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Reduced fetal growth velocity precedes antepartum fetal death

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CONTRIBUTION

What are the novel findings of this work?

Pregnancies that resulted in antepartum fetal death had significantly lower growth velocity of fetal head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL), and estimated fetal weight (EFW) than pregnancies with a live-born neonate, according to both PRB/NICHD and Hadlock fetal growth standards.

What are the clinical implications of this work?

Fetal growth velocity doubles the detection rate of antepartum fetal death compared to the last available scan before diagnosis. Fetuses with EFW percentile velocity $<10^{th}$ percentile value of pregnancies with live birth had 9.4-fold and 11.2-fold increased risk to die antepartum based on Hadlock and PRB/NICHD standards, respectively.

ABSTRACT

Objectives: 1) To determine whether decreased fetal growth velocity precedes antepartum fetal death, and 2) Evaluate if fetal growth velocity predicts better antepartum fetal death compared to a single, last available, ultrasound examination prior to diagnosis.

Methods: We conducted a retrospective, longitudinal study of 4,285 singleton pregnancies in African-American women who underwent at least two fetal ultrasound examinations between 14 and 32 weeks of gestation and delivered a live born neonate (controls; n=4,262) or experienced antepartum fetal death (cases; n=23). Fetal death was defined as the death of a fetus ≥ 20 weeks of gestation and confirmed by ultrasound examination. Exclusion criteria were: congenital anomalies, birth <20 weeks of gestation, multiple gestations, and intrapartum fetal death. The ultrasound examination performed at the time of fetal demise was not included in the analysis. Growth percentiles for estimated fetal weight (EFW) and individual biometric parameters were determined according to the Hadlock and Perinatology Research Branch / *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (PRB/NICHD) fetal growth standards. Fetal growth percentile velocity was defined as the slope of the regression line of the growth percentiles as a function of gestational age based on two or more measurements in each pregnancy.

Results: 1) Cases had significantly lower EFW and fetal head circumference (HC), biparietal diameter, abdominal circumference, and femur length percentile velocities compared to controls, according to both PRB/NICHD and Hadlock standards (all $p<0.05$); 2) Fetuses with EFW percentile velocity $<10^{th}$ percentile among controls had 9.4-fold and 11.2-fold increased risk to die antepartum based on Hadlock and customized PRB/NICHD standards, respectively; 3) at a 10% false-positive rate, the sensitivity for antepartum fetal death of EFW percentile velocity was

57%, compared to 26% for a single, last available, examination, according to the customized PRB/NICHD standard.

Conclusions: Given that 74% of antepartum fetal death cases were not small-for-gestational-age at the last ultrasound examination when they were alive (EFW>10th), alternative approaches are needed to improve detection of fetuses at risk for fetal death. Longitudinal sonographic evaluations to determine growth velocity doubles the sensitivity for prediction of antepartum fetal death compared to a single ultrasound examination, yet performance is still sub-optimal.

INTRODUCTION

Fetal death diagnosed after 20 weeks of gestation occurs in 6/1000 births and accounts for more than one-half of annual infant deaths in the United States.[1] More than 80% of fetal deaths occur prior to the onset of labor.[2-5]

Since birthweight has been considered as a surrogate of fetal growth, and a small-for-gestational-age (SGA) neonate was associated with fetal death,[6-10] fetal growth restriction (FGR) is frequently cited as a precedent of antepartum stillbirth.[7, 11-14]

The relationship between fetal growth and stillbirth is poorly understood for several reasons. First, the exact time of death is unknown and an overestimation of the gestational age leads to increased frequency of SGA among stillbirths.[6, 10, 14-18] Second, although, most cases of FGR and fetal overgrowth result in a live birth,[11, 19-21] abnormal fetal growth may still be a cause of fetal death.[6, 10, 14, 15, 22-24] Third, it is unclear whether impaired fetal growth is a cause of fetal death[2] or is a result of placental dysfunction. Fourth, maternal characteristics affecting growth of normal live-born neonates are also risk factors for stillbirth.[25] Finally, given that most reports examine maternal-fetal conditions present at the time of or after fetal death, longitudinal studies are needed to gain insight into causality.[7, 19, 26-29]

Although improvement in prediction of SGA at birth by serial ultrasound examinations compared to a single last available biometry is controversial,[30-32] the assessment of fetal growth velocity has been proposed to improve the detection of growth-restricted fetuses at increased risk of adverse perinatal outcome.[33-36]. Studies conducted in Sweden,[37] Norway,[38] and Denmark[22, 23] have reported that impairment of fetal growth was associated with fetal and/or neonatal death; yet growth velocity in fetal death was not assessed. Although Hirst et al.[4]

reported that a reduced fetal size doubled the risk of fetal death, the authors did not find evidence of decreased velocity in either HC or AC among cases with antepartum fetal death.

