were divided into the diapause group and the non-diapause group. Inclusion criteria: (1) because of different degrees of lower abdominal pain and or irregular vaginal bleeding; (2) Married, and there is no obvious abnormality in the indicators of premarital examination of both husband and wife; (3) 20-40 years old; (4) Singleton pregnancy, gestational age at diagnosis was 6-8 weeks (based on non-menstrual calculation and ultrasound verification). Exclusion criteria: (1) Have a history of adverse pregnancy such as abortion, embryo damage and biochemical pregnancy; (2) IVF or IVF assisted pregnancy; (3) those with endocrine diseases, autoimmune diseases and other diseases affecting fetal development; (4) Combined with uterine fibroids, ovarian cysts, uterine malformations and other uterine organic lesions; (5) Chromosome abnormality in either spouse; (6) Clear mycoplasma, chlamydia infection, TORCH infection; (7) Ectopic pregnancy or hydatidiform mole and other abnormal pregnancy; (8) There are bad behaviors such as smoking and drinking in daily life. This study has been approved by the Medical Ethics Committee of our hospital.

Research method

Material gathering: Log in to the hospital information system, Collect the age of the subjects when they came to the hospital, gestational age (based on non-menstrual calculation and ultrasound verification), whether there is malignant / vomiting in pregnancy reaction, is a timely pregnancy test conducted, whether there is vaginal bleeding, whether there is abdominal pain, ultrasound indicators at the time of treatment [Ratio of peak systolic velocity to end-diastolic velocity (S/D), resistance index (RI), pulsatility index (PI), the ratio of the mean diameter of the gestational sac to head and hip length of embryo (MSD/CRL)], blood indicators at the time of treatment [progesterone, estradiol, prolactin, pregnancy-associated protein A (PAPP-A), serum β-human chorionic gonadotropin (β-HCG), endothelial growth factor (VEGF)] and other data were collected.

Diagnosis of embryonic diapause. (1) First ultrasonic diagnosis: A. Gestational sac ≥ 20 mm, no ovarian sac and germ; B. Gestational sac ≥ 25 mm, no germ is found, Or germ head hip diameter > 7 mm without heart tube pulsation; C. Gestational age 5~6 weeks, Gestational sac ≤ 15 mm, No yolk sac was found. (2) Secondary ultrasound (reexamination after 1~2 weeks) diagnosis: A. The first ultrasound showed that the gestational sac was ≥ 20 mm, and there was no ovarian sac or embryo. After 1~2 weeks, the second ultrasound examination was still the original result. B. The first ultrasound showed that the diameter of the head and hip of the embryo was less than 7mm, and the second ultrasound examination after 1~2 weeks was still the original result; C. The gestational age was less than 6 weeks, the gestational sac was less than 20mm, and there was no yolk sac. After 1~2 weeks, no yolk sac and embryo were found again by ultrasound examination; D. The growth of the gestational sac was less than 0.7 mm per day, even without growth. Take the results of the second ultrasonic examination as the reference standard, that is, if the secondary ultrasound meets any one standard, It can be diagnosed as embryo development arrest.[11]

Statistical analysis

Qualitative data adoption rate and composition ratio [n (%)] description were analyzed using SPSS version 20.0 software (International Business Machines Corporation). Two inspections were conducted, and measurement data was expressed in (Mean±SD) using a *t* test. Multiple logistic regression analysis was used to identify the influencing factors of early pregnancy embryo development arrest. The difference was considered statistically significant at $P < .05$. To construct the model, Bootstrap was used to extract 70% of the sample size. The nomogram of the Logistic regression model was obtained by visualizing the influencing factors using R version 4.0.3 software (R found, Canada).

At the same time, the Random Forest package is run to realize the random forest algorithm to establish the random forest model. The model construction process mainly contains two important model parameters. They are the decision tree quantity (ntree) and the maximum depth of the number (mtry). Using grid search to optimize the parameters of ntree and mtry, Evaluate the importance of variables through the out-of-pocket error rate to explain the random forest model. Introduce the remaining 30% samples as testing validation, Evaluate the predictive performance of two statistical models by calculating their accuracy, sensitivity, specificity, recall, accuracy, and area under the subject operating characteristic (ROC) curve (AUC).

