Increased fetal fraction is associated with the incidence of birth weight discordance and selective fetal growth restriction:a retrospective cohort study

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Abstract

Objective To test the hypothesis that the fetal fraction in twin pregnancy would reflect birthweight discrepancy. **Design** Retrospective cohort study. **Setting** International Peace Maternity and Child Health Hospital in Shanghai, China **Population** We included 237 twin pregnancies undergoing cfDNA screening for aneuploidy and delivered at International Peace Maternity and Child Health Hospital in Shanghai, China between January 2018 to December 2021. Exclusion criteria including abnormal NIPT results, fetal demise or structural abnormalities. **Methods** All women with twin pregnancies were offered a scan to determine chorionicity as well as first-trimester nuchal translucency (NT) at 11 $^{+0}$-13 $^{+6}$ weeks. Dichorionic was confirmed when ultrasound assessment clearly indicates two placentas. The twin peak sign is used to distinguish chorionicity if only one placenta is visualized. **Main Outcome Measures** Fetal fraction and birth weight of the new borns were collected. Relationships between fetal fraction and birth weight discordance or sFGR were analysed. **Results** Fetal fraction was positively correlated with the difference of birth weight (β=0.004, 95%CI: 0.001˜0.006). Higher fetal fraction was significantly associated with the increased risk of birthweight discordance of 20% (adjusted OR:1.073, 95%CI: 1.009˜1.142) and 25% (adjusted OR:1.092, 95%CI: 1.006˜1.185) and sIUGR(adjusted OR:1.130, 95%CI: 1.038˜1.231). We obtained the optimum cut-off point of fetal fraction [?] 11.790, [?] 14.800 and [?] 14.800 for birthweight discordance of 20% and 25% and sFGR, respectively. **Conclusion** This study shown that fetal fraction was positively correlated with the difference of birth weight. Fetal fraction could be used as a biomarker in predating birth weight discordance and sFGR, and help to make individualized clinical monitoring plans for twin pregnancies.

Original article

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Keywords : twin pregnancy; Non-invasive prenatal testing; fetal fraction; selective fetal growth restriction

ABSTRACT

Objective

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International Peace Maternity and Child Health Hospital in Shanghai, China

Population

We included 237 twin pregnancies undergoing cfDNA screening for aneuploidy and delivered at International Peace Maternity and Child Health Hospital in Shanghai, China between January 2018 to December 2021. Exclusion criteria including abnormal NIPT results, fetal demise or structural abnormalities.

Methods

All women with twin pregnancies were offered a scan to determine chorionicity as well as first-trimester nuchal translucency (NT) at 11^{+0}-13^{+6} weeks. Dichorionic was confirmed when ultrasound assessment clearly indicates two placentas. The twin peak sign is used to distinguish chorionicity if only one placenta is visualized.

Main Outcome Measures

Fetal fraction and birth weight of the new borns were collected. Relationships between fetal fraction and birth weight discordance or sFGR were analysed.

Results

Fetal fraction was positively correlated with the difference of birth weight (β=0.004, 95%CI: 0.001˜0.006). Higher fetal fraction was significantly associated with the increased risk of birthweight discordance of 20% (adjusted OR:1.073, 95%CI: 1.009˜1.142) and 25% (adjusted OR:1.092, 95%CI: 1.006˜1.185) and sIUGR(adjusted OR:1.130, 95%CI: 1.038˜1.231). We obtained the optimum cut-off point of fetal fraction [?] 11.790, [?] 14.800 and [?] 14.800 for birthweight discordance of 20% and 25% and sFGR, respectively.

Conclusion

This study shown that fetal fraction was positively correlated with the difference of birth weight. Fetal fraction could be used as a biomarker in predating birth weight discordance and sFGR, and help to make individualized clinical monitoring plans for twin pregnancies.

Introduction

In recent years, there has been a significant rise in twin pregnancies due to the advancements in assisted reproductive technologies. However, these pregnancies are more susceptible to complications such as fetal growth restriction (FGR), premature birth, and perinatal loss. Twin pregnancies can also experience selective fetal growth restriction (sFGR), where one twin is compromised while the other grows normally. This condition puts the affected twins at a higher risk of perinatal mortality and morbidity[1]. Additionally, compared to non-sFGR pregnancies, those with sFGR tend to have earlier deliveries and higher rates of neonatal unit admission[2].

