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Values of hemodynamic changes of fetal vessels evaluated by color Doppler ultrasound for fetuses with growth restriction.

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Key words: color; Doppler ultrasound; fetal growth; growth restriction; restriction.

Abstract. We aimed to explore the values of hemodynamic changes of fetal vessels evaluated by color Doppler ultrasound (CDUS) for fetal growth restriction (FGR). A retrospective analysis was performed on clinical data of 75 pregnant women who received prenatal examination from January 2021 to August 2023 and whose fetuses were diagnosed with FGR (FGR group) and 75 pregnant women whose fetuses were healthy in the same period and were considered as the healthy group. CDUS was performed on the fetuses. The values of indicators of umbilical artery, middle cerebral artery and aortic arch isthmus for assessing pregnancy outcomes were investigated. The FGR group had significantly lowered arterial resistance index (RI), blood flow pulsatility index (PI), and systolic and diastolic velocity (S/D) levels of the middle cerebral artery and peak systolic velocity (PSV)/end-systolic reflux velocity (ESRV) level of aortic arch isthmus but significantly elevated RI, PI, and S/D levels of umbilical artery in comparison with those of the healthy group (p<0.05). The areas under the receiver operating characteristic curves (AUCs) of RI, PI, and S/D of the umbilical artery in diagnosing FGR were 0.893, 0.893 and 0.900, respectively, AUCs of RI, PI, and S/D of the middle cerebral artery were 0.812, 0.874 and 0.910, respectively, and AUC of PSV/ESRV was 0.857 (p<0.05). The incidence rate of severe hypoxia was significantly higher in the fetuses with a more significant RI value of the middle cerebral artery and a larger PSV/ESRV value than those with a smaller RI value of the middle cerebral artery and a smaller PSV/ESRV value (p < 0.05). The changes in umbilical artery RI, middle cerebral artery RI, and PSV/ESRV were unrelated to fetal survival rate (p>0.05). Fetal umbilical artery, middle cerebral artery and aortic arch isthmus parameters detected through CDUS are all sensitive indices for assessing FGR.

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Valores de los cambios hemodinámicos de los vasos fetales evaluados mediante ecografía Doppler a color en fetos con restricción del crecimiento.

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Palabras clave: color; ecografía Doppler; crecimiento fetal; parámetro; restricción de crecimiento, flujo sanguíneo arterial.

Resumen. Nuestro objetivo fue explorar los valores de los cambios hemodinámicos de los vasos fetales evaluados mediante ecografía Doppler a color (CDUS) en la restricción del crecimiento fetal (FGR). Se realizó un análisis retrospectivo de los datos clínicos de 75 mujeres embarazadas que recibieron un examen prenatal desde enero de 2021 hasta agosto de 2023 y cuyos fetos fueron diagnosticados con FGR (grupo FGR) y 75 mujeres embarazadas cuyos fetos estaban sanos en el mismo período y se consideraron como el grupo sano. Se realizó CDUS en los fetos. Se investigaron los valores de los indicadores de la arteria umbilical, la arteria cerebral media y el istmo del arco aórtico para evaluar los resultados del embarazo. El grupo FGR tuvo un índice de resistencia arterial (IR), un índice de pulsatilidad del flujo sanguíneo (IP) y niveles de velocidad sistólica y diastólica (S/D) significativamente reducidos de la arteria cerebral media y un nivel de velocidad sistólica máxima (PSV)/velocidad de reflujo telesistólico (ESRV) del istmo del arco aórtico, pero niveles significativamente elevados de IR, IP y S/D de la arteria umbilical en comparación con los del grupo sano (p<0,05). Las áreas bajo las curvas de características operativas del receptor (AUC) de RI, PI y S/D de la arteria umbilical en el diagnóstico de FGR fueron 0,893, 0,893 y 0,900, respectivamente, las AUC de RI, PI y S/D de la arteria cerebral media fueron 0,812, 0,874 y 0,910, respectivamente, y el AUC de PSV/ESRV fue 0.857 (p<0.05). La tasa de incidencia de hipoxia grave fue significativamente mayor en los fetos con un valor de RI más significativo de la arteria cerebral media y un valor de PSV/ESRV mayor que aquellos con un valor de RI menor de la arteria cerebral media y un valor de PSV/ESRV menor (p < 0.05). Los cambios en el RI de la arteria umbilical, el RI de la arteria cerebral media y el PSV/ESRV no se relacionaron con la tasa de supervivencia fetal (p>0.05). Los parámetros de la arteria umbilical fetal, la arteria cerebral media y el istmo del arco aórtico detectados mediante ecografía endoscópica son todos índices sensibles para evaluar la RCF.

