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Original Article

Predictive Value of the Novel Biomarker OS9 in Early-Onset and Late-Onset Fetal Growth Restriction

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Abstract:

Aim: To evaluate the potential predictive value of maternal serum OS9 for FGR and its subtypes.

Methods: In this prospective nested case-control study, OS9 levels were measured from recruitment to delivery in pregnant women undergoing Down syndrome screening. We analyzed the changes in OS9 levels across different gestational periods. The predictive performance of OS9 for FGR and its subtypes was evaluated using receiver operating characteristic (ROC) curves.

Results: In FGR pregnancies, OS9 levels showed a slight increase during early pregnancy but gradually decreased after 15 weeks with advancing gestational age. OS9 levels in the FGR group were significantly lower than those in the control group, particularly during the first and third trimesters. ROC curve analysis showed that OS9 alone had the highest AUC (0.815) for early-onset FGR in the third trimester, with a sensitivity reaching 0.923. Combining OS9 with maternal indicators markedly improved predictive performance. This was particularly evident in the third trimester, where the model incorporating all variables achieved an AUC of 0.869 for early-onset FGR, and the OS9-BMI or OS9-Age-BMI model achieved an AUC of 0.741 for late-onset FGR.

Conclusions: This study revealed the dynamic changes of OS9 levels. OS9 levels in the FGR group were significantly decreased in the first and third trimesters, suggesting that its potential as a novel biomarker for FGR prediction. ROC analysis demonstrated that OS9 alone had a certain predictive value for both early-onset and late-onset FGR, while its combination with maternal indicators significantly enhanced model performance, particularly in the third trimester.

Keywords: Fetal growth restriction, OS9, Serum biomarkers, Risk prediction

Introduction

Fetal growth restriction (FGR) refers to impaired fetal growth in the maternal uterus during pregnancy, meaning that the affected fetus fails to reach its genetically determined growth potential [1]. FGR is a complex disease influenced by multiple pathogenic factors, involving maternal, placental, and fetal components. Studies have shown that environmental factors, such as exposure to air pollution and low socioeconomic status, may also contribute to the development of FGR [2-4]. FGR is a major cause of increased risk of perinatal morbidity and mortality, as well as long-term health complications [5].

In developing countries, the prevalence of FGR is approximately 5-25% [6]. The occurrence of FGR not only affects an individual's health at birth but also has a profound impact on adult health through a programming effect [7]. Currently, clinical treatment options for FGR remain limited, and infants with severe FGR exhibit poor survival rates. The primary management strategies for such cases are timely delivery or termination of

pregnancy [8, 9]. The misdiagnosis rate of FGR is high, with 75% of FGR cases not being identified until delivery, and the detection rate in low-risk pregnancies is as low as 15% [10]. Therefore, developing effective predictive methods for FGR during pregnancy provides an opportunity to improve perinatal outcomes. Currently, FGR is primarily assessed through ultrasound examination [11]. Fetal biophysical parameters such as head circumference (HC), abdominal circumference (AC) and biparietal diameter (BPD) are measured directly by ultrasound. Alternatively, Doppler ultrasound of the umbilical artery and other placental vessels can be used to assess blood flow and placental function. However, the accuracy of these methods is susceptible to maternal obesity, gestational age, and the operator's experience. A retrospective study by Atallah *et al*. reported that between 2011 and 2017 in Lyon, France, the rate of missed diagnoses of FGR due to ultrasound examination was as high as 11.5% [12]. Given the high missed diagnosis rate associated with current prenatal diagnostic methods, researchers are actively investigating the diagnostic and prognostic value of biological markers. Previous studies have identified several potential predictors of FGR, including pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PIGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and beta-human chorionic gonadotropin (β-hCG) [13-15]. Among them, PIGF demonstrated low predictive ability at <20 weeks of gestation, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.58 (95% CI: 0.38 - 0.79) [13]. For sFlt-1, the sensitivity and specificity for predicting FGR were only 0.64 and 0.54, respectively during the second trimester [16]. The predictive values of these markers are suboptimal and insufficient to support clinical screening. Therefore, it is necessary to continue the search for novel predictive markers and models to improve clinical outcomes.

Osteosarcoma 9 (OS9) is a lectin that resides on the endoplasmic reticulum (ER). Studies have shown that OS9 recognizes and binds to misfolded glycoproteins, targeting them to the ER-associated degradation (ERAD) pathway [17, 18]. OS9 interacts with hypoxia-inducing factor 1α (HIF-1α) to promote migration and invasion of hepatocellular carcinoma cells [19, 20]. In a previous study, we performed RNA sequencing on the placentas of monochorionic twins with sFGR and matched control twins. The results showed a significant difference in OS9 expression levels in the placenta between the sFGR group and the control group, suggesting a potential association between OS9 and sFGR [21]. Since OS9 in the placenta can be released into maternal blood [22], it may serve as a potential biomarker for FGR prediction. Based on our research findings, this study aims to investigate the predictive value of maternal serum OS9 levels for FGR and its subtypes during pregnancy.

Materials and Methods

Study design and sample

This study adopted a prospective nested case-control method. Pregnant women who underwent Down syndrome screening at the Maternity and Child Healthcare Hospital of Nanshan District, Shenzhen City, Guangdong Province, China, between January 2018 and November 2022, were identified and followed up until delivery. Prenatal care and assessments of the pregnant women were performed according to the protocol of the hospital. A total of 510 women attended the study.

We followed the Delphi consensus criteria to classify FGR into early-onset FGR and late-onset FGR based on gestational age at diagnosis and ultrasound criteria. These parameters were different (see Table 1).

Table 1. Delphi method for the definition of early-onset and late-onset FGR

	Fetal Growth Restriction Parameters
Early-onset FGR (GA < 32 weeks)	Any of the following: AC/EFW < 3rd centile UtA Doppler velocimetry with absent end-diastolic flow Or AC/EFW < 10th centile Combined with either of the following: UtA-PI > 95th centile UA-PI > 95th centile

Late-onset FGR (GA ≥ 32 weeks)	Any of the following: AC/EFW < 3rd centile Or 2 of the following: AC/EFW < 10th centile AC/EFW crossing centile > 2 quartiles UA-PI > 95th centile CPR < 5th centile

AC, abdominal circumference; EFW, estimated fetal weight; GA, gestational age; UA, umbilical artery; UtA, uterine artery; CPR, cerebroplacental ratio; PI, pulsatility index.