We therefore aimed to 1) determine whether fetal growth velocity is decreased in pregnancies that experience antepartum fetal death compared to those that deliver a live-born neonate, and 2) evaluate if growth velocity predicts antepartum fetal death better than a single, last available evaluation prior to diagnosis.

METHODS

Study population

This was a retrospective study of 5,847 singleton pregnancies enrolled from August 2006 through April 2017 at the Center for Advanced Obstetrical Care and Research of Hutzel Women's Hospital, affiliated with Wayne State University (WSU) School of Medicine and the Detroit Medical Center, Detroit, Michigan. The clinical database is housed by the Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health, U.S. Department of Health and Human Services, Detroit, Michigan. All study participants provided written informed consent prior to the collection of demographic or clinical information, images, and samples. The use of demographic, clinical and ultrasound data for research purposes was approved by the Human Investigation Committee of Wayne State University and the Institutional Review Board of NICHD.

Given that most of the study participants self-reported as African American (92%) and we aimed at comparing growth velocity based on changes in growth percentiles obtained not only with Hadlock standard but also with the customized PRB/NICHD standard, which was

established in an African American population, we restricted our analysis to the African American population.

A retrospective, longitudinal study was designed based on the following inclusion criteria: 1) singleton pregnancy; 2) African-American maternal ethnicity; 3) at least two fetal ultrasound examinations performed between 14 and 32 weeks of gestation; and 4) availability of relevant perinatal information. Participants were classified according to pregnancy outcome: 1) delivery of a live-born neonate (controls) and 2) antepartum fetal death (cases). The outcome of antepartum fetal death included the death of a fetus diagnosed $\geq$ 20 weeks of gestation and confirmed by ultrasound examination prior to the onset of labor. Pregnancies with congenital anomalies, and intrapartum fetal death, and those who had been lost to follow-up, were not included in the study. Detailed demographic data, medical history, and pregnancy outcomes were extracted from the patients' electronic medical records.

Ultrasound examinations

Transabdominal ultrasound examinations to obtain the fetal biometric parameters were performed by using methods previously described by Altman and Chitty,[39-42] which are consistent with recommendations of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)[43] and the American Institute of Ultrasound in Medicine (AIUM).[44] Fetal biometric parameters included 1) BPD, outer edge to inner edge of the calvarium; 2) HC, ellipse around the outside of the calvarium; 3) AC, ellipse placed at the outer surface of the skin; and 4) FL, calipers placed at the ends of the ossified diaphysis.

Clinical definitions

Risk factors associated with fetal death[2, 4] that were considered, included: maternal medical chronic conditions, pregnancy complications, maternal age >35 years, and maternal age <20 years. *Obesity* was defined as a pre-pregnancy body mass index > 30 kg/m^2. *Maternal medical chronic conditions* included the presence of obesity, hypertension, diabetes mellitus, asthma, anemia, thyroid disease, epilepsy, liver disease, kidney disease, neurologic or psychiatric disease, and maternal syphilis. *Pregnancy complications* considered were the presence of any of the following conditions: preeclampsia, eclampsia, gestational hypertension, HELLP (hemolysis, elevated liver enzymes and low platelet count) syndrome, cervical insufficiency, placental abruption, preterm labor, preterm prelabor rupture of the fetal membranes (PPROM), clinical chorioamnionitis, and preterm delivery. *Medically indicated preterm delivery* were defined as birth < 37 weeks of gestation as a consequence of a medical intervention indicated to end the pregnancy in the presence of serious maternal or fetal compromise[45]. *Fetal death* was defined as the death of a fetus diagnosed ≥ 20 weeks of gestation and confirmed by ultrasound examination prior to delivery.[46]

Statistical analysis

Demographic variable data were compared between cases and controls using the Wilcoxon signed-rank test for continuous variables and the Fisher's exact test for categorical variables. EWF was calculated from fetal AC, FL, and HC using Hadlock's formula.[47] EFW percentiles were computed according to the PRB/NICHD,[48] and Hadlock,[49] fetal growth standards. The INTERGROWTH-21st growth standard was also considered but finally it was not included since percentiles could not be obtained for scans obtained prior to 22 weeks of gestation[50]. EFW percentiles for all standards were obtained using the fetal GPS calculator.[51]

Growth velocity was defined as the change in the percentile of EFW or individual fetal parameters per week of gestation, and was determined as the linear regression slope of growth percentiles with gestational age from all measurements from 14 to 30.6 weeks of gestation (see a spreadsheet calculator in File S1). The upper limit of gestational age corresponded to the highest gestational age at scan among cases before diagnosis of fetal death. Growth percentile velocity below zero represents *deceleration*, while positive values represent *acceleration*, and zero denoting no change in the percentile with gestational age.