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INTRODUCTION

Fetal growth restriction (FGR), a pregnancy complication, refers to the inability of fetuses to achieve their genetic growth potential, which has such characteristics as pathological lag of intrauterine growth rate in an intrauterine growth curve. FGR is also one of the common perinatal complications, accounting for about 30% of perinatal deaths, and is detected in 50% of perinatal infants with intrauterine hypoxia during delivery, which is the second leading cause of perinatal deaths 1,2. FGR has an association with various adverse perinatal outcomes, such as stillbirth, neonatal death, and neonatal diseases (intraventricular hemorrhage, neonatal hyperbilirubinemia, and hypoglycemia, among others.), probably having negative impacts on the neurobehavioral development of affected children in the long term and increasing the risk of such diseases as obesity, diabetes, and cardiovascular and cerebrovascular diseases in such children. The pathogenesis of FGR remains unclear, but previous studies have manifested that maternal nutrition, placental transfer, fetal inheritance and other relevant factors are implicated in the development of FGR. However, given that it is hard to diagnose FGR in the first trimester of pregnancy, FGR is usually diagnosed after delivery or in late gestation, which further highlights the importance of accurate ultrasound examination during the first trimester of pregnancy to assess fetal growth indicators dynamically. CDUS is a common imaging examination approach in clinical practice, characterized by good safety, non-invasion, simple operation and free-radiation. At 18-22 weeks of pregnancy, most morphological and structural abnormalities of fetuses can be screened out through ultrasound. As one of the crucial parameters in human physiological evaluation, arterial blood flow can illustrate the benefits of fetal metabolism, and blood flow velocity distribution is of great significance in clinical measurement ³. A study also denoted a close relationship between the pathological change of FGR and abnormal changes in uterine-placental-fetal blood circulation.⁴ For this reason, early assessment of blood flow changes in the umbilical artery, middle cerebral artery and fetal heart is conducive to early diagnosis and early intervention of FGR, which is significant for improving fetal prognosis.

In this study, 75 pregnant women who received prenatal examination and whose fetuses were diagnosed with FGR in our hospital from January 2021 to August 2023 were enrolled as subjects to analyze the value of relevant intraabdominal fetal parameters detected by CDUS in assessing FGR.

PATIENTS AND METHODS

General data

Seventy-five pregnant women with FGR fetuses receiving prenatal examinations in our hospital from January 2021 to August 2023 were selected as observation subjects (FGR group). The inclusion criteria were set as follows: 1) Pregnant women whose fetuses met the diagnostic criteria for FGR,⁵ 2) those who were singleton, naturally conceived and in the third trimester of pregnancy, 3) those whose fetuses had no response to fetal heart monitoring, 4) those with decreased fetal movement, 5) those who were healthy in the past, without history of genetic diseases, and 6) those with complete clinical data and no data loss. The exclusion criteria involved 1) pregnant women whose fetuses had structural malformations and chromosome abnormalities before delivery based on ultrasound examination, 2) those whose fetuses were complicated by severe congenital diseases, 3) those whose fetuses suffered from endogenous homologous FGR induced by fetal chromosomal abnormalities, 4) those with abnormities or spiral edema in umbilical cord insertion point, or single umbilical artery, 5) those complicated by prenatal complications or comorbidities, 6) those with placental morphological changes, including choriocarcinoma or other lesions, or 7) those with nervous system or mental disease. Meanwhile, 75 pregnant women with healthy fetuses undergoing prenatal examination in our hospital in the same period were selected as the healthy group. Pregnant women in the FGR group (n=75) were aged 22-35 years old, with an average of (28.85 ± 5.85) years old, and had a gestational age of 33-43 weeks, with a mean of (37.45 ± 4.15) weeks. In terms of parity, there were 46 primiparas and 29 multiparas. In the healthy group (n=75), pregnant women were aged 23-35 years old, with an average of 28.45 ± 5.85 years old, and had a gestational age of 35.42 weeks, with a mean of 37.85 ± 6.18 weeks. As to parity, there were 42 primiparas and 33 multiparas. No statistically significant differences between the two groups were found in age, parity and gestational age (p>0.05). This study was conducted with approval from the hospital's Ethics Committee.

