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

Silvia M. Lobmaier, MD, Nico Mensing van Charante, MD, Enrico Ferrazzi, Prof., Dino A. Giussani, Prof., Caroline J. Shaw, MD, Alexander Müller, MSc, Javier U. Ortiz, MD, Eva Ostermayer, MD, Bernhard Haller, MSc, Federico Prefumo, MD, Tiziana Frusca, MD, Kurt Hecher, Prof., Birgit Arabin, Prof., Baskaran Thilaganathan, Prof., Aris T. Papageorghiou, MD, Amarnath Bhide, MD, Pasquale Martinelli, Prof., Johannes J. Duvekot, MD, Jim van Eyck, MD, Gerard H.A. Visser, Prof., Georg Schmidt, Prof., Wessel Ganzevoort, MD, Christoph C Lees, MD, Karl T.M. Schneider, Prof., and TRUFFLE investigators*

*TRUFFLE investigators: Caterina M. Bilardo (Amsterdam/Groningen), Christoph Brezinka (Innsbruck), Anke Diemert (Hamburg), Jan B. Derks (Utrecht), Dietmar Schlembach (Graz/Jena), Tullia Todros (Turin), Adriana Valcamonico (Brescia), Neil Marlow (London), Aleid van Wassenaer-Leemhuis (Amsterdam)

We would like to publish Table 2 in the print issue.

Frauenklinik und Poliklinik, Technische Universität München, Munich, Germany (SM Lobmaier, JU Ortiz, E Ostermayer, KTM Schneider)

Department of Obstetrics and Gynecology, Academic Medical Centre Amsterdam, Netherlands (N Mensing van Charante, W Ganzevoort)

Children´s Hospital Buzzi, University of Milan, Milan, Italy (E Ferrazzi)

Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK (DA Giussani)

Department of Surgery and Cancer, Imperial College London, London, UK (CJ Shaw, CC Lees)

1. Medizinische Klinik und Deutsches Herzzentrum München der Technischen Universität München, Munich, Germany (A Müller, G Schmidt)

Institute for Medical Statistics and Epidemiology (IMSE), Klinikum rechts der Isar der Technischen Universität München, Munich, Germany (B Haller)

Maternal-Fetal Medicine Unit, University of Brescia, Brescia, Italy (F Prefumo, T Frusca)

Department of Obstetrics and Fetal Medicine, University Medical Center, Hamburg-Eppendorf, Germany (K Hecher)

Department of Perinatology, Isala Clinics, Zwolle, Overijssel, Netherlands (B Arabin, J van Eyck)

Fetal Medicine Unit, St George's Hospital, St George's University of London, London, United Kingdom (B Thilaganathan, AT Papageorghiou, A Bhide)

Department of Neuroscience, Reproductive Science and Dentistry, University of Naples Federico II, Napoli, Italy (P Martinelli)

Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, Netherlands (JJ Duvekot)

Department of Perinatal Medicine, University Medical Center, Utrecht, Netherlands (GHA Visser)

Department of Development and Regeneration, KU Leuven, Leuven, Belgium (CC Lees)

Corresponding author:

Silvia M. Lobmaier

Ismaninger Str. 22

D- 81675 München (Germany)

Telefone number: +49 89 4140 5417

Fax: +49 89 4140 4892

Email: silvia.lobmaier@tum.de

Word count abstract: 359 words
Word count main text: 2499 words

Condensation: We performed a secondary analysis of TRUFFLE trial CTG data to determine the longitudinal progression and prognostic accuracy of phase-rectified signal averaging indices in severely growth restricted fetuses.

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

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 better predicted values of Apgar<7 as well as antenatal death (area under the curve for prediction of antenatal death: delta average acceleration capacity .62 (.19 - 1.0 confidence interval), delta short term variation .54 (.13 - .97), p=.006; area under the curve for prediction Apgar<7: average deceleration capacity <24h before delivery .64 (.52 - .76 confidence interval), short term variation <24h before delivery .53 (.40 - .65), p=.015). Neither phase-rectified signal averaging

indices nor short term variation showed predictive power for developmental disability at 2 years of age (Bayley developmental quotient < 95 or < 85).