RESULTS

Single-factor analysis of early pregnancy-induced embryo development arrest

Among the 277 suspected embryo development arrest patients included, 88 were ultimately diagnosed with embryo development arrest (diapause group), and 189 cases of non-embryonic diapause (non-diapause group). There were

Table 1. Single-factor analysis of early pregnancy-induced embryo suspension

Factors	diapause group (n = 88)	Non-diapause group (n = 189)	t/χ^2	*P* value
Age (years)	28.07±3.65	26.79±2.94	3.117	.002
Gestational age (weeks)	7.14±0.49	7.23±0.58	1.261	.208
Timely pregnancy checkup			1.613	.204
Yes	45 (51.14)	112 (59.26)		
No	43 (48.86)	77 (40.74)		
Nausea and vomiting			0.245	.621
Have	56 (63.64)	126 (66.67)		
Not have	32 (36.36)	63 (33.33)		
Vaginal bleeding			0.304	.581
Yes	67 (76.14)	138 (73.02)		
No	21 (23.86)	51 (26.98)		
Lower abdominal pain			2.356	.125
Yes	25 (28.41)	38 (20.11)		
No	63 (71.59)	151 (79.89)		
Ultrasound S/D	6.09±1.51	5.85±1.48	1.248	.212
Ultrasonic RI	0.83±0.14	0.79±0.09	2.861	.004
Ultrasonic PI	2.11±0.37	2.18±0.41	1.364	.174
Ultrasonic MSD/CRL	5.16±0.83	4.67±0.72	5.019	<.001
Progesterone (ng/mL)	23.28±4.35	25.73±4.46	4.290	<.001
Estradiol (pg/mL)	790.52±95.79	811.43±101.32	1.627	.105
Prolactin (mmol/L)	4.19±0.62	4.28±0.67	1.065	.288
PAPP-A (ng/mL)	7.87±1.62	9.29±1.83	6.230	<.001
β-HCG (mIU/mL)	41899.32±7594.57	43271.59±7624.03	1.396	.164
VEGF (pg/mL)	96.64±23.75	131.16±32.84	8.839	<.001

significant differences in age, ultrasound RI, ultrasound MSD/CRL, progesterone, PAPP-A, and VEGF between the diapause and non-diapause groups ($P < .05$), See Table 1.

Multivariate Analysis of embryo development arrest in early pregnancy

Using $P < .05$ as the independent variable in univariate analysis (input actual value), The dependent variable is whether the subject has (0=no, 1=yes) experienced embryo development arrest. Through multiple-factor logistic regression analysis, it was found that: Age, high ultrasound RI, high ultrasound MSD/CRL, low progesterone, low PAPP-A expression, and low VEGF expression are all influencing factors for early embryo development arrest in pregnancy ($P < .05$). See Table 2.

Establishment of Logistic regression model of early pregnancy embryo development arrest

Based on the results of multiple factor regression analysis, the regression coefficients and constant terms are used to construct the model formula: Logit (*P*)=-5.097 + 0.815 × Age + 0.547 × Ultrasonic RI+0.652 × Ultrasonic MSD/CRL−0.576 × Progesterone −0.703 × PAPP-A−0.481 × VEGF. Among 277 suspected embryo suspension patients included, Randomly select 70% of the sample size using the Bootstrap method and visualized the logistic regression model using R software to obtain a column chart, The total score of early pregnancy embryo development arrest is obtained by adding the corresponding scores of each indicator value expressed in a graph, Translate the total score into the predicted probability of early pregnancy embryo discontinuation risk, See Figure 1.