It is estimated that 10-20% of twin pregnancies are affected by sFGR. Monochorionic twins have a higher incidence rate of 19.7% compared to dichorionic twins at 10.5%[3]. According to the latest guidelines from the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG), sFGR is characterized by

an estimated fetal weight (EFW) below the 10th percentile in one twin and an inter-twin EFW discordance of over 25%. Additionally, a discordance threshold of 20% may be used to identify pregnancies with a higher likelihood of negative outcomes[4]. Detecting pregnancies at risk of sFGR or birth weight discordance in the early stages can enhance counseling, intervention timing, and potentially enable preventive treatment trials. Various parameters identified during early pregnancy have been linked to the development of sFGR later in pregnancy. Although abnormal cord insertion is associated with sFGR in twins, it is present in more than 55% of all twin pregnancies[5]. A particularly isolated screening test is urgently needed in clinical practice.

Placental pathology often correlates with the onset and prognosis of sFGR. In cases of MC twins, sFGR is believed to be the result of an unequal distribution of blood through placental anastomoses due to uneven sharing of the placenta[6], whereas in DC twins, from placental insufficiency in one of the placentas[7]. Throughout gestation, the villous trophoblast in the placenta experiences ongoing turnover, which leads to the release of apoptotic debris and cell-free DNA (cfDNA) into the maternal bloodstream. Research suggests that levels of cfDNA may also indicate placental issues. The amount of cfDNA specifically from the fetus is known as fetal fraction, and it has been associated with negative perinatal outcomes, including FGR in single pregnancies[8, 9]. Here, we testing the hypothesis that the fetal fraction in twin pregnancy would reflect birthweight discrepancy.

Methods

Study design and data collection

We conducted a retrospective cohort study of all twin pregnancies undergoing cfDNA screening for aneuploidy and delivered at International Peace Maternity and Child Health Hospital in Shanghai, China between January 2018 to December 2021. All women with twin pregnancies were offered a scan to determine chorionicity as well as first-trimester nuchal translucency (NT) at 11^{+0}-13^{+6} weeks. Dichorionic was confirmed when ultrasound assessment clearly indicates two placentas. The twin peak sign is used to distinguish chorionicity if only one placenta is visualized. Following this, a thorough genetic consultation was offered to all future parents of the twin cohort. The characteristics of the tests and potential advantages and disadvantages of the different modalities of prenatal genetic testing will be discussed. Parents will also be informed that NIPT included information on the test being validated for the detection of only T21, T18 and T13, and the evidence of using NIPT in twin pregnancy is less than it is in singletons,thus offering limited information compared with invasive diagnostic testing. We used two platforms for cfDNA-BGI-Health (Shenzhen, China) and Berry Genomics. The screening was performed specific to the clinical site.

We abstracted maternal demographic characteristics, fetal fraction, obstetric outcomes, and neonatal outcomes from the medical record,including maternal age, race, parity, education levels, pre-pregnancy body mass index (BMI), method of conception, smoking status, gestational age delivery, and neonatal birthweight. Birth discordant was defined as an inter-twin EFW discordance greater than 20%. sFGR was defined as a condition in which the EFW of one twin is less than the 10th centile and an inter-twin EFW discordance greater than 25%. Cases with chromosome abnormalities were excluded from this study.

The exclusion criteria were the presence of twin-to-twin transfusion syndrome (TTTS), monoamniotic twins, twin anemia–polycythemia sequence (TAPS), and congenital, structural or genetic malformations in the fetus. To assure the placentas would be intact, only MC twins gestations delivered through cesarean section were included to ensure an intact placenta.

The Quintero criteria were used to diagnose TTTS, while TAPS was diagnosed based on a postnatal inter-twin hemoglobin difference of over 8 g/dL. Monochorionic twin pregnancies were identified in the first or early second trimester through ultrasonographic criteria, including the presence of a single placenta, a thin dividing membrane, and the absence of a twin peak (lambda) sign. Obstetricians confirmed monochorionicity through postpartum examination of the placenta, which showed a single placenta with inter-twin anastomoses[4].

Selective fetal growth restriction (sFGR) was characterized by two criteria: (I) a birth weight discordance of more than 25% and (II) the presence of an FGR twin with a birth weight below the 10th percentile. The

calculation for birth weight discordance involved subtracting the weight of the FGR twin from the weight of the appropriate-for-gestational-age (AGA) twin, dividing that difference by the weight of the larger twin, and multiplying the result by 100%[4, 10].