All procedures involving human participants in this study were conducted in accordance with the ethical standards of the institutional and/or national research councils, as well as the ethical principles outlined in the 1964 Declaration of Helsinki and subsequent amendments. The study protocol was approved by the Medical Ethics Committee of Maternity and Child Healthcare Hospital of Nanshan District (ethics approval number: NSFYEC-KY-2023015). All participants provided written informed consent, and patients' anonymity was ensured.

Clinical Data Collection

Demographic data of the pregnant women were recorded in detail, including age, height, weight, gestational age, parity, conception method and delivery method. Data on the neonates were collected after delivery, including neonatal sex, birth weight, Apgar score, and neonatal intensive care unit (NICU) admission. Maternal blood samples from FGR subjects and their matched controls were taken to measure OS9 levels.

Analysis of OS9 level by ELISA

OS9 levels in maternal serum were measured by ELISA kits (Baililai Biological, China). The collected maternal blood was centrifuged at 3000 rpm for 10 min to separate the serum from the red blood cells. The upper serum layer was carefully aspirated for further analysis. According to the instructions, first the serum sample was diluted five times with the sample diluent. Fifty μL standard/diluted sample and 100 μL horseradish peroxidase (HRP) labeled antibody were added into each reaction well, and incubated at 37°C for one-hour after sealing the wells with membrane. The 20× washing buffer was diluted with PBS (Sangon, China) at a 1:20 ratio. The plate was washed five times in duplicate with 1× washing buffer, then two substrates were added and incubated at 37°C in the dark for 15 min. Finally, the termination solution was added and the optical density was measured at 450 nm within 15 min.

Statistical Analysis

To exclude the influence of maternal characteristics (such as gestational age and maternal height) on OS9 concentration, the original concentration was $\log_{10}$ transformed based on the multiples of the median (MoM) to make it fit the normal distribution. The $\log_{10}$ MoM value was calculated according to the method described by Poon *et al* [23]. After transformation, we performed Pearson correlation analysis to examine the relationship between OS9 concentration and maternal characteristics. When no significant correlation was found ($P > 0.05$), the maternal characteristics were considered to have no influence on OS9 concentration. For parameters demonstrating significant correlations ($P < 0.05$), the OS9 concentration ($\log_{10}$ MoM value) was adjusted using a linear regression equation.

A logistic regression model was constructed to quantitatively assess the effect of OS9 on FGR risk while adjusting for the influence of other potential confounding factors. The predictive performance of OS9 for FGR was evaluated using the ROC curve. The evaluation metrics included AUC, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), detection rate (DR), and odds of being affected given a positive result (OAPR).

Statistical analysis was performed using R version 4.2.2. The Shapiro-Wilk test was used to evaluate the normality of continuous variables. Measurement data were normally distributed, expressed as mean ± standard deviation (SD), student's t test was used for comparison between the two groups, and experiments involving more than two groups were analyzed using analysis of variance (ANOVA). Measurement data of non-normal distribution were expressed as the median

(interquartile range), Kruskal-Wallis test was used for comparisons between groups, followed by pairwise comparisons using the Mann-Whitney U test with Bonferroni correction. Categorical variables were presented as frequencies and percentages, and comparisons were performed using the Chi-square test or Fisher's exact test, as appropriate. Significant difference was set as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Results

The Maternal and Fetal Clinical Characteristics of the Study Population

According to the inclusion and exclusion criteria, a total of 264 FGR cases and 246 matched control cases were recruited during the nearly three-year period from January 2018 to November 2022. Measurements and analyses were performed on 510 pregnant women, and the maternal characteristics of these cases were presented in Table 2. Comparing the baseline characteristics between the FGR group and the control group, we found no differences in maternal body mass index (BMI) at delivery, neonatal sex, and 5-min Apgar score among the control, early-onset FGR and late-onset FGR group. In the FGR group, maternal age, maternal BMI at the first trimester, maternal height, birthweight, birthweight Z score, EFW, BPD, HC, AC, and femur length (FL) were significantly lower than those in the control group, while nulliparous and gestation at delivery were significantly higher. The four measures of method of conception, gestation at sampling, gestation at diagnosis of FGR, and 1-min Apgar score, although no differences were observed between the FGR group and the control group, were significantly different in the two different subtypes of FGR (early-onset and late-onset).

Table 2. Demographic characteristics of 264 cases of FGR compared with 246 matched cases of normal pregnancies.

Variables	Control (n = 246)	All FGR (n = 264)	P^a	Early-onset FGR (n = 97)	Late-onset FGR (n = 167)	P^c
Maternal age (years)	31 (27 - 34)	29 (26 - 32)	0.023	28.9 ± 4.5	29.9 ± 4.4	0.193
Maternal BMI (Kg/m^2)						
At the first trimester	21.7 (19.9 - 24.3)	21.0 (19.2 - 23.0)	0.003	21.2 (19.4 - 23.5)	20.7 (19.0 - 23.0)	0.375
At delivery	25.9 (24.3 - 27.7)	25.9 (24.0 - 27.7)	0.526	25.9 (24.1 - 28.0)	25.8 (23.9 - 27.4)	0.482
Nulliparous	98 (39.8)	205 (77.7)	2.2e-16	77 (79.4)	128 (76.7)	0.681
Maternal height (cm)	159 (156.0 - 163.4)	157 (153.6 - 160.0)	1.8e-4	157.6 ± 0.1	157.2 ± 0.1	0.643
Method of conception						
Spontaneous	238 (96.8)	250 (94.7)	0.396	97 (100.0)	153 (91.6)	0.014
In vitro fertilization	8 (3.3)	14 (5.3)		0	14 (8.4)	
Gestation at sampling (weeks)	15.2 (14.2 - 19.1)	15.7 (14.4 - 19.3)	0.401	18.3 (14.5 - 20.2)	15.0 (14.2 - 19.1)	0.017
Gestation at diagnosis of FGR (weeks)	0	37.1 (34.8 - 42.6)	NA	34.6 (29.9 - 35.4)	42.5 (40.0 - 43.4)	2.2e-16
Gestation at delivery (weeks)	44.7 (44.6 - 45.6)	45.1 (44.5 - 45.9)	0.002	44.7 (44.4 - 45.9)	45.3 (44.7 - 45.9)	0.029
Mode of delivery						
Normal vaginal birth	121 (50.0)	122 (73.5)	6.1e-10	37 (66.1)	85 (77.3)	0.282
Assisted vaginal birth	4 (1.7)	6 (3.6)		3 (5.4)	3 (2.7)	
Cesarean delivery	67 (27.7)	38 (22.9)		16 (28.6)	22 (20.0)	
Neonatal sex						
Female	109 (44.3)	108 (40.9)	0.477	46 (47.4)	62 (37.1)	0.207
Male	137 (55.7)	156 (59.1)		51 (52.6)	105 (62.9)	
Birthweight (g)	3190 (2950 - 3500)	2630 (2422.5 - 2745)	8.9e-17	2600 (2210 - 2670)	2640 (2490 - 2760)	0.057
Birthweight Z score	0.006 (-5e-4 - 0.014)	-0.019 (-0.024 - -0.013)	5.4e-50	-0.021 (-0.028 - -0.015)	-0.018 (-0.023 - -0.013)	0.045