Examination methods

Routine prenatal ultrasound was measured using a real-time ultrasound imager with a linear or convex array probe (abdominal probe frequency of 3.0-3.5 MHz, with 5.0 MHz for thin pregnant women) as follows. After exposing the abdomen of pregnant women, couplant was smeared on the examination area, and the probe was placed on the area to observe whether there was any abnormality in fetal position, placental position and uterine appendages. Besides, a series of inspections were carried out on the fetuses from head to toe, and biological measurements were made on various standard cross sections, including biparietal diameter, head circumference, abdominal circumference, and femur length. In addition, the gestational age was predicted, fetal weight was estimated, and fetal position and fetal number were judged. Additionally, amniotic fluid and the position and maturity of the placenta were observed, and the depth of amniotic fluid and the thickness and area of the placenta were measured. Moreover, observations were made on physiological phenomena such as the fetal heart, fetal movement, and fetal swallowing. In our hospital, suspected FGR would be determined if the fetal weight assessed by ultrasound was less than the 10th percentile of the average weight of normal fetuses of the same gestational age or two standard deviations below the average weight.

CDUS was conducted with the DC-35Pro diagnostic system (Mindray Medical Equipment Co., Ltd., China). In brief, pregnant women were guided to lie in the supine position, and the fetal weight and head circumference were routinely measured. Then, the probe was placed vertically in the lower abdomen and tilted to one side to find and display the external iliac artery by the parasagittal section. Next, CDUS was employed to identify the uterine artery crossing the external iliac artery. Thereafter, considering that the uterine artery extends along one side of the uterus to the fundus of the uterus, the scanning direction of the probe was adjusted so that the main uterine artery was parallel to the sound beam as much as possible. Next, the spectrum Doppler sampling gate was placed on the main uterine artery 1 cm below the intersection point for measurement. After that, the contralateral uterine artery was measured in the same way. Thereafter, the middle cerebral artery was examined as follows. At the standard biparietal diameter section (the brain midline was perpendicular to the sound beam as far as possible), the probe was moved in parallel to the fetal skull base until a pair of great wings of sphenoid bone appeared between the anterior cranial fossa and the middle cranial fossa. The middle cerebral artery starts from the left and right sides of the middle part of the arterial ring, goes to both sides of the brain and slightly deviates to the forehead. The sampling volume was set to 2-3 mm, the probe was placed at the point 3-5 mm away from the starting point of the Willis arterial ring, and the Doppler angle was adjusted as close as possible to 0° (not more than 30°), and more than three continuous and stable pulse Doppler waveforms were obtained. Finally, the arterial resistance index (RI), blood flow pulsatility index (PI), and systolic/diastolic velocity (S/D) levels of the middle cerebral artery were measured.

Afterwards, the measurement of the umbilical artery was performed. In brief, the middle part of the umbilical cord floating in amniotic fluid was selected for examination, and the angle between the Doppler sound beam and umbilical blood vessel should be less than 30° to obtain the Doppler spectrum of the umbilical artery. After at least three continuous and stable waveforms appeared, the image was frozen to measure the RI, PI, and S/D levels. Afterwards, the three-vessel and trachea section of the fetal heart was found to measure the peak systolic velocity (PSV) and end-systolic reflux velocity (ESRV) levels of aortic arch isthmus, followed by calculation of PSV/ESRV.

Observation of indicators

The changes in RI, PI, and S/D levels of the fetal middle cerebral artery and umbilical artery and the PSV/ESRV level of fetal aortic arch isthmus were compared between the two groups.

Pregnancy outcomes were assessed. In brief, pregnancy outcomes were observed, and abnormal pregnancy outcomes, namely severe hypoxia (Apgar score ≤ 3 points after birth, stillbirth, neonatal death, and presence of hypoxic complications including cerebral palsy) and mild hypoxia (3 points < Apgar score ≤ 7 points, small for gestational age, relieving of hypoxia symptoms after birth, and absence of complications), were recorded.