Establishment of a random forest model for embryo development stagnation in early pregnancy

Among 277 suspected embryo development arrest patients included, randomly selected 70% of the sample size used the Bootstrap method, the Random Forest package using R software to implement the random forest algorithm, and establish a random forest model. Optimize the ntree and mtry parameters of the random forest model through grid search, When the mtty is 3 and ntree is 500, The error rate of out-of-bag data is the lowest, See Figure 2. Finally, the optimal random forest model's overall prediction accuracy changes are determined, and the sequence of variables affecting early pregnancy embryo development arrest is VEGF > Ultrasound MSD/CRL > Progesterone > PAPP-A > Age > Ultrasound RI.

Table 2. Multifactor analysis results of embryo development arrest

Variable	β	SE	Waldχ^2	*P* value	*OR*(95%CI)
Age	0.815	0.341	5.712	.017	2.259 (1.158~4.406)
Ultrasonic RI	0.547	0.264	4.293	.038	1.728 (1.030~2.898)
Ultrasonic MSD/CRL	0.652	0.242	7.259	.007	1.919 (1.195~3.083)
Progesterone	-0.576	0.227	6.439	.011	0.562 (0.360~0.877)
PAPP-A	-0.703	0.309	5.176	.023	0.495 (0.270~0.908)
VEGF	-0.481	0.171	7.912	.005	0.618 (0.442~0.864)
Constant	-5.097	1.318	14.955	<.001	-

Figure 1. Nomogram expression of the risk prediction model for embryo development arrest

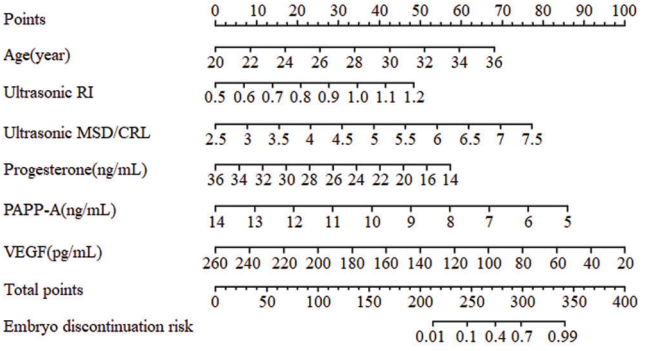


Figure 2. Random forest prediction model for early pregnancy embryo development arrest: the relationship between model error and random number

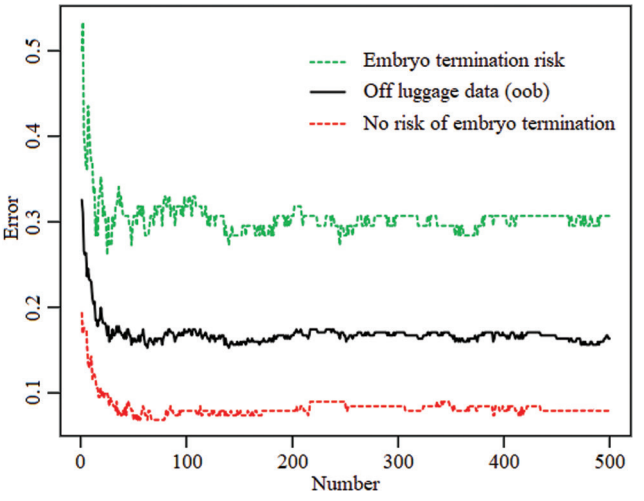
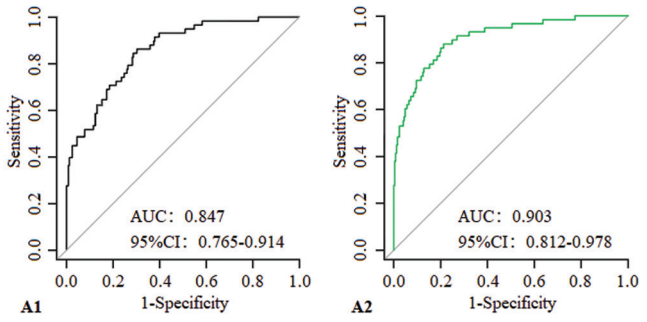