EFW (g)	3117 (2900.0 - 3340.5)	1611.5 (1278.0 - 2437.3)	2.5e-38	1264.5 (767.5 - 1342.5)	2394.5 (2006.5 - 2580.5)	1.3e-20
BPD (cm)	9.3 (9.0 - 9.5)	8.7 (7.6 - 9.1)	2.4e-16	7.4 (6.7 - 7.7)	8.9 (8.6 - 9.2)	2.2e-13
HC (cm)	33 (32.3 - 33.6)	31 (27.4 - 32.4)	1.6e-17	26.9 (25.0 - 27.9)	31.7 (30.7 - 32.6)	6.3e-13
AC (cm)	33.6 (32.7 - 34.5)	30 (24.8 - 31.8)	9.5e-24	24.4 (22.7 - 25.2)	30.8 (29.0 - 32.5)	4.4e-13
FL (cm)	7.1 (6.9 - 7.2)	6.7 (5.6 - 7.0)	1.0e-15	5.4 (5.1 - 5.6)	6.8 (6.5 - 7.1)	3.4e-13

All continuous variables are presented as median (interquartile range), and categorical variables are presented as number (percentage).

BMI, body mass index; EFW, estimated fetal weight; BPD, biparietal diameter; HC, head circumference; AC, abdominal circumference; FL, femur length; NICU, neonatal intensive care unit; NA, not applicable.

Changes in OS9 Levels Across Gestational Age

In 246 normal maternal cases, serum OS9 concentration showed an increasing trend in early pregnancy (8^{+0} to 14^{+6} weeks). With the progression of gestation, OS9 concentration gradually decreased (15^{+0} to 20^{+6} weeks), reaching its lowest point at 17 weeks, followed by a slow increase, peaking around 35 weeks. In late pregnancy (35^{+0} weeks to delivery), OS9 concentration significantly decreased (Figure 1A). In FGR cases, OS9 concentration exhibited a slight increasing trend in early pregnancy. After 15 weeks, OS9 levels decreased with advancing gestational age (Figure 1B).

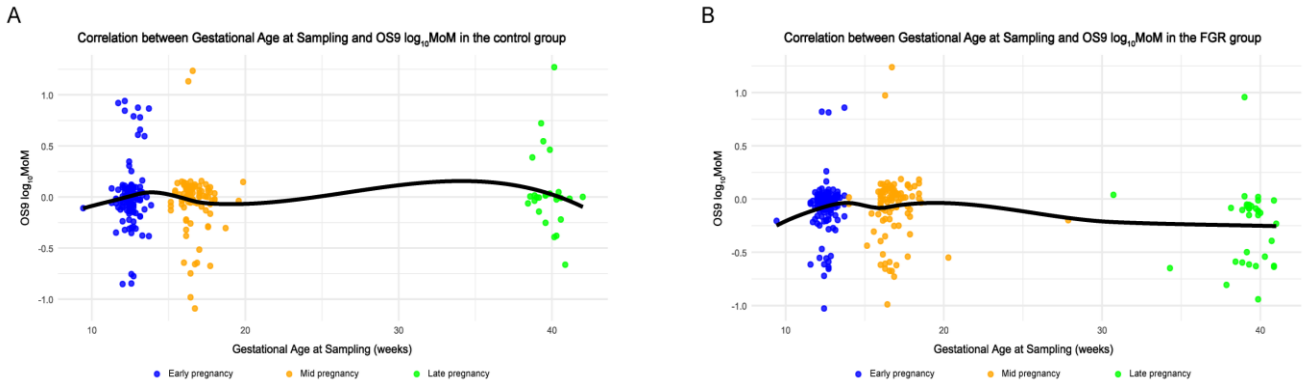

Figure 1. The concentration of maternal serum OS9 changes with gestational age in the control group (A) and the FGR group (B). Blue circles represent samples from the first trimester, orange circles represent samples from the second trimester, and green circles represent samples from the third trimester.

Comparison of the OS9 between the Normal and FGR Groups

To explore the specific association between OS9 and FGR, we first compared the serum OS9 concentrations between FGR and normal pregnant women throughout pregnancy. ELISA results indicated that OS9 levels were significantly decreased in the FGR group (Figure 2A). This finding suggested that the abnormal expression of OS9 is associated with the occurrence and progression of FGR and may serve as a potential biomarker for FGR diagnosis and fetal monitoring.

To determine the specific period of OS9 alterations, pregnancy was divided into the first, second, and third trimesters, and OS9 concentrations were compared separately. During the first and third trimesters, OS9 concentrations were significantly decreased in the FGR group compared to the control group (Figure 2B and D). Notably, no significant difference in OS9 concentration was observed between the FGR and control groups during the second trimester (Figure 2C). These results revealed that OS9 expression in

FGR may be period-specific and play a crucial regulatory role in the first and third trimesters.