This study was approved by the Ethics Review Board of the International Peace Maternity and Child Health Hospital Affiliated to the Shanghai Jiao Tong University School of Medicine (No.GKLW2019-20).

Statistical Analysis

Categorical variables were described by percentage, and continuous variables were described by mean and standard deviation. The Kolmogorov-Smirnov test was used to test the normality of the continuous variables. The group differences were examined using Chi-squared test, Student t test, or Mann Whitney U test where appropriate.

The linear regression models were used to test the association of fetal fraction with birthweight difference. Logistic regression was used to obtain the odds ratio (OR) and 95% confidence interval (CI), and examine the effects of fetal fraction on birth weight discordance of 20% and 25% and sFGR.

The Optimal Cutpoints package was used to perform a receiver operator characteristics curve (ROC) analysis for fetal fraction and birth outcomes that have significance in above regression analysis, and determine the optimum cut-off points. Then fetal fraction was converted into according to the optimal cut-off points, and the association of fetal fraction (categorical variable) on birth outcomes was assessed using logistic regression.

The multivariate analyzes between fetal fraction and birth outcomes were carried out on using multiple logistic regression and multiple linear regression model. Model adjusted for maternal age, weight, primipara, history of abortion, chorionicity, pregnancy via ART and physical conditions (gestational hypertension, gestational diabetes).

All analyses were performed with the Statistical Package for the Social Sciences (version 24; SPSS Inc, Chicago, IL) and the Optimal Cutpoints and verification packages for R statistical software (version 3.5.1; http://www.R-project.org). Significance was defined as a 2-tail probability value of <0.05.

Gestational Age Notation

The authors follow the World Health Organization's evaluation of the gestational age. Under this notation, the first day of the last menstrual period (LMP) is day 0 of week 0. Therefore, days 0 to 6 are completed week 0, days 7 to 13 are completed week 1, etc.

Results

Study population

A total of 237 twin pregnancies who had normal NIPT results and delivered [?]28 weeks were included in study period. 5 cases of intrauterine death of one fetus, 8 structural abnormality in one or both foetuses were excluded from this study. The proportions of DC and MC twins were 77.6% (174) and 22.3% (50) respectively. Compared to MC group, DC group had higher maternal age (p =0.006), longer gestational weeks at delivery (p <0.001), larger birth weight of the foetuses (p =0.001) and higher percentage of pregnancies conceived by Assisted Reproductive Technology (p <0.001). The incidence of preterm delivery was higher in MC group (p =0.004). There was no difference in other pregnancy complications such as gestational hypertension, gestational diabetes or thyroid diseases. (Table 1).

The association of Fetal fraction with birthweight discordance and sIUGR

As shown in Table 2, the adjusted model demonstrated that fetal fraction was positively correlated with the difference of birth weight (β=0.004, 95%CI: 0.001˜0.006). That is, for every one percent increase in fetal fraction, the difference of birth weight between twins increases by 0.4 percent.

The results of logistic analysis were shown in Table 3, indicating that higher fetal fraction was significantly associated with the increased risk of birthweight discordance of 20% (adjusted OR:1.073, 95%CI: 1.009˜1.142)

and 25% (adjusted OR:1.092, 95%CI: 1.006˜1.185) and sIUGR (adjusted OR:1.130, 95%CI: 1.038˜1.231).

The optimum cut-off points for fetal fraction in birthweight discordance and sIUGR were obtained with a ROC analysis (Table 4). Using ROC analysis, we obtained the optimum cut-off point of fetal fraction [?] 11.790, [?] 14.800 and [?] 14.800 for birthweight discordance of 20% and 25% and sIUGR, respectively. For birthweight discordance of 20%, the AUC was 0.591, the sensitivity was 52.9% and the specificity was 66.3%(p =0.017). The AUC of sIUGR was 0.620, with 37.5% sensitivity and 86.5% specificity(p =0.024).

Compared with women with fetal fraction of < 11.790, there were a 1.091-fold higher risk of birth weight discordance of 20% (adjusted OR: 2.091, 95%CI: 1.218˜3.591) among these with fetal fraction of [?]11.790, and significantly increased risks of birth weight discordance of 25% (adjusted OR: 3.045, 95%CI: 1.297˜7.149) and sIUGR (adjusted OR: 3.526, 95%CI: 1.443˜8.618) among these with fetal fraction of [?]14.800.