Receiver operating characteristic (ROC) curves were used to compare prediction of fetal death based on the following parameters: 1) velocity of EFW percentiles according to the PRB/NICHD and Hadlock standards; 2) velocity of percentiles defined based on the non-customized PRB/NICHD and Hadlock for HC, BPD, AC, and FL and 3) EFW percentile at last available scan in cases and matched controls.

A p-value less than 5% was considered statistically significant. All statistical analyses were performed using the R programming language (version 3.5.1) (https://www.r-project.org).

RESULTS

Among 5,846 singleton pregnancies enrolled during the study period, 5,375 (91.2%) were of African-American women. Of these, 4,290 underwent two or more ultrasound examinations between 14 and 32 weeks of gestation. Among these pregnancies, 28 cases had antepartum fetal death and 4,262 delivered a live-born neonate.

Of the 28 cases of antepartum fetal death, only 29% (8/28) had an EFW <10th percentile at the last ultrasound examination, according to either of the two standards considered. **Figure 1** depicts the longitudinal EFW percentiles of the 28 cases according to the two standards, and a downward trend with gestational age was observed, suggesting a decline in EFW percentile with advancing gestational week. Given that five of the 28 cases with antepartum fetal death had only two ultrasound examinations, with the latter examination being after fetal demise was diagnosed, these five cases were excluded from further analysis. The clinical characteristics of the study group (4,262 controls and 23 cases) are shown in **Table 1**. The median gestational age at delivery and neonatal weight were lower in cases than in controls ($p<0.001$, for both). The frequency of induction of labor was higher in cases than in controls ($p<0.001$), and the frequency of spontaneous vaginal delivery was lower in cases than in controls ($p<0.001$). There were no differences in maternal age, maternal height, and body mass index, parity, smoking status, cesarean delivery, and fetal sex between cases and controls. Cases had a significantly higher frequency of placental abruption (Relative risk: 16.4, 95% CI: 5.4-49.5, $p=0.001$), preterm delivery (Relative risk: 7.0, 95% CI: 6.1-8.2, $p<0.001$), and indicated preterm delivery (Relative risk: 10.6, 95% CI: 6.1-8.2, $p<0.001$) than controls.

An EFW <50th percentile at the last scan before diagnosis carried a 3-fold increase in risk of fetal death using the PRB/NICHD standard ($p<0.05$), yet it did not reach significance according to the Hadlock standard ($p=0.08$).

Estimated fetal weight percentiles at the first and last ultrasound examinations before 31 weeks of gestation

We compared the EFW percentiles between cases and controls in two gestational age intervals corresponding to the range of gestational age at the first (14.1week - 26.6 weeks) and last (19.4 weeks - 30.6 weeks) ultrasound examination of the cases (n=23). There were 4,115 controls that matched with cases at the first ultrasound examination, and 2,634 controls that matched with cases at the last ultrasound examination. The median gestational age of the first ultrasound examination was 17.4 weeks (interquartile range, IQR: 15.9−19.9 weeks), and the median gestational age at the last ultrasound examination was 28.9 weeks (IQR: 26.9-30.1 weeks) (**Figure 2**). At the first ultrasound examination, there was no differences between cases and controls, regardless the growth standard considered. [PRB/NICHD: median (IQR) percentile: controls 49.61(33.8-66.84) versus cases 49.12(25.18-72.86); p = 0.709 (Figure 2A); and Hadlock standard: median (IQR) percentile: controls 49.3(32.45-61.65) versus cases 43.4(24.3-67.2); p = 0.556 (Figure 2B)]. The median EFW percentiles at the first ultrasound examination was close to 50^{th} for both standards in cases and control alike (**Figure 2**). However, the EFW percentiles at the last ultrasound examination were lower in cases than in gestational age matched controls according to both standards [PRB/NICHD: median (IQR) percentile: controls 46.02(22.41-71.25) versus cases 22.22(6.12-38.14); p = 0.002 (**Figure 2A**); and Hadlock: median (IQR) percentile: controls 32.5(16.8-54.7) versus cases 21.5(7.55-35.85); p = 0.015 (**Figure 2B**)]. Of note, at the last scan before 30.6 weeks, the median EFW percentiles of controls using PRB/NICHD standard was at 46^{th} (and not 50^{th}) percentile because the control group included all pregnancies with and without complications, but delivered a live birth neonate. In addition, the median EFW percentile at last scan among controls according to the Hadlock standard was even lower (32.5^{th}) than that obtained with the PRB/NICHD standard (46^{th}), which is in agreement with previous