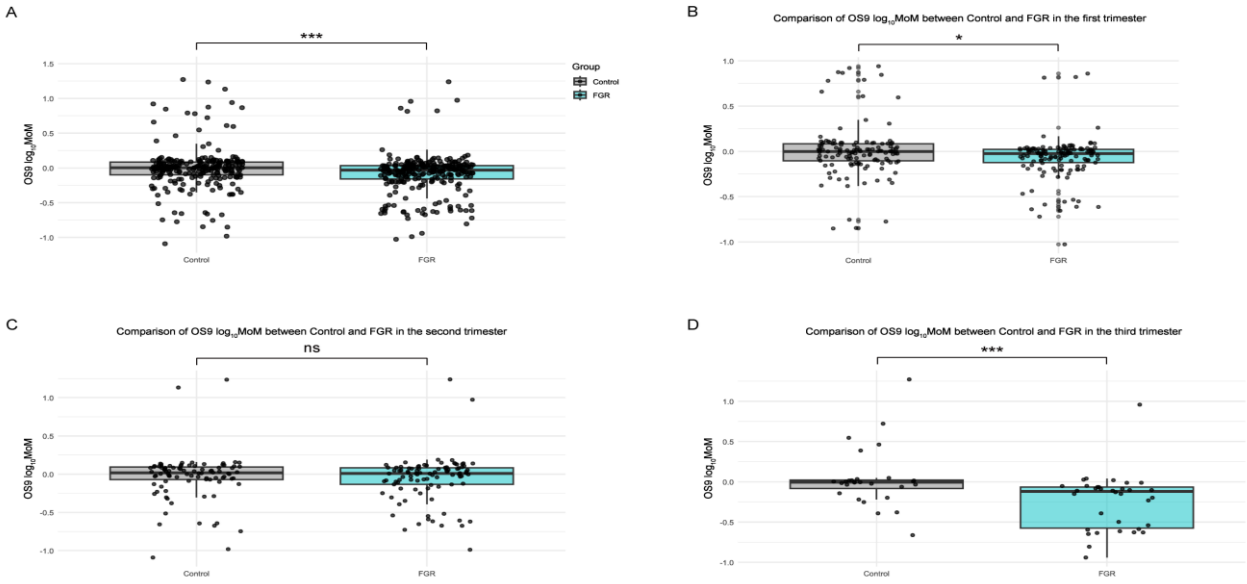

Figure 2. The serum OS9 concentration of FGR pregnant women was lower compared to the control group. (A) Comparison throughout the entire pregnancy. ******P* **< 0.001, assessed by Mann-Whitney U test. (B) Comparison during the first trimester. ****P* **< 0.05, assessed by Mann-Whitney U test. (C) Comparison during the second trimester. Assessed by Mann-Whitney U test, ns, no significance. (D) Comparison during the third trimester. ******P* **< 0.001, assessed by Mann-Whitney U test.

Comparison of the OS9 between the Normal Group and FGR Subtypes

We initially found that the serum levels of OS9 exhibited significant differences between early and late pregnancy. Therefore, the subsequent research primarily focused on these two periods. FGR was further classified into early-onset and late-onset FGR based on the timing of onset. The results showed that in the first trimester, compared to the control group, there was a decreasing trend in OS9 concentration in both the early-onset FGR and late-onset FGR groups, but no significant difference was observed among the three groups (P = 0.07241) (Figure 3A). In the third trimester, a significant difference was detected (P = 0.002431). The OS9 levels were significantly reduced in both the Early-onset FGR and Late-onset FGR groups, with a more pronounced decline in the Early-onset FGR group (Figure 3B).

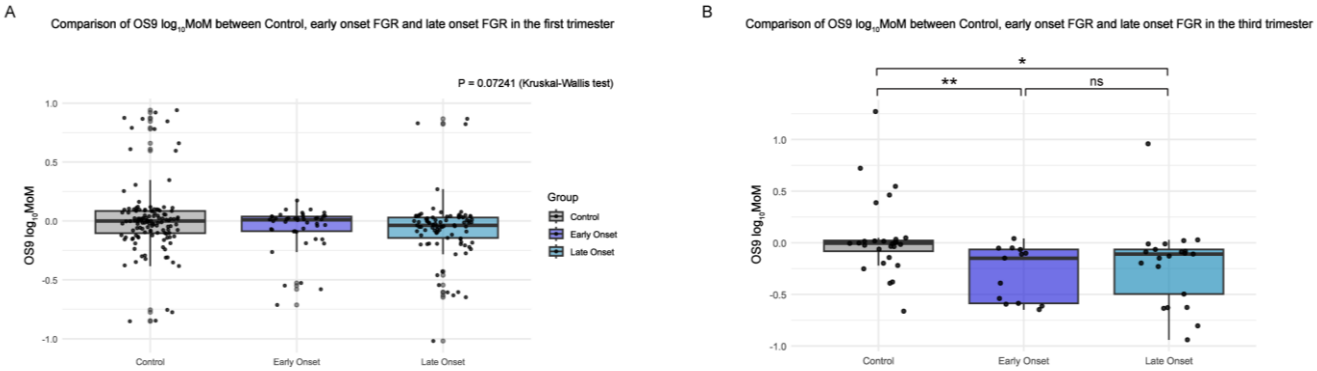

Figure 3. The serum OS9 concentration in different subtypes of FGR. (A) Comparison during the first trimester. Assessed by Kruskal-Wallis test, ns, no significance. (B) Comparison during the third trimester. Assessed by Kruskal-Wallis test, ****P* **< 0.05, *****P* **< 0.01, ns, no significance.

The Predictive Ability of OS9 for FGR and its Subtypes

The ROC curve plays a critical role in the evaluation of biomarkers and disease diagnostic tests [24]. In this study, we employed ROC curve analysis to assess the predictive performance of different variable combinations (single-variable and multivariable) for FGR and its subtypes.

We first predicted FGR. In the first trimester (Figure 4A), OS9 alone yielded an AUC of 0.589. The model incorporating all variables (OS9-Age-BMI-Height) achieved the highest AUC (0.654), outperforming other combinations. For all variables, the sensitivity and specificity were 0.707 and 0.575, respectively (Table 3). In the third trimester (Figure 4B), OS9 alone exhibited improved predictive capability with an AUC of 0.769, accompanied by higher sensitivity (0.818) and specificity (0.741). The OS9-Age model showed the highest AUC (0.775). This model demonstrated a sensitivity of 0.765 and a specificity of 0.778. The data indicated that OS9 had greater predictive value for FGR in the third trimester compared to the first trimester.

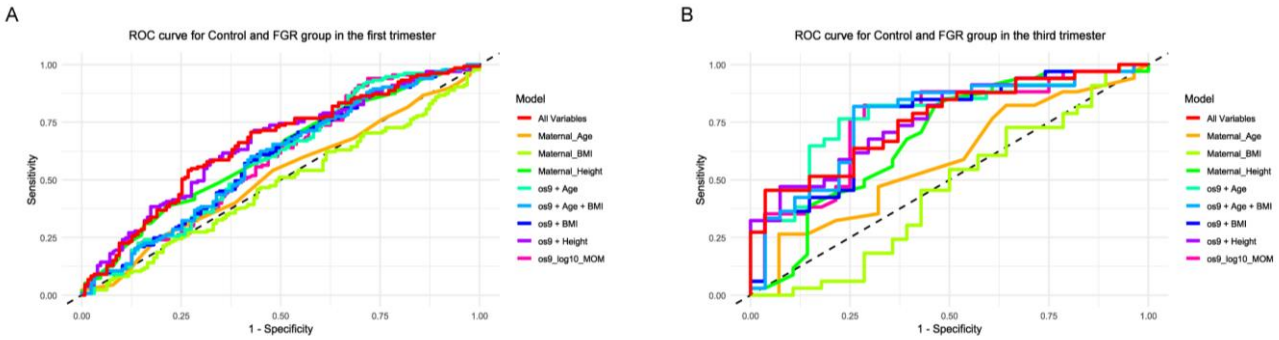

Figure 4. Predictive model for FGR. ROC curves of All Variables, Maternal Age, Maternal BMI, Maternal Height, OS9-Age, OS9-Age-BMI, OS9-BMI, OS9-Height, and OS9 model for the first trimester (A) and the third trimester (B).

