**Title page**

**Phase-rectified signal averaging method to predict perinatal outcome in infants with very preterm fetal growth restriction- a secondary analysis of TRUFFLE-trial**

Contributors: All authors have been involved in the conception or the study design of the project. Lobmaier SM and Schneider KTM devised and proposed the study. Mensing van Charante N, Lees CC and Schmidt G contributed to design. Lobmaier SM, Müller A and Haller B undertook mathematical and statistical analysis. Lobmaier SM, Ganzevoort W, Giussani DA, Shaw CJ, Ortiz JU, Ostermayer E, Prefumo F, Frusca T, Hecher K, Arabin B, Thilaganathan B, Papageorghiou AT, Bhide A, Martinelli P, Duvekot JJ, van Eyck J, Visser GHA, Ferrazzi E, Lees CC, Schneider KTM and the TRUFFLE investigators contributed to data acquisition and drafted the submitted article and revised critically for important intellectual content.

Declaration of interests: CCL is supported by the UK National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare National Health Service Trust and Imperial College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. All other authors declare no competing interests.

**Short title: Phase-rectified signal averaging method applied to TRUFFLE raw data**

**Phase-rectified signal averaging method to predict perinatal outcome in infants with very preterm fetal growth restriction- a secondary analysis of TRUFFLE-trial**

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**Abstract**

Background: Phase-rectified signal averaging, an innovative signal processing technique, can be used to investigate quasi-periodic oscillations in noisy, non-stationary signals obtained from fetal heart rate. Phase-rectified signal averaging is currently the best method to predict survival after myocardial infarction in adult cardiology. Application of this method to fetal medicine has established significantly better identification than with short term variation by computerized cardiotocography of growth restricted fetuses.

Objective: The aim of this study was to determine the longitudinal progression of phase-rectified signal averaging indices in severely growth restricted human fetuses and the prognostic accuracy of the technique in relation to perinatal and neurological outcome.

Study design: Raw data from cardiotocography monitoring of 279 human fetuses were obtained from eight centers taking part in the multicenter European “TRUFFLE” trial on optimal timing of delivery in fetal growth restriction. Average acceleration and deceleration capacities were calculated by phase-rectified signal averaging to establish progression from 5 days to 1 day prior to delivery and compared with short term variation progression. The receiver operating characteristic curves of average acceleration and deceleration capacities and short term variation were calculated and compared between techniques for short- and intermediate-term outcome.

Results: Average acceleration and deceleration capacities and short term variation showed a progressive decrease in their diagnostic indices of fetal health from the first exam five days prior to delivery to one day before delivery. However, this decrease was significant three days before delivery for average acceleration and deceleration capacities, but two days before delivery for short term variation. Compared with analysis of changes in short term variation, analysis of delta average acceleration and deceleration capacities showed a tendency to better predict infant acidosis (pH<7.10) and values of Apgar<7 as well as antenatal death.

Conclusion: Phase-rectified signal averaging method seems to be not inferior to short term variation to monitor progressive cardiovascular dysfunction of severely growth restricted fetuses. Our findings suggest that for short term outcomes such as Apgar score, phase-rectified signal averaging indices could even be a better test than short term variation. Overall our findings confirm the possible value of prospective trials based on PRSA indices of autonomic nervous system of severely growth restricted fetuses.

**Key words:** fetal growth restriction, FGR, intrauterine growth restriction, IUGR, short- term variation, STV, phase-rectified signal averaging, PRSA, CTG, **Introduction**

The variability in heart rate is determined by several mechanisms including opposing sympathetic and vagal influences of the autonomic nervous system in addition to respiratory, baroreflex and circadian processes 1. Its analysis has long been established as a useful predictor of cardiovascular health in the fetal, newborn and adult periods 2,3. Human fetuses affected with severe growth restriction show a decrease in fetal heart rate (FHR) variability. Short term variation (STV), a calculated measure designed to make assessment of FHR variability quantitative, has proven predictive of fetal distress in the antenatal setting. Values for STV below 2.6 ms are known to be highly associated with fetal asphyxia and/or intrauterine death whereas STV values above 3 ms are rarely associated with adverse outcome 4.