reports on disparity between the study population (African American) and the population used to derive the Hadlock standard (White).[48, 52]

Association between EFW percentile velocity and fetal death based on two or more longitudinal scans before 31 weeks of gestation

Next, we analyzed growth percentile velocity from all available ultrasound examinations performed before 30.6 weeks of gestation in each pregnancy. The distributions of EFW percentile velocities in cases and controls are shown in **Figure 3**. The median EFW percentile velocity was −0.14 (IQR: −1.66 to 1.23) percentile/week in controls and −4.53 (IQR:−8.56 to −0.38) percentile/week ($p<0.001$) in cases, according to the customized PRB/NICHD standard (**Figure 3A**). The growth deceleration among controls is expected given the cross-sectional EFW percentile results described above (median EFW percentile was 50th at first scan and decreased to 46th at last scan before 31 weeks). Similarly, when EFW percentiles were derived using the Hadlock standard, the median EFW percentile velocity was -0.8 (IQR: -2.28 to 0.39) percentile/week among controls and −4.27 (IQR: −8.82 to −1.13) percentile/week among cases ($p < 0.001$) (**Figure 3B**). Overall, these results suggest that a reduced EFW percentile velocity precedes diagnosis of fetal death according to both standards. As an example, the median EFW percentile velocity (about 4.5 percentile/week) would correspond to a pregnancy destined to have fetal death that had an EFW in the 50th percentile at 20 weeks but decreased to the 5th percentile at 30 weeks.

Although the analyses presented above were based on data collected in women self-identified as African American, the decline in EFW percentile velocity preceding fetal death is likely not particular to this ethnic group. Expanding the analysis to include data from 379 additional

women (148 White, 28 Hispanic, 20 Asian, and 183 other), including one additional case of fetal death, resulted in similar results. While the median Hadlock standard percentile velocity of African-American controls was declining by -0.8 percentiles per week, it was declining slightly less (-0.5 percentiles per week) in all other pregnancies (p=0.002), yet the decline in percentile velocity among the cases remained substantially steeper (-4.15 percentiles per week) (p<0.001) (**Figure S1**).

Association between individual fetal biometric parameter percentile velocity and fetal death based on two or more longitudinal scans before 31 weeks

We have presented above differences in the customized PRB/NICHD and non-customized Hadlock EFW percentile velocities between cases and controls. To assess differences in percentile velocity for individual fetal biometric measurements we used the Hadlock standard and PRB/NICHD African American standards that were not customized for additional maternal characteristics and fetal sex.

The fetal HC, BPD, AC, and FL percentile velocity calculated using the non-customized PRB/NICHD standard showed significantly lower medians in cases than in controls (p<0.05 for all). Similarly, fetal HC, BPD, AC, and FL percentile velocity calculated using the Hadlock standard showed significantly lower medians in cases than in controls (p<0.05 for all).

Prediction of antepartum fetal death by fetal growth velocity

ROC curves for prediction of fetal death by EFW percentile velocity were similar among the growth standards: PRB/NICHD 0.74 (95% CI, 0.57-0.85), and Hadlock 0.73 (95% CI, 0.57-0.85) (Figure 4). At a 10% false-positive rate, the sensitivity of EFW percentile velocity for prediction of antepartum fetal death using the customized PRB/NICHD, and Hadlock standards

were 56.5% (34.8%-78.3%) for both. When the percentile velocity was calculated using only the first and last available scans from 14-32 weeks, as opposed to two or more scans, the AUC for prediction of fetal death for the PRB/NICHD standard decreased slightly from 0.74(0.57−0.86) to 0.73(0.59−0.86). If the same two scans from each patients were used to calculate the velocity percentile using the NICHD calculator (https://www.nichd.nih.gov/fetalvelocitycalculator)[32] as opposed to the percentile velocity based on the customized PRB/NICHD standard, the point estimate of AUC for prediction of fetal death decreased further to 0.7(0.56−0.82), although not significantly (**Figure S2**).