Early-onset FGR affects 1%-2% of neonates and is frequently associated with complications such as preeclampsia, abnormal fetal umbilical Doppler findings, fetal hypoxia, and increased perinatal mortality [25]. As shown in Figure 5, we subsequently evaluated the predictive performance of different models for early-onset FGR. In the first trimester, the all variables model achieved the highest AUC (0.656), with sensitivity and specificity of 0.659 and 0.614, respectively (Figure 5A). The AUC for OS9 alone was only 0.466. However, during the third trimester (Figure 5B), the predictive value of OS9 significantly increased, with an AUC of 0.815, sensitivity of 0.923, specificity of 0.741, and NPV of 0.952 (Table 3). When integrated with additional parameters, the all variables model had the highest AUC (0.869), followed by OS9-Age (0.843), OS9-Height (0.838), and OS9-Age-BMI (0.838). These multifactorial combined models demonstrated excellent diagnostic value in late pregnancy, showing significant discriminative advantages.

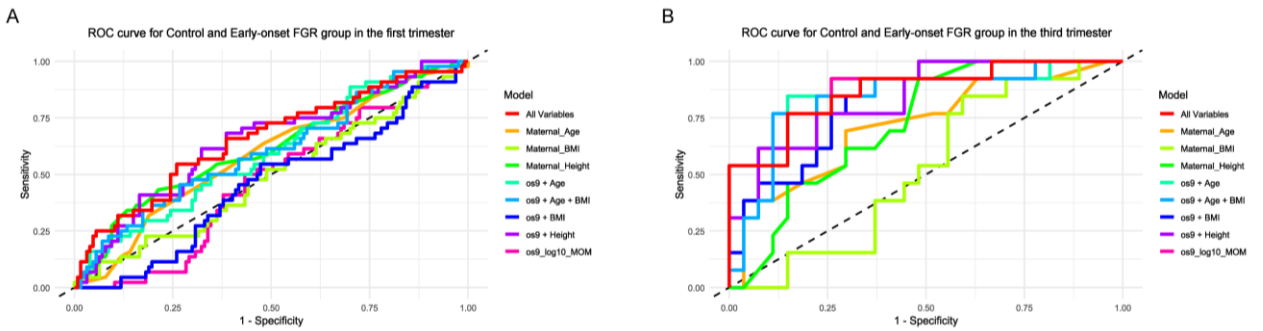

Figure 5. Predictive model for early-onset FGR. ROC curves for the first trimester (A) and the third trimester (B).

Late-onset FGR accounts for 3%-5% of births, although associated with a lower risk of fetal hypoxia compared to early fetal growth failure, has a significant impact on the occurrence of

stillbirth and neonatal morbidity [26]. We finally investigated the diagnostic performance of different models for late-onset FGR. The results showed that in the first trimester, OS9 alone achieved an AUC of 0.591. The All variables model achieved the highest AUC (0.659), with a sensitivity and specificity of 0.697 and 0.598, respectively (Figure 6A). The difficulty in predicting late-onset FGR in early pregnancy suggested the need to incorporate additional biological markers to improve predictive performance. In the third trimester (Figure 6B), OS9 alone showed an AUC of 0.737, with a PPV of 0.714 and an NPV of 0.808 (Table 3). The OS9-BMI and OS9-Age-BMI models performed best, both achieving an AUC of 0.741, with a sensitivity of 0.800 and a specificity of 0.741. Compared to the first trimester, these two models demonstrated a higher detection rate and better predictive performance for late-onset FGR.

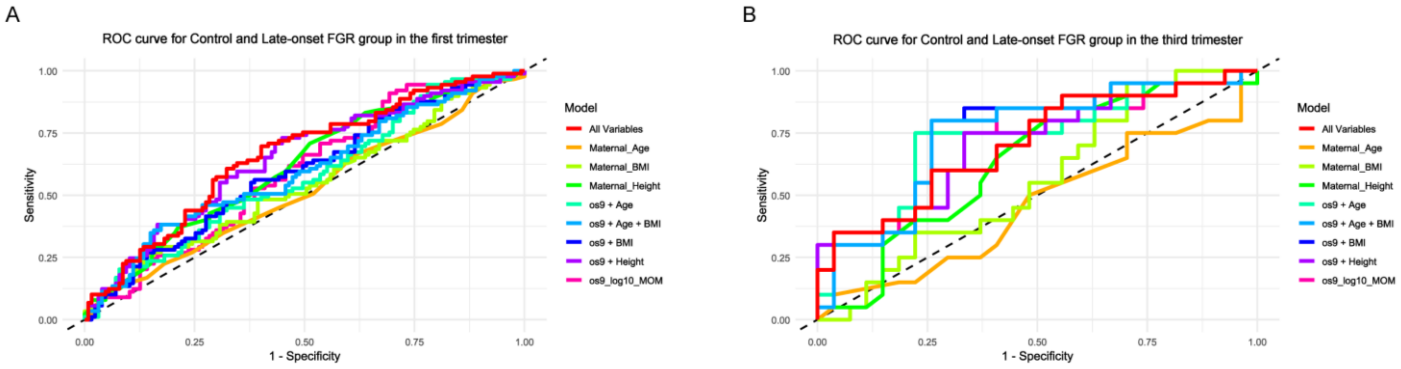

Figure 6. Predictive model for late-onset FGR. ROC curves for the first trimester (A) and the third trimester (B).

Table 3 presented multiple performance metrics of various models for FGR and its subtypes during both the first and third trimesters. The variables included OS9 concentration, maternal age, BMI, and height.