Table 3. Comparison of predictive performance between logistic regression model and random forest model

Model	Accuracy (%)	Sensitivity (%)	Specificity (%)	Recall (%)	Accuracy (%)	AUC
Logistic regression model	0.805	0.839	0.759	0.832	0.794	0.847
Random forest model	0.864	0.897	0.814	0.901	0.868	0.903

Figure 3. ROC curve analysis of the model in test validation (A1: Logistic regression model, A2: Random forest model)



Verification of predictive models for early pregnancy embryo development arrest

Consider whether embryo development arrest occurs in the early stages of pregnancy as a state variable. Using the remaining 30% sample size as a test, The results of verifying the effectiveness of the two models show that the random forest model is significantly superior to the logistic regression model in predicting the effectiveness of actual early pregnancy embryo development arrest, The accuracy of the random forest model is 0.864, sensitivity is 0.897, specificity is 0.814, recall is 0.901, accuracy is 0.868, and AUC is 0.903, Both are higher than the logistic regression model, See Table 3 and Figure 3. This indicates that the random forest model has a more ideal performance in predicting early pregnancy embryo development arrest.

DISCUSSION

Embryo discontinuation is mainly common in the early stages of pregnancy.[12] In the era of precision medicine, Find the relevant influencing factors of early embryo development arrest during pregnancy, using this to construct a predictive model to evaluate the potential risk of embryo development arrest in early pregnancy, This has important guiding significance for decision-making related to subsequent pregnancy maintenance and fetal protection treatment.

The results of this study indicate that older age contributes to the occurrence of embryo development arrest in early pregnancy. Perhaps due to an increase in female age, especially in older pregnancies (referring to pregnant women ≥35 years old), The quality of its oocytes will gradually decrease with age, The function of the adhesion complex between chromatids and the decline of endometrial receptivity,[13] Affects embryo development, Increase the risk of early embryo development arrest during pregnancy. Ultrasound examination is a routine examination in the early stages of pregnancy. Through color Doppler ultrasound detection, information such as blood flow RI, PI, S/D index, gestational sac diameter, and fetal head and buttock length can be obtained. It is currently known that blood flow perfusion in uterine arteries is crucial for embryonic development.[14] The measurement of blood flow RI index using vaginal color Doppler ultrasound can effectively reflect the resistance of uterine artery blood flow.

This study found that a high ultrasound RI value is a contributing factor to the occurrence of embryo development arrest in early pregnancy. It may be due to an abnormal increase in uterine artery blood flow resistance, insufficient blood flow perfusion, affects endometrial receptivity, and increases the risk of early embryo development arrest during pregnancy. Second, insufficient remodeling of uterine spiral arterioles can lead to an abnormal increase in uterine arterial blood flow resistance, During the normal development of the fetal sac, it may be affected by blood circulation disorders in the trophoblast and placenta. This increases the risk of embryo development arrest. The normal gestational sac shows a double ring sign, and there is a certain regularity between the size of the gestational sac and the changes in embryonic development, Because the gestational sac increases with gestational age, The embryo will also gradually grow with changes in the gestational sac.[15] Research has found that, When the MSD measured by vaginal ultrasound is equal to 25 mm If no germ is detected, The probability of false positive embryo development arrest is 0; If the CRL is equal to 7 mm and there is no heartbeat, The probability of false positive embryo development arrest in early pregnancy is 0.[16] This suggests that the size of the gestational sac can predict spontaneous abortion.[17] This study found that a high ultrasound MSD/CRL ratio is a contributing factor to the occurrence of early embryo development arrest during pregnancy. It may be that the size of the early pregnancy sac is inconsistent with the growth of the head and buttocks, Increasing the risk of adverse pregnancy outcomes. The growth of the gestational sac is closely related to progesterone levels.[18] Progesterone is the most biologically active progesterone secreted by trophoblasts and the corpus luteum of ovarian pregnancy, it can measure luteal function and placental development. This study found that low progesterone levels are a contributing factor to the occurrence of embryo development arrest in early pregnancy. Low progesterone refers to insufficient progesterone, therefore it is unable to suppress the permeability of various ions on the uterine muscle cell membrane, enhances the excitability of uterine muscles, is not conducive to embryo survival, and causes an increased risk of embryo development arrest. PAPP-A is a maternal plasma high-molecular-weight glycoprotein synthesized and secreted into the blood circulation by placental syncytiotrophoblast cells, decidual cells, and normal menstrual cycle endometrial stromal cells.[19] It can be detected in the 5th week of pregnancy, And it will gradually increase with the increase of gestational age.[20,21]