The prediction of antepartum fetal death based on different EFW percentile velocity cut-offs for the PRB/NICHD and Hadlock growth standards is displayed in **Table 2**. Fetuses with growth velocity < 50th percentile among controls had a 4.7-fold increased risk of antepartum fetal death for both growth standards considered. An EFW percentile velocity less than the 40th, 30th, 20th, 10th, and 5th percentiles carried a 3.4-, 4.3-, 6.1-, 11.2-, and 13.6-fold increased risk of antepartum death, respectively. Similarly, according to the Hadlock standard, EFW percentile velocity less than the 40th, 30th, 20th, 10th, and 5th percentiles carried a 3.4-, 3.6-, 5.1, 9.4-, and 13.6-fold increased risk of anterpartum death, respectively (**Table 2**).

The prediction of antepartum fetal death by percentiles of HC, BPD, AC, and FL growth velocities based on non-customized PRB/NICHD and Hadlock charts are displayed in **Figure 5**. Regardless of the non-customized standard considered, a low percentile velocity of HC and EFW predicted antepartum fetal death with an AUC of about 0.73 for both, estimate that was higher than that for BPD, AC, or HC. Among these parameters, BPD percentile velocity also predicted antepartum fetal death [AUC=0.67(0.51-0.8)] based on the PRB/NICHD standard (**Figure 5A**)

while AC percentile velocity also predicted fetal death [AUC=0.7(0.54−0.84)] based on the Hadlock standard (**Figure 5B**).

Comparison of prediction performance for antepartum fetal death between EFW percentile velocity and EFW percentile at the last scan before 32 weeks

To assess the benefit of EFW growth velocity relative to a single EFW determination, we retained the last ultrasound examination of controls within the same range of gestational age as the last available scan in cases. The AUC of EFW percentile velocity for prediction of fetal death was slightly higher compared to that of the EFW percentile at the last scan for both standards, although the difference did not reach statistical significance: PRB/NICHD standard, EFW percentile velocity AUC= 0.72 (95% CI, 0.57-0.86) versus last scan EFW percentile AUC= 0.69 (95% CI, 0.58-0.79), p-value=0.46; Hadlock standard, EFW percentile velocity AUC=0.71 (95% CI, 0.56-0.85) versus EFW percentile AUC= 0.65 (0.53-0.76), p-value = 0.16. However, for both standards, the sensitivity (10% false positive rate) of EFW percentile velocity rate was two times higher than that of the last EFW percentile evaluation: PRB/NICHD standard sensitivity (95% confidence interval): EFW percentile velocity 56.5% (34.8%-76.2%) versus EFW percentile last scan 26.1% (8.7%-43.5%), p value=0.02; Hadlock standard sensitivity (95% confidence interval): EFW percentile velocity 52.2%(30.4%-69.6%) versus EFW percentile last scan 26.1% (8.7%-43.5%), p value =0.03.

DISCUSSION

Results in the context of what is known

The current study demonstrates, for the first time, that a reduced fetal growth velocity, expressed as change in growth percentile per week for individual fetal biometry (HC, BPD, AC, and FL) and EFW, precedes antepartum fetal death. This concept complements four previous studies[22, 23, 37, 38] that reported that impaired fetal growth was associated with perinatal death; however, none of these studies evaluated EFW and fetal biometric parameter velocity. Of note, when data from only two scans are analyzed, the growth percentile regression slope is the same as the difference in percentiles between scans divided by the difference in gestational ages. For the purpose of ranking fetuses from the lowest to highest growth velocity based on two scans, our approach is equivalent to the one based on the difference in Z-scores[34, 53].

Most cases of antepartum fetal death are not small-for-gestational-age fetuses

Only 26% (6/23) of cases of fetal death herein were SGA (EFW<10^{th} percentile) at the last scan prior to diagnosis of fetal demise. Moreover, an EFW<50^{th} percentile at the last available examination carried a three- fold increased risk of fetal death using PRB/NICHD growth standard, which is consistent with Williams et al. who found that birthweight <50^{th} or >90^{th} percentile were associated with fetal death[54]. Others have reported that the stillbirth was associated with birthweight <40^{th} or ≥95^{th} percentiles[55], <75^{th} or ≥95^{th} percentiles[56] and <80^{th} or >95^{th} [57].