		OS9	Age	BMI	Height	OS9-Age	OS9-BMI	OS9-Height	OS9-Age-BMI	OS9-Age-BMI-Height
Sensitivity	Prediction of FGR in the first trimester	0.602	0.541	0.496	0.677	0.564	0.609	0.707	0.602	0.707
	Prediction of FGR in the third trimester	0.818	0.485	0.697	0.818	0.765	0.818	0.727	0.818	0.758
	Prediction of early-onset FGR in the first trimester	0.500	0.636	0.523	0.545	0.500	0.455	0.682	0.568	0.659
	Prediction of early-onset FGR in the third trimester	0.923	0.692	0.769	0.615	0.846	0.846	0.769	0.769	0.769
	Prediction of late-onset FGR in the first trimester	0.663	0.663	0.483	0.708	0.483	0.562	0.708	0.461	0.697
	Prediction of late-onset FGR in the third trimester	0.750	0.500	0.650	0.700	0.750	0.800	0.750	0.800	0.600
Specificity	Prediction of FGR in the first trimester	0.535	0.520	0.496	0.488	0.591	0.559	0.575	0.575	0.575
	Prediction of FGR in the third trimester	0.741	0.704	0.444	0.519	0.778	0.741	0.667	0.741	0.630
	Prediction of early-onset FGR in the first trimester	0.433	0.520	0.512	0.638	0.654	0.472	0.614	0.583	0.614
	Prediction of early-onset FGR in the third trimester	0.741	0.704	0.444	0.704	0.852	0.704	0.778	0.889	0.852
	Prediction of late-onset FGR in the first trimester	0.504	0.386	0.606	0.488	0.638	0.614	0.567	0.732	0.598

	Prediction of late-onset FGR in the third trimester	0.778	0.481	0.444	0.556	0.778	0.741	0.667	0.741	0.741
AUC	Prediction of FGR in the first trimester	0.589	0.516	0.468	0.615	0.591	0.589	0.651	0.589	0.654
	Prediction of FGR in the third trimester	0.769	0.609	0.548	0.668	0.775	0.758	0.758	0.767	0.760
	Prediction of early-onset FGR in the first trimester	0.466	0.585	0.490	0.615	0.591	0.456	0.645	0.602	0.656
	Prediction of early-onset FGR in the third trimester	0.815	0.719	0.524	0.724	0.843	0.812	0.838	0.838	0.869
	Prediction of late-onset FGR in the first trimester	0.591	0.518	0.542	0.615	0.574	0.593	0.647	0.597	0.659
	Prediction of late-onset FGR in the third trimester	0.737	0.463	0.563	0.632	0.722	0.741	0.704	0.741	0.707
95% CI	Prediction of FGR in the first trimester	0.520 - 0.659	0.445 - 0.586	0.398 - 0.539	0.547 - 0.683	0.522 - 0.661	0.520 - 0.659	0.585 - 0.718	0.520 - 0.659	0.587 - 0.720
	Prediction of FGR in the third trimester	0.643 - 0.895	0.465 - 0.753	0.393 - 0.703	0.524 - 0.812	0.651 - 0.898	0.630 - 0.885	0.637 - 0.879	0.640 - 0.893	0.639 - 0.880
	Prediction of early-onset FGR in the first trimester	0.373 - 0.559	0.487 - 0.682	0.390 - 0.590	0.515 - 0.714	0.495 - 0.687	0.359 - 0.552	0.550 - 0.741	0.503 - 0.700	0.559 - 0.753
	Prediction of early-onset FGR in the third trimester	0.669 - 0.960	0.543 - 0.895	0.342 - 0.707	0.567 - 0.880	0.700 - 0.987	0.674 - 0.950	0.710 - 0.965	0.696 - 0.979	0.746 - 0.992
	Prediction of late-onset FGR in the first trimester	0.516 - 0.667	0.439 - 0.597	0.463 - 0.621	0.540 - 0.691	0.496 - 0.651	0.516 - 0.669	0.572 - 0.721	0.519 - 0.674	0.586 - 0.733
	Prediction of late-onset FGR in the third trimester	0.586 - 0.888	0.291 - 0.635	0.396 - 0.730	0.469 - 0.796	0.568 - 0.877	0.591 - 0.890	0.548 - 0.859	0.591 - 0.890	0.554 - 0.860
P value	Prediction of FGR in the first trimester	< 0.05	> 0.05	> 0.05	< 0.001	< 0.05	< 0.05	< 0.001	< 0.05	< 0.001
	Prediction of FGR in the third trimester	< 0.001	> 0.05	> 0.05	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Prediction of early-onset FGR in the first trimester	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05	> 0.05	< 0.01	< 0.05	< 0.01
	Prediction of early-onset FGR in the third trimester	< 0.001	< 0.05	> 0.05	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Prediction of late-onset FGR in the first trimester	< 0.05	> 0.05	> 0.05	< 0.01	> 0.05	< 0.05	< 0.001	< 0.05	< 0.001
	Prediction of late-onset FGR in the third trimester	< 0.01	> 0.05	> 0.05	> 0.05	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01
PPV	Prediction of FGR in the first trimester	0.576	0.541	0.508	0.581	0.591	0.591	0.635	0.597	0.635
	Prediction of FGR in the third trimester	0.794	0.667	0.605	0.675	0.813	0.794	0.727	0.794	0.714
	Prediction of early-onset FGR in the first trimester	0.234	0.315	0.271	0.343	0.333	0.230	0.380	0.321	0.372
	Prediction of early-onset FGR in the third trimester	0.632	0.529	0.400	0.500	0.733	0.579	0.625	0.769	0.714
	Prediction of late-onset FGR in the first trimester	0.484	0.431	0.462	0.492	0.483	0.505	0.534	0.547	0.549
	Prediction of late-onset FGR in the third	0.714	0.417	0.464	0.538	0.714	0.696	0.625	0.696	0.632