This study has found that the low expression level of PAPP-A can contribute to the occurrence of embryo

development arrest in early pregnancy. This may be due to the poor function of trophoblasts. The level of PAPP-A in pregnant women does not increase significantly with the increase of gestational age, leading to abnormal development of placental villi, and increasing the risk of embryo development arrest. However, due to the unique nature of pregnancy, the pathological process of embryo development arrest cannot be fully explained by progesterone levels and PAPP-A content alone. VEGF is the most specific regulatory factor known to promote angiogenesis. It can induce the formation of new capillaries and maintain the integrity and permeability of blood vessel walls.[22] VEGF can be secreted by trophoblasts and participates in the formation of the placental vascular network in early pregnancy.[23] It plays an important role in maintaining embryonic development. Studies have shown that The expression level of VEGF is related to the occurrence of embryo development arrest in early pregnancy, [24] consistent with the results of this study. It may be due to a lack of VEGF expression, reducing the migration and proliferation function of endothelial cells, affecting the invasion and differentiation of villous trophoblasts, causing poor early villous formation, and affecting embryonic development. Second, the expression level of VEGF affects the formation of vascular network in the endometrial matrix, Reduced angiogenesis, causing an imbalance between the proliferation and apoptosis of trophoblasts, This in turn increases the risk of early embryo development arrest during pregnancy.

Traditional logistic regression models have excellent interpretability but cannot fit the distribution of high-dimensional data points well. Visualizing the characteristics of influencing factors through software to obtain the expression form of column charts, Enables physicians to calculate the probability of patient or event outcomes when using intuitively and conveniently.[25] But the model is simple, has poor stability, and overall efficiency is not high. In recent years, new machine learning models such as random forests, support vector machines, decision trees, and other algorithms have made significant progress in predicting other diseases, showing good stability performance.[26,27] This study constructed a logistic regression model and a random forest model for the risk of embryo development arrest in early pregnancy. After testing and verification, it was found that: The random forest model outperforms the logistic regression model in predicting the risk of early pregnancy embryo development arrest. The accuracy, sensitivity, specificity, recall, accuracy, and AUC of the random forest model are all higher than those of the logistic regression model. This indicates that the random forest model has a more ideal performance in predicting early pregnancy embryo development arrest. Perhaps the random forest algorithm has the advantage of introducing randomness and processing high-dimensional data, Therefore, the model is not prone to overfitting, with high accuracy and strong stability.

In summary, early embryo development arrest during pregnancy is related to age, ultrasound RI, progesterone, pregnancy-related protein A, ultrasound MSD/CRL ratio, and VEGF markers. Using this to construct a random forest model, Can effectively assess the risk of early embryo development arrest during pregnancy.

The limitation of this study is a single-center retrospective study, which included a relatively small sample size; and there are many uncontrollable factors in early pregnancy embryo development arrest, such as changes in the psychological condition of pregnant women, living environment, awareness of pregnancy precautions, sleep quality, diet, etc., which may all have an impact on embryo development. Therefore, in the future, large-scale multicenter research is needed, combined with a comprehensive analysis of pregnant women's psychology, diet, sleep, and other aspects, to provide medical personnel with a more reliable early pregnancy embryo development arrest risk screening model.

AUTHORS' DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest related to the study.

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DATA-SHARING STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Nevertheless, all data that is essential to the understanding of our manuscript is included in the Results section and the Tables.

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