Discussion

In this study, we assessed the association between fetal fraction and birth weight discordant and sIUGR. It is showed that fetal fraction was positively correlated with the difference of birth weight. For every one percent increase in fetal fraction, the difference of birth weight between twins increases by 0.4 percent. Higher fetal fraction was significantly associated with the increased risk of birthweight discordance of 20% and 25% and sIUGR. Using ROC analysis, we obtained the optimum cut-off point of fetal fraction [?] 11.790, [?] 14.800 and [?] 14.800 for birthweight discordance of 20% and 25% and sIUGR, respectively. For birthweight discordance of 20%, the AUC was 0.591, the sensitivity was 52.9% and the specificity was 66.3%(p =0.017). The AUC of sIUGR was 0.620, with 37.5% sensitivity and 86.5% specificity(p =0.024). Compared with women with fetal fraction of < 11.790, there were a 1.091-fold higher risk of birth weight discordance of 20% among these with fetal fraction of [?]11.790, and significantly increased risks of birth weight discordance of 25% and sFGR among these with fetal fraction of [?]14.800.

Cell-free fetal DNA actually originates from placenta[11]. Throughout a typical pregnancy, cffDNA experiences a gradual increase, comprising roughly 13% of all cell-free DNA found in maternal plasma towards the end of gestation, before swiftly decreasing to undetectable levels after childbirth[12, 13]. The size of the placenta and the rate of trophoblast apoptosis are factors that affect the release of cffDNA[9]. According to reports, cffDNA triggers the secretion of type 1 interferons such as IFN-β and IFN-α, along with other pro-inflammatory agents[14]. The introduction of CpG resulted in gestational hypertension and heightened vasoconstriction in rats. This suggests that cffDNA has the ability to initiate inflammatory cascades as a pro-inflammatory trigger.

Research has demonstrated that oxidative stress results in trophoblast apoptosis and elevated cffDNA release[15]. The liberation of cffDNA can be affected by doxorubicin and high-mobility group box protein-1, which are inflammatory agents. Conversely, lipopolysaccharide does not affect the release of early or term cffDNA[16], suggesting that cffDNA is involved in sterile inflammation as opposed to infectious processes. Sterile inflammation may lead to placental dysfunction and consequently cause pregnancy complications.

In order to predict negative outcomes earlier and non-invasively, many studies have linked pregnancy complications with cffDNA plasma concentrations. Pregnancies affected by pre-eclampsia, preterm delivery, and FGR were found to have higher cffDNA concentrations during the second trimester. Additionally, there was a noticeable increase in cffDNA levels three weeks prior to the onset of symptoms[17]. In twins with birth weight discordant, we were able to see an increase in cffDNA which was represented by fetal frection. Increased levels of cffDNA are also a result of intrauterine surgeries, such as laser ablation, used to treat twin-twin transfusion syndrome[18]. It is possible that the rise in cffDNA levels observed in twins with discordant birth weight and selective fetal growth restriction (sFGR) is a result of the repair process. In cases where there is discordant growth in DC pregnancies, the incidence of preeclampsia can be as high as 37.5%, which is significantly greater than the 21.9% rate observed in MC sFGR pregnancies[19, 20]. It is known that placental insufficiency affects only one fetus and results in selective growth restriction in DC pregnancies[10]. After corrected for both preeclampsia and gestational diabetes, increased cffDNA is still associated with the incidence of birth weight discordance and sFGR, which showed that there may be other pathological mech-

anisms involved. Placentae that are affected by uneven sharing have fetal circulations that are more tightly connected with larger and more frequent arterio-arterial (AA) anastomoses. This can be helpful for growth-restricted fetuses, as it allows their co-twin to compensate to some extent for the inadequacy of their own placenta. However, this close connection also poses risks to appropriately grown fetuses, who are vulnerable to sudden changes in the blood pressure of their smaller co-twin. Although unequal placental sharing is the primary cause of sFGR in MC pregnancies, the interdependent feto-placental circulations play a crucial role in the prognosis and perinatal outcome. In cases of sFGR, especially those with abnormal umbilical artery Doppler, glucose transporters (GLUTs) were found to be elevated due to hypo-perfusion. We suspect that increased cffDNA in MC twins with sFGR is a result of apoptosis in such hypoxic environments. Additionally, hypermetabolism may also be a factor in elevated cffDNA levels since the placenta attempts to increase glucose transport to the fetal circulation as an adaptive response[21]. We suspect that increased cffDNA in MC twins with sFGR is a consequence of apoptosis in such hypoxic environment. Further, hypermetabolism may also be a reason for elevated cffDNA since the placenta attempts to increase glucose transport to the fetal circulation as an adaptive response.