Statistical analysis

The SPSS 20.0 software was employed for statistical analysis. Measurement data were expressed by ($\bar{\mathbf{x}} \pm$ SD) and subjected to the *t*-test. Count data were expressed by % and subjected to the χ^2 test. The receiver operating characteristics (ROC) curves were plotted to assess the diagnostic value of ultrasound parameters in FGR, p<0.05 suggested that the difference was statistically significant.

RESULTS

Middle cerebral artery parameters

The RI, PI, and S/D levels of the middle cerebral artery were obviously lower in the FGR group than those in the healthy group (p<0.05) (Table 1).

Table 1Middle cerebral artery parameters.

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Creare	n	Middle cerebral artery					
Group		RI	PI	S/D			
Healthy	75	0.72 ± 0.16	1.60 ± 0.37	4.56 ± 0.59			
FGR	75	0.51 ± 0.12	1.33 ± 0.41	3.26 ± 0.57			
t		9.093	4.234	13.724			
р		< 0.001	< 0.001	< 0.001			

Measurement data were expressed by $(\bar{\mathbf{x}} \pm \text{SD})$ and subjected to the *t*-test. FGR: Fetal growth restriction; PI: pulsatility index; RI: resistance index; S/D: systolic/diastolic velocity.

Umbilical artery indicators

The RI, PI, and S/D levels of umbilical artery were significantly higher in the FGR group than those in the healthy group (p<0.05) (Table 2).

Table 2	
Umbilical artery parameter	S.

C		Umbilical artery					
Group	n	RI	PI	S/D			
Health	75	0.56 ± 0.12	0.75 ± 0.16	2.01 ± 0.34			
FGR	75	0.87 ± 0.24	1.19 ± 0.48	2.89 ± 0.36			
t		10.005	7.531	15.391			
р		< 0.001	< 0.001	< 0.001			

Measurement data were expressed by $(\bar{\mathbf{x}} \pm \text{SD})$ and subjected to the *t*-test. FGR: Fetal growth restriction; PI: pulsatility index; RI: resistance index; S/D: systolic/diastolic velocity.

Aortic arch isthmus indicators

The PSV/ESRV level of the aortic arch isthmus was markedly lower in the FGR group than in the healthy group $[(2.86\pm0.62) vs. (3.85\pm0.78)]$ (*t*=8.605, p<0.05).

Diagnostic value of umbilical artery, middle cerebral artery and aortic arch isthmus indicators in FGR

As shown in ROC curves, the area under the ROC curves (AUCs) of RI, PI, and S/D of the umbilical artery in diagnosing FGR were 0.893, 0.893 and 0.900, respectively (p<0.05). The AUCs of RI, PI, and S/D of middle cerebral artery in diagnosing FGR were 0.812, 0.874 and 0.910, respectively (p < 0.05). The AUC of PSV/ESRV of aortic arch isthmus in diagnosing FGR was 0.857 (p < 0.05) (Table 3 and Fig. 1).

Evaluation of pregnancy outcomes based on umbilical artery, middle cerebral artery and aortic arch isthmus indicators

Combined with the ability to assess pregnancy outcomes by simply comparing blood flow parameters in clinical practice, ROC curves were adopted for the analysis of pregnancy outcomes based on the RI values of the umbilical artery and middle cerebral artery and the cutoff value of PSV/ESRV. It was found that the incidence rate of severe hypoxia was higher in fetuses with a more significant RI value of the middle cerebral artery and a larger PSV/ESRV value than those with a smaller RI value of the middle cerebral artery and a smaller PSV/ESRV value (p<0.05) (Table 4).

Table 3							
Diagnostic value of umbilical artery,	, middle cerebral artery and	l aortic arch isthmus i	indicators in FGR.				

Indi	cator	Area under the curve	95% confidence interval	р	Sensitivity	Specificity	Cut-off	Youden index
Umbilical	RI	0.893	0.839~0.946	< 0.001	76.00	86.00	0.66	0.720
artery	PI	0.893	0.843~0.942	< 0.001	93.33	70.67	1.02	0.640
	S/D	0.900	0.850~0.950	< 0.001	86.67	81.33	2.76	0.680
Middle cerebral artery	RI	0.812	0.735~0.890	< 0.001	76.00	86.67	0.69	0.627
	PI	0.874	0.815~0.933	< 0.001	74.67	88.00	1.38	0.627
	S/D	0.910	0.857~0.963	< 0.001	84.00	89.33	3.38	0.733
Aortic arch isthmus	PSV/ESRV	0.857	0.797~0.917	< 0.001	84.00	73.33	3.36	0.573

ESRV: End systolic reflux velocity; PI: pulsatility index; PSV: peak systolic velocity; RI: resistance index; S/D: systolic/diastolic velocity.