	trimester									
NPV	Prediction of FGR in the first trimester	0.562	0.520	0.485	0.590	0.564	0.577	0.652	0.579	0.652
	Prediction of FGR in the third trimester	0.769	0.528	0.545	0.700	0.724	0.769	0.667	0.769	0.680
	Prediction of early-onset FGR in the first trimester	0.714	0.805	0.756	0.802	0.790	0.714	0.848	0.796	0.839
	Prediction of early-onset FGR in the third trimester	0.952	0.826	0.800	0.792	0.920	0.905	0.875	0.889	0.885
	Prediction of late-onset FGR in the first trimester	0.681	0.620	0.626	0.705	0.638	0.667	0.735	0.660	0.738
	Prediction of late-onset FGR in the third trimester	0.808	0.565	0.632	0.714	0.808	0.833	0.783	0.833	0.714
Accuracy	Prediction of FGR in the first trimester	0.569	0.531	0.496	0.585	0.577	0.585	0.642	0.588	0.642
	Prediction of FGR in the third trimester	0.783	0.583	0.583	0.683	0.771	0.783	0.700	0.783	0.700
	Prediction of early-onset FGR in the first trimester	0.450	0.550	0.515	0.614	0.614	0.468	0.632	0.579	0.626
	Prediction of early-onset FGR in the third trimester	0.800	0.700	0.550	0.675	0.850	0.750	0.775	0.850	0.825
	Prediction of late-onset FGR in the first trimester	0.569	0.500	0.556	0.579	0.574	0.593	0.625	0.620	0.639
	Prediction of late-onset FGR in the third trimester	0.766	0.489	0.532	0.617	0.766	0.766	0.702	0.766	0.681
OAPR	Prediction of FGR in the first trimester	1.356	1.180	1.031	1.385	1.442	1.446	1.741	1.481	1.741
	Prediction of FGR in the third trimester	3.857	2.000	1.533	2.077	4.333	3.857	2.667	3.857	2.500
	Prediction of early-onset FGR in the first trimester	0.306	0.459	0.371	0.522	0.500	0.299	0.612	0.472	0.592
	Prediction of early-onset FGR in the third trimester	1.714	1.125	0.667	1.000	2.750	1.375	1.667	3.333	2.500
	Prediction of late-onset FGR in the first trimester	0.937	0.756	0.860	0.969	0.935	1.020	1.145	1.206	1.216
	Prediction of late-onset FGR in the third trimester	2.500	0.714	0.867	1.167	2.500	2.286	1.667	2.286	1.714
DR	Prediction of FGR in the first trimester	0.535	0.512	0.500	0.596	0.488	0.527	0.569	0.515	0.569
	Prediction of FGR in the third trimester	0.567	0.400	0.633	0.667	0.525	0.567	0.550	0.567	0.583
	Prediction of early-onset FGR in the first trimester	0.550	0.520	0.497	0.409	0.386	0.509	0.462	0.456	0.456
	Prediction of early-onset FGR in the third trimester	0.475	0.425	0.625	0.400	0.375	0.475	0.400	0.325	0.350
	Prediction of late-onset FGR in the first trimester	0.565	0.634	0.431	0.593	0.412	0.458	0.546	0.347	0.523
	Prediction of late-onset FGR in the third trimester	0.447	0.511	0.596	0.553	0.447	0.489	0.511	0.489	0.404

With $P < 0.05$ considered statistically significant.

AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; OAPR, odds of being affected given a positive result; DR, detection

rate.

Discussion

Main findings

FGR is a severe perinatal disorder that significantly impacts the health of pregnant women and newborns. Therefore, prenatal screening and early identification of FGR are particularly crucial. In this study, we first observed that OS9 levels in FGR pregnancies were slightly elevated in early pregnancy but gradually decreased with gestational age after 15 weeks, which differed from the fluctuating pattern of OS9 levels in normal pregnancies. During both the first and third trimesters, OS9 levels in the FGR group were significantly lower than those in the control group, suggesting that OS9 may serve as a potential biomarker for predicting FGR. Further analysis revealed that OS9 alone had a certain predictive value for FGR in both the first and third trimesters. However, when FGR was classified into different subtypes, OS9 and multivariable models exhibited enhanced predictive efficacy for early-onset and late-onset FGR. Notably, compared to the first trimester, OS9 alone demonstrated higher AUCs for FGR and its subtypes in the third trimester (FGR: first trimester 0.589 vs. third trimester 0.769; early-onset FGR: first trimester 0.466 vs. third trimester 0.815; late-onset FGR: first trimester 0.591 vs. third trimester 0.737). In the first trimester, OS9 and combined models showed the best performance in predicting late-onset FGR, while in the third trimester, they performed best for early-onset FGR. At both gestational periods, the all variables model (OS9-Age-BMI-Height) achieved the highest AUC. These findings may open a new perspective for the prediction of FGR.

Findings in the Context of the Existing Literature

According to previous studies, FGR prediction models are primarily based on clinical evaluation, Doppler ultrasound and biomarkers [1]. Among these, maternal serum biomarkers are one of the most obvious ways to identify FGR, mainly including the measurements of placenta-related proteins and circulating factors, such as PAPP-A and the sFlt-1/PIGF ratio [27, 28]. Abnormal expression of these markers often indicates placental dysfunction, which is highly associated with the occurrence of FGR. Although these methods have improved the predictive ability of FGR to a certain extent, their sensitivity and specificity remain suboptimal, making them unsuitable for clinical recommendation. Therefore, the search for new biomarkers for the diagnosis and treatment of FGR remains critically important.

The dynamics of placental and fetal growth are intimately interrelated. Placental dysfunction is the most common cause of FGR, as evidenced by insufficient angiogenesis of the placenta, abnormal trophoblast cell invasion and differentiation, destruction of villous structures, and decreased ability to transport nutrients and oxygen [29-31]. When the placenta is in a hypoxic or nutrient-depleted environment, the burden of protein folding in the ER increases, and unfolded or misfolded proteins gradually accumulate, leading to ER stress [32, 33]. At the same time, hypoxia or placental ischemia-reperfusion induces excessive production of reactive oxygen species (ROS), which disrupts the redox balance of the ER lumen and further aggravates the stress response [34]. In addition, placental dysfunction such as trophoblast cell invasion and insufficient angiogenesis in the villous spaces, reduces placental blood perfusion and nutrient transport capacity, thereby enhancing the persistent state of oxidative stress [32, 35]. This vicious cycle of ER oxidative stress severely impairs the normal development of the fetus and is a key pathological mechanism of FGR and maternal complications such as preeclampsia.

Based on the relationship between ER stress and FGR, changes in the levels of ER stress-related molecules may serve as potential biomarkers for the early diagnosis of FGR or preeclampsia. OS9 is a glycan-binding chaperone protein in the ER. By associating with the ubiquitin ligase complex on the ER membrane and glycoprotein substrates, OS9 directs misfolded proteins to the proteasome for degradation, thereby maintaining endoplasmic reticulum homeostasis [36]. OS9 is closely related to ER stress. Previous studies have shown that deletion of Sel1L in mice leads to pancreatic ER stress and activation of the unfolded protein response (UPR) pathway. During this process, OS9 protein levels are significantly elevated, enhancing ERAD capacity to relieve the stress burden [37]. However, silencing of OS9 expression delays the delivery of misfolded polypeptides to

the proteasome for degradation, exacerbates ER stress, and ultimately induces apoptosis [38]. Previous studies have not reported an association between OS9 and pregnancy complications. Our results showed that serum OS9 levels were significantly reduced in FGR pregnancies during the first and third trimesters. The downregulation of OS9 expression may impair the function of the ERAD pathway and lead to the accumulation of ROS within the ER reticulum, thereby causing dysfunction in placental trophoblast cells [38-41]. The placenta serves as a bridge between the mother and the developing fetus. The first trimester is a critical period for the establishment of the placenta and the maternal-fetal interface, during which trophoblast cells play a particularly important role [42-44]. The placenta undergoes aging as pregnancy progresses [45]. Once senescence is prematurely triggered by stress factors, it may become pathological, as seen in conditions such as preeclampsia and FGR [46, 47]. FGR pregnancies are characterized by increased trophoblast cells apoptosis and impaired proliferation [48, 49]. This may explain the significant changes in OS9 levels in maternal serum during the first and third trimesters in FGR cases.