Conclusion:

High fetal fraction is associated with poor placental development and dysfunction. Cutoff of both 11.790 and 14.800 can be use as potential biomarker in predict birth weight discordance and sFGR.

Author contributions

Renyi Hua: conceptualisation, methodology, investigation, resources, writing – original draft, visualisation, project administration. Yuanqing Xia: formal analysis, data curation.Shan Wang and Jinling Sun: conceptualization, methodology, visualisation. Li Gao, Yi Wu and Xinrong Zhao: conceptualisation, methodology, formal analysis, investigation, resources, data curation, project administration. Yanlin Wang: methodology, writing – review & editing, supervision.

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Conflict of interests:

None declared.

Ethics approval:

This study was approved by the Ethics Review Board of the International Peace Maternity and Child Health Hospital Affiliated to the Shanghai Jiao Tong University School of Medicine (No.GKLW2019-20).

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Table 1. Demographics of DC and MC groups

	Overall	**DC**	**MC**	p
	mean±SD/n(%)	mean±SD/n(%)	mean±SD/n(%)	
Maternal characteristic	n=224	n=174	n=50	
Age(year), n(%)				**0**

mean±SD	31.97 ±3.75	32.30 ±3.49	30.82 ±4.40	
<35	180 (80.4)	137 (78.7)	43 (86.0)	?,
35	44 (19.6)	37 (21.3)	7 (14.0)	
Maternal weight, kg	59.09 ±8.98	59.52 ±9.38	57.59 ±7.28	0.
First gestation, n (%)				0.
Yes	141 (62.9)	111 (63.8)	30 (60.0)	
No	83 (37.1)	63 (36.2)	20 (40.0)	
Primipara, n (%)				0.
Yes	199 (88.8)	158 (90.8)	41 (82.0)	
No	25 (11.2)	16 (9.2)	9 (18.0)	
History of abortion, n (%)				0.
No	155 (69.2)	118 (67.8)	37 (74.0)	
Yes	69 (30.8)	56 (32.2)	13 (26.0)	
Pregnancy via ART, n (%)				<
No	72 (32.1)	42 (24.1)	30 (60.0)	
Yes	152 (67.9)	132 (75.9)	20 (40.0)	
Fetal fraction	10.73 ±4.15	10.57 ±4.25	11.30 ±3.78	0.
Gestational hypertension, n (%)				0.
No	184 (82.1)	143 (82.2)	41 (82.0)	
Yes	40 (17.9)	31 (17.8)	9 (18.0)	
Gestational diabetes, n (%)				0.
No	180 (80.4)	141 (81.0)	39 (78.0)	
Yes	44 (19.6)	33 (19.0)	11 (22.0)	
Thyroid disease, n (%)				0.
None	191 (85.3)	147 (84.5)	44 (88.0)	
Subclinical hypothyroidism	5 (2.2)	4 (2.3)	1 (2.0)	
Hypothyroidism	16 (7.1)	13 (7.5)	3 (6.0)	
Subclinical hyperthyroidism	7 (3.1)	6 (3.4)	1 (2.0)	
Hyperthyroidism	5 (2.2)	4 (2.3)	1 (2.0)	
Fetal characteristic	n=448	n=348	n=100	
Gestational age at delivery, week	35.87 ±1.99	36.08 ±1.85	35.16 ±2.27	<
Preterm birth, n (%)				**0**
No	200 (44.6)	168 (48.3)	32 (32.0)	
Yes	248 (55.4)	180 (51.7)	68 (68.0)	
Birth weight, g	2413.55±466.54	2456.14±436.53	2265.35±535.05	**0**
Difference of birth weight	0.12 ±0.11	0.11 ±0.11	0.12 ±0.12	0.
Birth-weight discordance of 20%, n (%)	Birth-weight discordance of 20%, n (%)			0.
No	380 (84.8)	296 (85.1)	84 (84.0)	
Yes	68 (15.2)	52 (14.9)	16 (16.0)	
Birth-weight discordance of 25%, n (%)	Birth-weight discordance of 25%, n (%)			0.
No	412 (92.0)	320 (92.0)	92 (92.0)	
Yes	36 (8.0)	28 (8.0)	8 (8.0)	
sFGR, n (%)				0.
No	416(92.9)	324 (93.1)	92 (92.0)	
Yes	32 (7.1)	24 (6.9)	8 (8.0)	