Conclusion: Phase-rectified signal averaging method seems to be at least as good as short term variation to monitor progressive deterioration 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 phase-rectified signal averaging indices of autonomic nervous system of severely growth restricted fetuses.

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 metabolic acidaemia (defined as umbilical artery base deficit greater than 12 mmol/L) 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, typical signals represented by the 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 for first time the longitudinal changes ofPRSA and STV and the prognostic value for 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. Therefore four time windows were selected. 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. According to protocol c-CTGs were recorded using Sonicaid System 8002 (Oxford Instruments Medical Ltd, Surrey, UK). Data were analyzed directly for STV using the original Dawes/Redman algorithm [21,23] 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 samples, L = 100 samples, anchor points were defined as increases (AAC) or decreases (ADC) <5%. CTG data do not reflect real beat-to-beat heart rates. CTG technique works with a sample frequency of 4 Hz. That means that 4 times per second the fetal heart rate is detected by ultrasound, so, the used filter of T = 10 samples corresponds to 2,5 seconds and T= 100 samples to 25 seconds. Not all centers disposed of a computer connected to the CTG device. That´s the reason for missing CTG raw data from the centers not providing data.

Delta (Δ) values of AAC, ADC and STV were calculated, taking 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/). 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). Confidence intervals for the AUCs were estimated based on 2000 bootstrap samples, each. Comparisons between AUCs was performed using the test proposed by Hanley et al. [24], with standard deviation of the difference between the AUCs estimated from 2000 bootstrap samples. The function *roc.test* provided in the R library *pROC* [25] was used therefore. 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 (3.4 CTG records per fetus) 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.

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.

The area under the ROC curve (AUC) value was calculated for each main perinatal outcome. Table 2 shows the results of the ROC curve comparisons considering 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).

Comment

The aim of this study based on data obtained from severely growth restricted human fetuses in the TRUFFLE study was to assess the predictive value of average acceleration and deceleration capacities (AAC, ADC) calculated by phase-rectified signal averaging (PRSA) analysis compared to 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. Comparing these data to published reference curves we can see that these changes seem to be due to impairment of the intrauterine situation. In healthy fetuses AAC and ADC remain relatively constant during pregnancy [8]. 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 [26], thereby limiting the precision of the estimated measures of 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 [15,27,28] 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 [29], increased fetal renal sympathetic nerve discharge [30] and fetal treatment with sympathetic antagonists markedly impairs fetal cardiovascular responses to acute hypoxia [31,32]. 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 [33,34].

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 fall in fetal heart rate with advancing gestation [34].

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 [35]. The initial increase in FHR variability is likely caused by the increase of fetal plasma catecholamine concentration [30] but heart rate variability decreases in spite of persistent elevations in fetal plasma norepinephrine and arterial blood pressure during chronic hypoxia [35]. 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. [36] 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 [36]. Temporary reductions in STV have been linked with decreased

sympathetic control of FHR variability [37] and reductions in the sympathetic control of FHR variability have been observed in chronically hypoxic and growth restricted human fetuses [38], 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 [36], 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 speedof 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.

These findings cast a stronger link between cardiovascular and neuroendocrine damages in fetal life and cardiovascular events later in adult life [39,40]. Long term follow-up studies could prove or disprove the possible role of PRSA as a better predictor of impaired cardiovascular outcome at later age. On the other hand PRSA analysis might be of interest in other population like pregnancies affected by gestational diabetes. These fetuses are at high risk for developing hypertension in later life and PRSA might help to identify those fetuses.

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.

Acknowledgment

References

Uncategorized References

1.   Jensen EC, Bennet L, Guild SJ, Booth LC, Stewart J, Gunn AJ. The role of the neural sympathetic and parasympathetic systems in diurnal and sleep state-related cardiovascular rhythms in the late-gestation ovine fetus. *Am J Physiol Regul Integr Comp Physiol* 2009; **297**(4): R998-R1008.

2.   Henson GL, Dawes GS, Redman CW. Antenatal fetal heart-rate variability in relation to fetal acid-base status at caesarean section. *Br J Obstet Gynaecol* 1983; **90**(6): 516-21.