In contrast to other methods of analysis of FHR variability, phase-rectified signal averaging (PRSA) permits the detection of quasi-periodicities in non-stationary, noisy variables, such as fetal heart rate, thereby allowing complex oscillatory modulations of multiple frequency drivers to be determined, rather than simply describing the degree of variability from the baseline. PRSA predicts survival after myocardial infarction in adult cardiology 5 and it has been successfully investigated in fetal medicine 6, despite the challenges of a non-stationary signal, with more interference in the signal obtained than the post-infarct adult. PRSA, in short, calculates not only the variation of the fetal heart rate, but the speed of changes in fetal heart rate, described as the average acceleration (AAC) and deceleration (ADC) capacities. The novel parameter AAC better differentiates growth restricted fetuses from controls than analysis by STV 6-8. Accurate prediction of fetal growth restriction by PRSA analysis has also been confirmed by investigators comparing data both from Doppler 9,10 and trans-abdominal fetal ECG 11,12 signals.

Even acute intra-partum hypoxia might be better predicted using PRSA than STV 13-15 analysis. Therefore, analysis of alterations in FHR by PRSA holds potential in predicting value of acute as well as chronic fetal hypoxia in complicated pregnancy. However, this has not been tested systematically in large cohorts.

The most comprehensive, multi-center study of human early fetal growth restriction (FGR) is the Trial of Umbilical and Fetal Flow in Europe (TRUFFLE) 16. In TRUFFLE, more than 500 pregnancies were monitored for fetal health surveillance using Doppler indices in the ductus venosus or STV determined by computerized cardiotocography (c-CTG). Perinatal outcome as well as intermediate neurological outcome at two years of age were also determined. The aim of this study was to apply PRSA analysis to FHR data obtained from the TRUFFLE cohort to compare the prognostic value between PRSA and STV in predicting adverse perinatal and neurological outcome in severely growth restricted human fetuses.

**Materials and Methods**

The TRUFFLE clinical trial was a prospective, multicenter randomized study performed in five European countries and 20 tertiary care centers. Women were eligible for inclusion if they had a singleton pregnancy between 26 and 31+6 weeks of gestation affected by fetal growth restriction defined as a fetal abdominal circumference below the 10th percentile and abnormal umbilical artery Doppler with a pulsatility index (PI) above the 95th percentile. Exclusion criteria were ultrasound appearances suggestive of congenital fetal abnormality, abnormal karyotype on invasive testing or women younger than 18 years of age. The study protocol was approved by the institutional ethics committee and patients provided written informed consent. Participants were randomly assigned to one of three groups (c-CTG STV reduction, early or late ductus venous changes) to establish the timing of delivery. Baseline maternal and fetal characteristics were collected at study entry.

In the CTG randomization arm the timing of delivery was decided on the following cut-off values: STV < 3.5 ms at < 29 weeks of gestation or STV < 4 ms at ≥ 29 weeks of gestation. In cases where maternal corticosteroids were given to accelerate fetal lung maturation, no decision regarding delivery was made on the grounds of reduced STV up to 72h after the first intramuscular dose, as maternal corticosteroids are known to produce short term reductions in FHR variability 17-20. Monitoring in all three groups included umbilical artery Doppler and c-CTG was recommended at least once a week. However, most centers performed c-CTGs more frequently, subject to local policies. “Safety net” criteria, which prompted delivery regardless of any other measures, including spontaneous fetal heart rate decelerations, or assigned to a study group with a STV < 2.6 ms at 26+0 - 28+6 weeks or STV < 3 ms at ≥ 29 weeks of gestation. Furthermore, delivery was recommended if reversed umbilical artery end diastolic flow occurred ≥ 30 weeks of gestation or if there was absent umbilical artery end diastolic flow at ≥ 32 weeks gestation. Further details about the study protocol can be derived from the original publication 16.

All participating centers were invited to provide c-CTG raw data for this secondary analysis, and all registrations available in the five days preceding delivery or antenatal fetal death were selected for inclusion. From all participating centers, eight of twenty were able to provide appropriate c-CTG raw data (Amsterdam, Brescia, Hamburg, London, Munich, Naples, Rotterdam, Zwolle). The complete c-CTG signal was used for analysis. Data were analyzed by Sonicaid FetalCare software for STV 21,22 and by the PRSA method for AAC and ADC calculation previously described in detail by Lobmaier et al. 6. For PRSA, data were analyzed off-line after computer download, and the following parameters were used: T = 10 s, L = 100 s, anchor points were defined as increases (AAC) or decreases (ADC) <5%.