Table 2 Linear regression analyses of fetal fraction and difference of birth-weight

Outcome	**β(95%˜I)**	p	**β(95%˜I)** [a]	p
Difference of birth-weight	1.076(1.046˜1.107)	**0.000**	1.004(1.001˜1.006)	**0.003**

[a]Model adjusted for maternal age, weight, history of abortion, primipara, pregnancy via ART, chorionicity, gestational hypertension and gestational diabetes

Table 3 Logistic regression analyses of fetal fraction and birth-weight discordance and sFGR

Outcome	**OR(95%CI)**	p	**aOR(95%CI)** [a]	p
Birth-weight discordance-20%	1.079(1.018˜1.144)	**0.011**	1.073(1.009˜1.142)	**0.025**
Birth-weight discordance-25%	1.082(1.005˜1.166)	**0.036**	1.092(1.006˜1.185)	**0.035**
sFGR, n (%)	1.117(1.035˜1.205)	**0.004**	1.13(1.038˜1.231)	**0.005**

[a]Model adjusted for maternal age, weight, history of abortion, primipara, pregnancy via ART, chorionicity, gestational hypertension and gestational diabetes

Table 4 ROC analyses for fetal fraction and birth outcomes

Outcome	**Cut-off value**	**AUC (95%CI)**	p
birth-weight discordance of 20%	11.790	0.591(0.516˜0.666)	**0.017**
birth-weight discordance of 25%	14.800	0.569(0.466˜0.673)	0.083
sFGR	14.800	0.620(0.514˜0.725)	**0.024**

Table 5 Logistic regression analyses of fetal fraction and birth-weight discordance and sFGR

Outcome	**Fetal fraction**	**OR(95%CI)**	p	**aOR(95%**
Birth-weight discordance of 20%	Birth-weight discordance of 20%	Birth-weight discordance of 20%		
	[0, 11.79%)	1		1
	[11.79%, 100%]	2.215(1.315˜3.731)	**0.003**	2.091(1.21
Birth-weight discordance of 25%	Birth-weight discordance of 25%	Birth-weight discordance of 25%		
	[0, 11.79%)	1		1
	[11.79%, 14.80%)	0.985(0.379˜2.559)	0.976	0.843(0.30
	[14.80%, 100%]	3.167(1.444˜6.944)	**0.004**	3.045(1.29
sFGR				
	[0, 11.79%)	1		1
	[11.79%, 14.80%)	1.286(0.48˜3.445)	0.617	1.211(0.43
	[14.80%, 100%]	4.133(1.815˜9.411)	**0.001**	3.526(1.44

[a]Model adjusted for maternal age, weight, history of abortion, primipara, pregnancy via ART, chorionicity, gestational hypertension and gestational diabetes

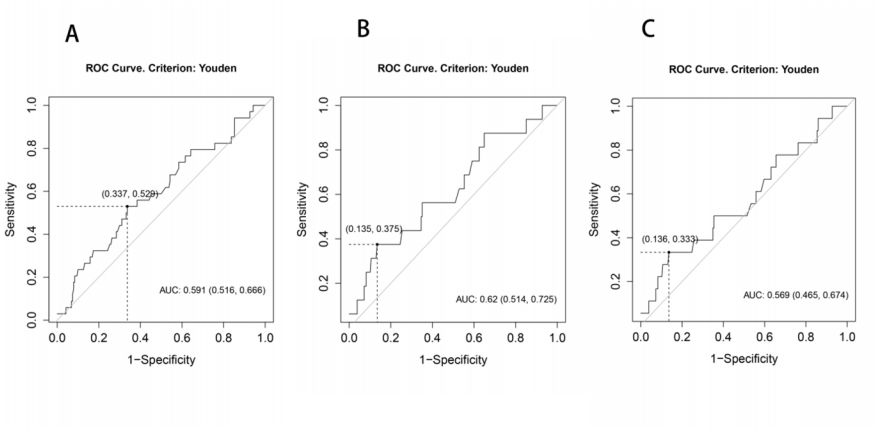

Figure legends :

Figure 1: ROC Curves for fetal faction and birth outcomes.

A: ROC Curve for fetal faction and birth weight discordance of 20%; B: ROC Curve for fetal faction and birth weight discordance of 25%; C: ROC Curve for fetal faction and sFGR