Customized versus non-customized fetal growth standards for prediction of antepartum fetal death

Sovio et al.[34] have found that the association between birthweight percentile and adverse neonatal outcome was similar when customized or non-customized birthweight or fetal growth

standards were applied in nulliparous women. Similarly, others observed no improvements by customized fetal growth standards relative to non-customized ones for the prediction of neonatal morbidity[58-60] and stillbirth,[59, 60]. However, we have recently demonstrated that there was a modest benefit in customized evaluation of fetal growth for prediction of perinatal mortality, yet the choice of the customized and non-customized standard being compared can also be a factor[58]. Although the PRB/NICHD standard was superior to Hadlock standard when a single ultrasound scan was considered for prediction of perinatal death[58], the two standards performed similarly when velocity of the percentiles were evaluated in the current study. Possible explanations are that 1) fetal growth velocity already accounts for some of the effects of maternal factors on fetal growth, and 2) the current study involved growth evaluation at earlier gestational ages compared to the previous report[58].

Clinical Implications

In the current study, 74% of cases with antepartum fetal death occurred in fetuses that were not SGA. Given that a meta-analysis of randomized control trials reported no reduction of perinatal death or perinatal morbidity by routine ultrasound examination in late pregnancy,[61] and the routine ultrasound examination is associated with a high iatrogenic prematurity rate among pregnancies incorrectly suspected with an SGA neonate,[62] the strategy to prevent antepartum fetal death must change, and the use of fetal growth velocity can be a useful tool for detection and prevention of this complication.

We found that a fetus with a decline of EFW percentile velocity <50th percentile among controls have a 4.7-fold increased risk to die antepartum using Hadlock or PRB/NICHD growth standards. These findings are in line with a recent definition of late FGR by 56 participating

experts[63] which consider that a decline of more than two quartiles in a growth chart is a criterion for late FGR. Previous studies have demonstrated that a decline in fetal growth velocity is a major determinant of adverse perinatal outcome both in small[31, 34, 53] and appropriately grown[64-67] fetuses. According to Chatzakis et al,[67] a fetal growth deceleration $\geq 50^{th}$ percentile in non-SGA fetuses was associated with increased risk for neonatal intensive care unit (NICU) admission (OR 1.8) and perinatal death (OR 3.8).

The result of the current study is also in line with reported relationship between low growth velocity and intrapartum operative delivery and admission to the NICU in both low-risk[33] and high-risk populations[53, 64, 68-70]. In addition, growth velocity of the fetal AC in the lowest decile distinguished SGA newborns who experience increased morbidity.[71] These observations indicate that fetal growth velocity has more clinical utility for identifying adverse perinatal outcomes or neonatal anthropometric features of FGR. [32, 36, 72]

Research Implications

The current study strengthens the importance of considering reduction in fetal growth velocity as a herald of antepartum fetal death. Given the moderate sensitivity (57% at 10% false positive rate) and low prevalence of fetal death, the prediction performance based on ultrasound alone is sub-optimal; hence, additional biochemical markers are needed to improve the prediction of fetal death. For instance, an abnormal low maternal plasma Angiogenic index-1 (ratio of placental growth factor [PlGF] to soluble fms-like tyrosine kinase 1 [sFlt-1]) determined at 24-28 weeks[29] or 30-34 weeks[28] of gestation carries a 29-fold and 23-fold increased risk for stillbirth, respectively. Doppler uterine velocimetry[73] or maternal serum alpha fetoprotein/pregnancy-associated plasma protein-A ratio[74] during the first trimester of pregnancy have been also proposed as potential predictors of stillbirth. Future studies that combine maternal

risk factors, placental biomarkers, and fetal growth velocity in pregnancy to predict stillbirth are warranted.

Strengths and Limitations

The strengths of the current study are: 1) this is the largest study of fetal growth velocity in fetal death in an African-American population; 2) patient enrollment took place at a single ultrasound unit and a consistent protocol was implemented to acquire ultrasound data by sonographers blinded to the clinical information, and 3) the use of both customized and non-customized standards to determine percentile velocity provided additional generalization to the findings. Since fetal growth velocity was expressed as the regression line slope of growth percentiles with gestational age based on two or more serial measurements prior to 32 weeks, a possible limitation is the irregularity in the distribution of gestational ages.

Conclusion

Antepartum fetal death is preceded by a significant decrease in fetal growth velocity. Given that three out of four antepartum fetal death cases had EFW > 10^{th} percentile at last scan when they were alive, fetal growth velocity percentile may be a useful tool for improving prediction of pregnancies at risk for antepartum fetal death. Longitudinal sonographic evaluations to determine growth velocity doubles the sensitivity for predicting antepartum fetal death compared to a single ultrasound examination.