Fig. 1. ROC curves of the umbilical artery, middle cerebral artery and aortic arch isthmus are indicators of FGR. A: Diagnostic value of umbilical artery in FGR. B: Diagnostic value of middle cerebral artery in FGR. C: Diagnostic value of aortic arch isthmus in FGR. AUC: Area under the curve; FGR: fetal growth restriction; PI: pulsatility index; ROC: receiver operator characteristic; RI: resistance index; S/D: systolic/diastolic velocity.

Evaluation of pregnancy outcomes based on various parameters.								
Indicator	n	Abnormal preg	χ2	р				
mulcator		Mild hypoxia	Severe hypoxia					
RI of umbilical artery >0.66	60	23 (38.33)	37 (61.67)	1.114	0.291			
RI of umbilical artery ≤0.66	15	8 (53.33)	7 (46.67)					
RI of middle cerebral artery >0.69	55	15 (27.27)	40 (72.73)	16.816	< 0.001			
RI of middle cerebral artery ≤0.69	20	16 (80.00)	4 (20.00)					
PSV/ESRV >3.36	53	17 (32.08)	36 (67.92)	6.386	0.012			
$PSV/ESRV \le 3.36$	22	14 (63.64)	8 (36.36)					

 Table 4

 Evaluation of pregnancy outcomes based on various parameters.

Count data were expressed by % and subjected to the χ^2 test. ESRV: End systolic reflux velocity; PSV: peak systolic velocity; RI: resistance index.

Indiantan		Fetal out	χ2	р				
Indicator	11	Survival	Death					
RI of umbilical artery >0.66	60	53 (88.33)	7 (11.67)	1.930	0.165			
RI of umbilical artery ≤ 0.66	15	15 (100.00)	0 (0.00)					
RI of middle cerebral artery >0.69	55	48 (87.27)	7 (12.73)	2.808	0.094			
RI of middle cerebral artery ≤0.69	20	20 (100.00)	0 (0.00)					
PSV/ESRV >3.36	53	46 (86.79)	7 (13.21)	3.205	0.073			
$PSV/ESRV \leq 3.36$	22	22 (100.00)	0 (0.00)					

 Table 5

 Evaluation of fetal survival rate based on various parameters.

Count data were expressed by % and subjected to the χ^2 test. ESRV: End systolic reflux velocity; PSV: peak systolic velocity; RI: resistance index.

Evaluation of fetal survival rate based on umbilical artery, middle cerebral artery and aortic arch isthmus indicators

The fetal survival rates were further evaluated based on the umbilical artery, middle cerebral artery, and aortic arch isthmus indicators. The changes in umbilical artery RI, middle cerebral artery RI, and PSV/ESRV were not related to fetal survival rate (p > 0.05) (Table 5).

DISCUSSION

As one of the common perinatal complications, FGR is closely related to placental dysfunction and decreased fetal reserve capacity, leading to high perinatal fetal mortality and a high incidence rate of long-term complications ⁶. Therefore, early screening of FGR and early intervention are significant for improving the prognosis of fetuses with FGR.

CDUS has no significant effect on fetal growth and development due to noninvasive and radiation-free operation, and it can be used to evaluate the blood perfusion of the fetal-placental circulation by observing fetal vascular hemodynamic changes ^{7,8}. Normally, with increasing gestational week, the diastolic blood flow of the umbilical artery increases, and S/D, PI, and RI levels decrease^{9,10}. However, FGR may occur when there is a decrease in villous vascular branches, an increase in circulatory resistance, and a decrease in the total cross-sectional area of the vascularized lumen in the placenta, and an elevation in S/D, PI, and RI levels of the umbilical artery. In severe cases, the risk of adverse events, such as intrauterine distress, asphyxia, and even death of the fetus, may increase ^{11,12}. In this study, the RI, PI, and S/D levels of umbilical artery in the FGR group were significantly higher than those of the healthy group (p<0.05), indicating that the blood flow in the umbilical artery of fetuses with FGR was in a high resistance state.