Delta (Δ) values of AAC, ADC and STV were calculated, including the difference between first (5-4 days prior to delivery) and last (<24h prior to delivery) value before delivery or intrauterine fetal death.

Statistical analysis was performed using SPSS for Windows (version 22.0, SPSS Inc., Chicago, IL, USA) and R (version 3.2.2, R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>) with its package pROC. (pROC: an open-source package for R and S+ to analyze and compare ROC curves). For comparison of mean values at different time points an analysis of variance for repeated measurements was performed. If a significant change of mean values over time was observed in the ANOVA, Student’s t- test for paired data was used for comparison of consecutive time points and for a comparison of the first (5-4 days before delivery) to the last (within 24 hours before delivery) measurement. The diagnostic effectiveness of the different c-CTG parameters for outcome prediction was analyzed using the area under the receiver operating characteristic (ROC) curve (AUC). For estimation of confidence intervals and to test the difference between two AUCs, 2000 bootstrap samples stratified for the outcome of interest were drawn. All statistical comparisons were conducted two-sided and a p value <0.05 was considered statistically significant.

**Results**

A total of 279 fetuses and 947 c-CTG records were available for secondary analysis for this study. From one center (Rotterdam) STV data could not be extracted for technical reasons, so that only PRSA indices were available for Rotterdam patients. Data for the study population demographic characteristics, obstetric and neonatal outcome are summarized in Table 1. Considering adverse outcomes, 11.1% of the neonates had a 5- minute Apgar below 7 and 3.2% had an umbilical artery pH below 7.1, suggestive of poor condition at birth in these infants. A Bayley score developmental quotient (DQ) < 95 was observed in 22.9% and a score below 85 in 5.0% at the two years follow-up, suggestive of moderate developmental disability in these infants.

Table 1: Study population characteristics. Data are reported as number and percentage in brackets, and as mean and SD in brackets.

|  |  |
| --- | --- |
| **Study population :** | Total n=279 |
| **Demographic and clinical characteristics:** |   |
| Mean maternal age (years) | 30.5 (5.6) |
| Caucasian ethnicity | 221 (79.2%) |
| Nulliparous | 174 (62.4%) |
| Mean BMI (kg/m²) | 25.4 (6.0) |
| Smoking | 50 (17.9%) |
| Diabetes | 2 (0.7%) |
| Chronic hypertension | 28 (10.0%) |
| Renal morbidity | 5 (1.8%) |
| Other medical disease | 46 (16.5%) |
| Any gestational hypertensive disease  | 53 (19.0%) |
| Pre-eclampsia/HELLP | 122 (43.7%) |
| Mean gestational age at entry (weeks) | 29.1 (10.5) |
| Mean EFW by ultrasound (grams) | 886.2 (210.1) |
| Mean UA PI | 2.01 (0.58) |
| Umbilical artery AREDF | 111 (39.8%) |
| Mean U/C ratio | 1.48 (0.61) |
| Mean DV PI | 0.60 (0.02) |
|  |  |
| **Obstetric outcome:** |   |
| Mean GA at delivery (weeks) | 30.6 (2.0) |
| Mean interval to delivery (days) | 10.2 (10.2) |
| Cesarean delivery | 272 (97.5%) |
| Mean birthweight (grams) | 1000.8 (280.9) |
| Male sex | 137 (49.1%) |
| Apgar score <7 | 31 (11.1%) |
| UA pH |  |
| Data available | 202 (72.4%) |
| Mean pH | 7.26 (0.08) |
| <7.0 | 4 (1.4%) |
| <7.1 | 9 (3.2%) |
|  |  |
| **Neonatal outcome:** |   |
| Livebirth | 255 (91.4%) |
| Neonatal death | 18 (6.4%) |
| Antenatal death | 6 (2.2%) |
| Bayley III test performed | 219 (78.5%) |
| DQ<85 | 14 (5.0%) |
| DQ<95 | 60 (22.9%) |
|  |  |
|  |  |
| EFW: estimated fetal weight; UA: umbilical artery; PI: pulsatility index; AREDF: Absent or reversed end diastolic ﬂow; U/C ratio: umbilical artery pulsatility index to median cerebral artery pulsatility index ratio; DV: ductus venosus; GA: gestational age; DQ: developmental quotient |