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Table 1. Clinical Characteristics of the Study Population

Characteristics	Controls (n=4,262)	Cases (n=23)	p- value
Age in years old, median (IQR)	23(20-27)	20(19-28.5)	0.23
Height in centimeters, median (IQR)	162.56(157.48-167.64)	160.02(157.48-170.18)	0.92
Weight in kilograms , median (IQR)	73.03(60.78-89.81)	77.11(62.37-90.72)	0.77
Body mass index in kg/m2, median (IQR)	27.4(22.90-33.6)	30.45(22-35.78)	0.9
Parous women in %, (n)	63 (2683/4262)	52.2 (12/23)	0.29
Gestation age at delivery in weeks, median (IQR)	39.1(38-40.1)	28.6(23.7-30.35)	<.001
Neonatal weight in grams, median (IQR)	3155(2811.25-3475)	930(408.5-1295)	<.001
Cesarean delivery in % (n)	31.1 (1326/4262)	13 (3/23)	0.07
Spontaneous labor in % (n)	56.9 (2425/4261)	17.4 (4/23)	<.001
Induction of labor in % (n))	32.9 (1401/4262)	78.3 (18/23)	<.001
Fetal male sex in % (n)	51.2 (2181/4262)	60.9 (14/23)	0.41
Medical chronic conditions in % (n)	64.1 (2732/4262)	73.9 (17/23)	0.39
Smoking in % (n)	17.9 (764/4262)	17.4 (4/23)	1.0
Drugs abuse in % (n)	27.1 (1153/4262)	30.4 (7/23)	0.81
Alcohol abuse in % (n)	3.1 (133/4262)	4.3 (1/23)	0.52
Chronic hypertension in % (n)	6.0 (255/4262)	8.7 (2/23)	0.65
Gestational diabetes in % (n)	3.9 (165/4262)	8.7 (2/23)	0.23
Preeclampsia in % (n)	6.1 (260/4262)	13 (3/23)	0.16
Chronic hypertension with preeclampsia in % (n)	2.8 (120/4262)	8.7 (2/23)	0.14
Gestational hypertension in % (n)	12.2 (521/4262)	13 (3/23)	0.76
Placental abruption in % (n)	0.8 (34/4262)	13 (3/23)	0.001
Preterm labor in % (n)	6.5 (278/4262)	13 (3/23)	0.19
Preterm prelabor rupture of membranes in % (n)	3.3 (140/4262)	4.3 (1/23)	0.54
Clinical chorioamnionitis in % (n)	6 (257/4262)	8.7 (2/23)	0.65
Preterm delivery in % (n)	13.0 (552/4262)	91.3 (21/23)	<.001
Medically -indicated preterm delivery in % (n)	7.4 (316/4262	78.3 (18/23)	<.001
Spontaneous preterm labor with preterm delivery in % (n)	5.5 (236/4262)	13 (3/23)	0.13

%: percentage; IQR: Interquartile range

Table 2. Prediction performance for antepartum fetal death by estimated fetal weight percentile velocity. For each standard, the estimated fetal weight (EFW) percentile velocity are determined in cases and controls. Test positive is defined as EFW percentile velocity <p^{th} percentile of velocities among controls. Statistics are shown with 95% confidence intervals.