We utilized OS9 concentration in combination with other factors to predict FGR and its subtypes, and the results indicated that the predictive performance of the model varied depending on the combination of variables used. OS9 exhibited stronger predictive performance for FGR and its subtypes in the third trimester compared to the first trimester. This difference might have been attributed to more pronounced changes in placental function and maternal cardiovascular dynamics in late pregnancy [50]. Under endoplasmic reticulum stress, abnormal OS9 expression was more readily detectable in serum. OS9 and combined models showed the best performance in predicting late-onset FGR in the first trimester, while their performance was better for early-onset FGR in the third trimester. This may be attributed to the heterogeneity of FGR subtypes. In the first trimester, OS9 levels may reflect impaired placental development, which later manifests as late-onset FGR as placental function progressively declines. In the third trimester, OS9 may be directly involved in acute placental hypoxia and closely associated with early-onset FGR [51].

The morbidity of neonates in FGR cases remains challenging to accurately predict prenatally. The most widely discussed parameter is the sFlt-1/PIGF ratio. A recent study reported that the sFlt-1/PIGF ratio had an overall sensitivity of 0.63, a specificity of 0.84, and an AUC of 0.8354 for predicting FGR at 19 weeks of gestation [52]. It was considered a useful parameter in the diagnosis and prognostic assessment of FGR [28, 53]. In the third trimester (27^{+0} weeks), the predictive performance of PIGF alone for early-onset FGR demonstrated an AUC of 0.661 (95% CI: 0.593 - 0.730), with a PPV of only 0.31 [54]. However, when combined with umbilical artery Doppler parameter, the AUC increased to 0.89 (95% CI: 0.83 - 0.94) [55]. Our findings showed that OS9 alone had a certain predictive value for FGR. However, the predictive performance improved when it was combined with multiple maternal indicators. In the third trimester, OS9 alone demonstrated an AUC of 0.815 for early-onset FGR, with high specificity (0.923), outperforming PIGF in predictive performance. After combining maternal age, height, and BMI, the model achieved the highest AUC of 0.869. For late-onset FGR, the study by Tonyali *et al*. reported that the delta neutrophil index (DNI) had an AUC of 0.677 (95% CI: 0.642 - 0.711) in predicting late-onset FGR during the third trimester, with a sensitivity of 0.7841 and a specificity of 0.5297 [56]. In our study, OS9 alone in the third trimester yielded an AUC of 0.737 and an NPV of 0.808. The predictive value further improved after combining maternal age and BMI. Therefore, we considered that integrating multiple biomarkers and clinical parameters provided a more comprehensive assessment of placental function and fetal growth potential, thereby enhancing the sensitivity and specificity of FGR prediction.

Sensitivity refers to the probability of testing positive among individuals with the disease. A high sensitivity indicates fewer false-negative results, whereas low sensitivity may lead to erroneous reassurance [57]. Our results showed that in the third trimester, OS9 alone exhibited sensitivities of 0.818 and 0.923 for predicting FGR and early-onset FGR, respectively. The use of OS9 as a predictive marker substantially reduced the rate of missed diagnoses. AUC is an effective metric for summarizing the overall diagnostic accuracy of a model. Its value ranges from 0 to 1, with AUC values between 0.8 and 0.9 generally considered excellent and those

exceeding 0.9 classified as outstanding [58]. The measured AUC of OS9 for early-onset FGR in the third trimester was 0.815, indicating its robust predictive performance and potential applicability in clinical diagnostics. NPV refers to the proportion of individuals with a negative result who don't have the disease [59]. In the third trimester, OS9 alone yielded NPVs of 0.952 and 0.808 for early-onset and late-onset FGR, respectively, reflecting its high true-negative rate and low false-positive rate in subtypes prediction during the third trimester.

Strengths and Limitations

The OS9 we focused on had been rarely reported in terms of its predictive utility and underlying pathophysiological mechanisms in placenta-mediated pregnancy complications. This was the first study to report the predictive value of OS9 for FGR in a Chinese population. Our study has several strengths. A significant proportion of the pregnant women recruited for the experiment completed the study and donated blood samples during the first, second and third trimesters. This allowed us to clearly observe the changes in OS9 concentrations throughout pregnancy in both the FGR and control groups, and to evaluate the predictive value of OS9 for FGR at different time points. We individually excluded the influence of maternal and neonatal clinical data confounding variables on OS9 concentrations. The protein biomarkers examined in this study were analyzed only after the collection of outcomes, thus their levels had no influence on pregnancy management. However, our study has certain limitations. OS9 expression levels were not detected in the placenta. This was a historical cohort study, and some data from the centers were incomplete, such as clinical information including Apgar scores and neonatal intensive care unit records.

Conclusions

This study demonstrated that decreased maternal serum OS9 levels were closely associated with an elevated risk of FGR, with this correlation being particularly pronounced during both the first and third trimesters of pregnancy. In terms of prediction, OS9 alone showed a certain performance in predicting FGR and its subtypes. However, a multivariable model combining OS9 with maternal characteristics significantly improved diagnostic accuracy. The optimal prediction time for OS9 and combined models was in the third trimester. In the first trimester, OS9 and combined models showed the better predictive performance for late-onset FGR, whereas in the third trimester, the all variables model achieved the highest AUC (0.869) for predicting early-onset FGR. Our findings indicated that OS9 may serve as a novel parameter for predicting FGR and its subtypes, potentially helping to mitigate adverse outcomes associated with FGR.

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Author Contributions

Wei Li: Conceptualization, funding acquisition, methodology, resources, writing-original draft, writing-review and editing. Benli Gong: Resources. Jianli Li: Resources. Youdan Hu: Methodology, data curation. Yuchen Chen: Supervision, project administration. Sifan Zeng: Project administration. Zhaoyi Guo: Resources. Zhuohuan Cai: Resources.

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Statement

Research ethics approvals were granted at all centers (approval reference number NSFYEC-KY-2023015) and conducted in accordance with the Declaration of Helsinki. All participants provided informed consent prior to inclusion in the study.

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