Mean values of AAC, ADC and STV at 4 different time points (5-4 days prior to delivery, 72-48 h, 48-24 h, <24 h) were calculated and compared as displayed in Figure 1. At 5-4 days compared to <24 h prior to delivery AAC was reduced from 1.97 (SD 0.39) to 1.69 (0.45), ADC from 1.95 (0.40) to 1.69 (0.48) and STV from 6.07 (2.14) to 4.71 (2.14). Although a progressive decrease in all three indices of fetal health was obtained towards delivery, the decrease for AAC and ADC became significant 72 hours prior to performed delivery, while the decrease in STV became statistically significant < 48 h prior to delivery.







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Figure 1 a-c: Longitudinal changes of AAC (A), ADC (B) and STV (C) during the 5 days prior to delivery or intrauterine death.

The area under the curve (AUC) value was calculated for each main perinatal outcome. Table 2 shows the results of the ROC curve comparisons calculated by means of the delta values for each variable between the 5-4 days to 24 hours prior to delivery time interval or using the last index within 24 h prior to delivery. Although changes in AAC and ADC showed a general trend towards better predictive performance for adverse outcomes than STV, this was significant only for ∆AAC in the prediction of antenatal death and for ADC in the 24 h before delivery to predict an Apgar score < 7. Neither ∆AAC, ∆ADC, ∆STV nor AAC, ADC or STV in the last 24 h before delivery showed predictive power for developmental disability at 2 years of age (Bayley DQ < 95 or < 85).

Table 2: Comparison of Areas under the ROC for indices of fetal heart rate variability. (95% confidence interval in brackets)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | ∆AAC | ∆ADC | ∆STV | AAC1 | ADC1 | STV1 |
| **Apgar < 7** | .67 (.50-.82) | .64 (.47-.79) | .61 (.43-.78) | .63 (.49-.76) | .64 (.52-.76)\* | .53 (.40-.65) |
| **pH < 7.1** | .72 (.40-.95) | .71 (.38-.96) | .70 (.40-.96) | .68 (.45-.88) | .68 (.44-.90) | .74 (.56-.89) |
| **Antenatal death** | .62 (.19-.1.0)\* | .54 (.06-.1.0) | .56 (.13-.97) | .72 (.40-.96) | .72 (.38-.95) | .51 (.28-.86) |
|  |  |  |  |  |  |  |

∆ = AUC obtained for each parameter when differences between first CTG available (5-4 days prior to delivery) and last CTG (within 24 hours prior to delivery). Only cases with valid AAC, ADC AND STV values were compared.

1 = AUC obtained for each parameter within 24h prior to delivery

\* = significant difference at p<0.05 versus STV

**Comment**

The aim of this study based on data obtained from severely growth restricted human fetuses in the TRUFFLE study was to determine if average acceleration and deceleration capacities (AAC, ADC) calculated by phase-rectified signal averaging (PRSA) analysis could predict adverse outcome better than analysis of fetal heart rate short term variation (STV) measured by computerized CTG (c-CTG).

The main finding of this analysis is represented by the longitudinal progression of AAC and ADC compared with STV. All three parameters showed a decrease in their diagnostic indices from the first exam, five days prior to delivery to one day before delivery. However, this decrease became statistically significant three days prior to delivery for AAC and ADC, and two days prior to delivery for STV. PRSA method proved not only to compare well with a standard of care such as STV, but to yield even earlier signs of a worsening control of cardiovascular function by fetal autonomic nervous system (ANS).

Further, fetuses with more pronounced decrements from baseline in AAC and ADC as measured by greater ∆AAC and ∆ADC showed a tendency for increased risk for adverse perinatal outcome. Areas under the Receiver Operator Curves (ROC) of PRSA indices were significantly higher for the Apgar score <7 and antenatal death compared to conventional c-CTG calculation.

The analysis in the present study has some limitations. The total number of adverse events in this study was not very high 23, thereby limiting the prognostic accuracy of the results regarding perinatal outcome. Furthermore, raw data were available in a subset of 279 out of 503 TRUFFLE patients.