Figure Legends

Standard	Cut-off (% ile)	EFW percentile velocity in controls	Relative risk	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio
PRB/ NICHD	50th	-0.115	4.7 (1.6-13.77)	0.83 (0.61-0.95)	0.5 (0.48-0.52)	1.36 (1.65-2)	0.35 (0.14-0.85)
PRB/ NICHD	40th	-0.712	3.39 (1.4-8.21)	0.7 (0.47-0.87)	0.6 (0.58-0.62)	1.32 (1.74-2.29)	0.51 (0.27-0.94)
PRB/ NICHD	30th	-1.395	4.31 (1.84-10.13)	0.65 (0.43-0.84)	0.7 (0.68-0.72)	1.6 (2.17-2.95)	0.5 (0.28-0.87)
PRB/ NICHD	20th	-2.291	6.08 (2.65-13.98)	0.61 (0.39-0.8)	0.8 (0.78-0.82)	2.17 (3.04-4.26)	0.49 (0.29-0.81)
PRB/ NICHD	10th	-3.662	11.17 (4.94-25.23)	0.57 (0.34-0.77)	0.9 (0.89-0.91)	3.87 (5.64-8.22)	0.48 (0.3-0.77)
PRB/ NICHD	5th	-5.223	13.62 (6.08-30.53)	0.43 (0.23-0.66)	0.95 (0.94-0.96)	5.29 (8.68-14.23)	0.6 (0.42-0.85)
Hadlock	50th	-0.765	4.7 (1.6-13.77)	0.83 (0.61-0.95)	0.5 (0.48-0.52)	1.36 (1.65-2)	0.35 (0.14-0.85)
Hadlock	40th	-1.33	3.39 (1.4-8.21)	0.7 (0.47-0.87)	0.6 (0.58-0.62)	1.32 (1.74-2.29)	0.51 (0.27-0.94)
Hadlock	30th	-1.989	3.59 (1.56-8.25)	0.61 (0.39-0.8)	0.7 (0.68-0.72)	1.45 (2.03-2.83)	0.56 (0.34-0.93)
Hadlock	20th	-2.881	5.1 (2.25-11.56)	0.57 (0.34-0.77)	0.8 (0.78-0.82)	1.96 (2.83-4.08)	0.54 (0.34-0.87)
Hadlock	10th	-4.139	9.41 (4.19-21.13)	0.52 (0.31-0.73)	0.9 (0.89-0.91)	3.46 (5.21-7.83)	0.53 (0.35-0.81)
Hadlock	5th	-5.467	13.62 (6.08-30.53)	0.43 (0.23-0.66)	0.95 (0.94-0.96)	5.29 (8.68-14.23)	0.6 (0.42-0.85)

Figure 1. Longitudinal estimated fetal weight (EFW) percentiles as a function of gestational age. The figure shows EFW percentiles according to the customized PRB/NICHD growth standard (A), and the Hadlock growth standard (B) for 28 cases of fetal death. There was a downward trend of EFW percentiles with advancing gestation. Only 28.6% (8/28) of cases had an EFW <10th percentile at the last scan, using either of the two fetal growth standards. The red horizontal line shows the 10th percentile line.

Figure 2. Estimated fetal weight (EFW) percentiles at the first and last ultrasound examinations. The figure shows EFW percentiles before 31 weeks in the study group according to the PRB/NICHD growth standard (A), and the Hadlock growth standard (B). There was no significant difference in the median percentile between cases and controls at the first ultrasound examination. However, the EFW percentiles of cases were lower than those of controls at the last ultrasound examination. IQR: Interquartile range.

Figure 3. Differences in estimated fetal weight (EFW) percentile velocity between cases and controls. The figure shows EFW percentile velocity according to the PRB/NICHD standard (A), and the Hadlock standard (B). EFW velocity was calculated as the change in the EFW percentile per week by fitting a linear regression model to the percentile values of each patient. Cases had significantly lower EFW velocity compared to controls, according to the two growth standards.

Figure 4. Receiver Operating Characteristic (ROC) curve for the prediction of antepartum fetal death by a low growth velocity. The ROC curves are constructed from EFW percentile velocity data based on the PRB/NICHD growth standard, and the Hadlock growth standards. AUC: area under the ROC curve. 95% confidence intervals are provided.

Figure 5. Prediction of antepartum fetal death by non-customized percentiles velocity. ROC curves were obtained based on percentile velocity of estimated fetal weight (EFW), fetal head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) based on non-customized PRB/NICHD (A) and Hadlock (B) standards. AUC: area under the ROC curve. 95% confidence intervals are provided.

Figure S1. Differences in estimated fetal weight (EFW) percentile velocity between cases and controls by ethnicity. The figure shows EFW percentile velocity according to the Hadlock

standard. EFW velocity was calculated as the change in the EFW percentile per week by fitting a linear regression model to the percentile values of each patient. Overall cases had significantly lower EFW velocity compared to controls. Note, the only non-African American case shown in the figure had two scans at 15 and 19 weeks, and hence the velocity calculation was likely less reliable.

Figure S2. Prediction of antepartum fetal death by percentile velocity (PRB/NICHD standard) and velocity percentile (NICHD standard). ROC curves were obtained based on percentile velocity of estimated fetal weight (EFW) calculated based on first and last scan in the interval from 14 to 32 weeks (PRB/NICHD). The same data was used as input in the NICHD velocity percentile calculator for African American women (https://www.nichd.nih.gov/fetalvelocitycalculator). AUC: area under the ROC curve. 95% confidence intervals are provided.