According to the hemodynamic principle of fetal placental circulation, villous vascular bed increases in the second and third trimesters of pregnancy, the resistance of fetal placental circulation and the S/D decrease, and the placental blood flow increases accordingly, which is conducive to the growth and development of fetuses.¹³ The results of this study revealed that the S/D value of FGR fetuses significantly increased, probably due to prolonged hypoxia and nutritional deficiencies. Moreover, fetal development is closely related to placental blood flow. As a result, the S/D value indirectly reflects the fetal-placental circulation state and intrauterine conditions. Also, the ROC curve analysis results showed that the AUCs of RI, PI, and S/D of the umbilical artery in the diagnosis of FGR were 0.893, 0.893 and $0.900 \ (p < 0.05)$, respectively, indicating that abnormal umbilical artery hemodynamics can affect the supply of nutrients to fetuses. Regular monitoring of umbilical artery hemodynamic changes is favorable for the early diagnosis and clinical management of FGR.

As an important branch of the internal carotid artery, the middle cerebral artery markedly affects the changes in fetal cerebral circulation, and its hemodynamic alterations are closely related to fetal cranial blood circulation and hypoxia ^{14,15}. In the case of insufficient cerebral blood supply and oxygenation, the hypoxia and ischemia of the fetus are aggravated, causing damage to other organs and affecting the prognosis ^{16,17}. In this study, the RI, PI, and S/D levels of the middle cerebral artery in the FGR group were significantly lower than

those in the healthy group (p < 0.05), probably because FGR activated the cerebral protective effect to self-regulate and contract peripheral vasculature to increase the blood supply to the heart, brain, and other vital organs. As a result, monitoring the changes in the resistance parameters of the middle cerebral artery can evaluate the effect of fetal hypoxia on FGR. The results of ROC curve analysis herein revealed that the AUCs of RI, PI, and S/D of the middle cerebral artery in the diagnosis of FGR were 0.812, 0.874, and 0.910 (p<0.05), with high specificity and Youden index. Moreover, the analysis of pregnancy outcomes based on the cut-off value of RI showed that a high proportion of fetuses with severe hypoxia had an RI > 0.69. Therefore, the blood flow parameters of the middle cerebral artery can be used as indicators for the prenatal ultrasound diagnosis of FGR.

PSV/ESRV can reflect the blood flow of the aortic arch isthmus. When a fetus has a reduced blood supply, the body activates the compensatory mechanism to protect important organs such as the heart and brain and increase the perfusion of such organs, increasing ESRV level and decreasing PSV/ ESRV ^{18,19}. In this study, the PSV/ESRV level of the aortic arch isthmus in the FGR group was significantly lower than that in the healthy group (p < 0.05), suggesting that FGR can also be evaluated based on hemodynamic changes in the aortic arch isthmus. Probably, the decreased blood oxygen level during the increase in the resistance to fetal peripheral blood flow cannot meet the needs of fetal growth and development, so the body initiates a compensatory mechanism to promote dilatation to increase the perfusion of blood flow. Also, local anaerobic glycolysis increases in a state of hypoxia, producing metabolites such as lactate and adenosine, which can dilate blood vessels and reduce cardiac output ²⁰. Additionally, the results of ROC curve analysis revealed that the AUC of PSV/ESRV of aortic arch isthmus in diagnosing FGR was 0.857 (p<0.05), with the sensitivity and specificity of 84.00% and 73.33%, respectively. The infants with diagnostic value >3.36 accounted for a significantly high proportion. This indicates that the blood flow changes in the aortic arch isthmus are valuable for diagnosing FGR. The early monitoring of the PSV/ESRV level changes is conducive to diagnosing FGR at an early stage and can help guide the treatment.

In conclusion, fetal umbilical artery, middle cerebral artery, and aortic arch isthmus parameters detected by CDUS are all sensitive indicators for evaluating FGR, and the determination of optimal diagnostic value for each flow parameter is valuable for the clinical determination of FGR and intrauterine hypoxia, and for improving the prognosis. However, due to the short duration of this study, the values of fetal parameters detected by CDUS in evaluating the severity and prognosis of FGR have not yet been analyzed. In the future, the research duration will be increased, and the source of subjects will be expanded for in-depth investigation.

Conflicts of interest

The authors declare they have no conflicts of interest.

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