The PRSA derived indices AAC and ADC describe the speed of changes in fetal heart rate, triggered by sympathetic and vagal branches, reflecting fetal ANS capacity. Although changes in STV, and now PRSA, have been demonstrated signal deteriorating fetal condition, the corresponding changes in the developing fetal ANS that underlie this reduction in heart rate variability have not been fully elucidated. It is well established from data derived from human pregnancy 13-15 and from animal models 15,24,25 that acute fetal hypoxia leads to activation of the fetal ANS. For instance, acute fetal hypoxia in ovine pregnancy leads to an elevation in fetal plasma catecholamine levels 26, increased fetal renal sympathetic nerve discharge 27 and fetal treatment with sympathetic antagonists markedly impairs fetal cardiovascular responses to acute hypoxia 28,29. Conversely, the effect of chronic fetal hypoxia on fetal sympathetic activity is less clear. However, accumulated evidence is beginning to suggest that chronic fetal hypoxia leads to marked alterations in sympathetic ANS activity. For instance, two studies have reported elevated basal values and alterations in the developmental decline of fetal heart rate with advancing gestation in the chronically hypoxic sheep fetus 30,31. One very recent study of in vivo continuous wireless recording of fetal cardiovascular function in fetal sheep exposed to significant chronic hypoxia for 10 days in late gestation confirmed a delayed ontogenic fall in fetal heart rate with advancing gestation in the chronically hypoxic sheep fetus 31.

In contrast, acute fetal hypoxia causes an immediate increase in short-term fetal heart rate variation followed by a gradual decrease when fetal hypoxia becomes chronic 32. The initial increase in FHR variability is likely caused by the increase of fetal plasma catecholamine concentration 27 but heart rate variability decreases in spite of persistent elevations in fetal plasma norepinephrine and arterial blood pressure during chronic hypoxia 32. Murotsuki et al. hypothesized that chronic fetal hypoxia might alter the normal maturational control of fetal heart rate and its variability. Data of wireless recording of fetal cardiovascular function in fetal sheep exposed to significant chronic hypoxia for 10 days in late gestation is in keeping with this hypothesis. Shaw et al. 33 reported that in normoxic pregnancy the fetal heart rate decreased and SDNN (an index of total fetal heart rate variability) increased with advancing gestation. Conversely, advancing gestation in hypoxic pregnancy led to a greater fall in fetal heart rate, a decrease in sympathetic activity, and a loss of total heart rate variation.

Therefore, chronic significant fetal hypoxia appears to be associated with a loss of total power and increased parasympathetic dominance in the fetal heart rate variation power spectra 33. Temporary reductions in STV have been linked with decreased sympathetic control of FHR variability 34 and reductions in the sympathetic control of FHR variability have been observed in chronically hypoxic and growth restricted human fetuses 35, although no evaluation of STV was made in these studies. Rivolta et al. 15 reported the first *in vivo* evaluation of PRSA on FHR analysis in late gestation fetal sheep: acute fetal hypoxia led to an increase in the AAC and ADC values, confirming activation of the fetal ANS by PRSA. Data in the present study show that fetal growth restriction in human pregnancy is associated with a progressive fall in AAC and ADC values. This finding is not only in keeping with the chronically hypoxic sheep fetus showing impaired or perhaps exhausted activation of the sympathetic component of the ANS influences on fetal heart rate variation 33, but it highlights that PRSA may be useful in predicting fetal deterioration associated with chronic fetal hypoxia. In this context, it is interesting that PRSA predicted earlier deterioration of fetal health associated with adverse outcome than STV. It is possible that the use of PRSA to quantify the speed, rather than magnitude, of changes in the fetal heart rate could indicate reduced responsiveness of the sympathetic nervous system. This would precede exhaustion of sympathetic nervous control and acute reduction in STV, as in our data, however this remains conjecture.

In summary, analysis of fetal heart rate by PRSA identifies progressive cardiovascular dysfunction in severely growth restricted human fetuses slightly earlier than STV. These data suggest that further investigation of the value and implementation of PRSA in monitoring fetal health in human clinical practice is warranted, perhaps with a particular relevance for alterations in autonomic nervous system function in severely growth restricted human fetuses.

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