3.   La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 1998; **351**(9101): 478-84.

4.   Dawes GS, Moulden M, Redman CW. Short-term fetal heart rate variation, decelerations, and umbilical flow velocity waveforms before labor. *Obstet Gynecol* 1992; **80**(4): 673-8.

5.   Bauer A, Kantelhardt JW, Barthel P, et al. Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: cohort study. *Lancet* 2006; **367**(9523): 1674-81.

6.   Lobmaier SM, Huhn EA, Pildner von Steinburg S, et al. Phase-rectified signal averaging as a new method for surveillance of growth restricted fetuses. *J Matern Fetal Neonatal Med* 2012; **25**(12): 2523-8.

7.   Huhn EA, Lobmaier S, Fischer T, et al. New computerized fetal heart rate analysis for surveillance of intrauterine growth restriction. *Prenat Diagn* 2011; **31**(5): 509-14.

8.   Graatsma EM, Mulder EJ, Vasak B, et al. Average acceleration and deceleration capacity of fetal heart rate in normal pregnancy and in pregnancies complicated by fetal growth restriction. *J Matern Fetal Neonatal Med* 2012; **25**(12): 2517-22.

9.   Fanelli A, Magenes G, Campanile M, Signorini MG. Quantitative assessment of fetal well-being through CTG recordings: a new parameter based on phase-rectified signal average. *IEEE J Biomed Health Inform* 2013; **17**(5): 959-66.

10.   Signorini MG, Fanelli A, Magenes G. Monitoring fetal heart rate during pregnancy: contributions from advanced signal processing and wearable technology. *Comput Math Methods Med* 2014; **2014**: 707581.

11.   Stampalija T, Casati D, Montico M, et al. Parameters influence on acceleration and deceleration capacity based on trans-abdominal ECG in early fetal growth restriction at different gestational age epochs. *Eur J Obstet Gynecol Reprod Biol* 2015; **188**: 104-12.

12.   Stampalija T, Casati D, Monasta L, et al. Brain sparing effect in growth-restricted fetuses is associated with decreased cardiac acceleration and deceleration capacities: a case-control study. *BJOG* 2015.

13.   Aye CY, Redman CW, Georgieva A. The effect of augmentation of labour with syntocinon on the fetal CTG using objective computerised analysis: a nested case-control study. *Eur J Obstet Gynecol Reprod Biol* 2014; **176**: 112-8.

14.   Georgieva A, Papageorghiou AT, Payne SJ, Moulden M, Redman CW. Phase-rectified signal averaging for intrapartum electronic fetal heart rate monitoring is related to acidaemia at birth. *BJOG* 2014; **121**(7): 889-94.

15.   Rivolta MW, Stampalija T, Casati D, et al. Acceleration and deceleration capacity of fetal heart rate in an in-vivo sheep model. *PLoS One* 2014; **9**(8): e104193.

16.   Lees CC, Marlow N, van Wassenaer-Leemhuis A, et al. 2 year neurodevelopmental and intermediate perinatal outcomes in infants with very preterm fetal growth restriction (TRUFFLE): a randomised trial. *Lancet* 2015; **385**(9983): 2162-72.

17.   Derks JB, Mulder EJ, Visser GH. The effects of maternal betamethasone administration on the fetus. *Br J Obstet Gynaecol* 1995; **102**(1): 40-6.

18.   Koenen SV, Mulder EJ, Wijnberger LD, Visser GH. Transient loss of the diurnal rhythms of fetal movements, heart rate, and its variation after maternal betamethasone administration. *Pediatr Res* 2005; **57**(5 Pt 1): 662-6.

19.   Mulder EJ, Derks JB, Zonneveld MF, Bruinse HW, Visser GH. Transient reduction in fetal activity and heart rate variation after maternal betamethasone administration. *Early Hum Dev* 1994; **36**(1): 49-60.

20.   Mulder EJ, de Heus R, Visser GH. Antenatal corticosteroid therapy: short-term effects on fetal behaviour and haemodynamics. *Semin Fetal Neonatal Med* 2009; **14**(3): 151-6.

21.   Dawes GS, Visser GH, Goodman JD, Redman CW. Numerical analysis of the human fetal heart rate: the quality of ultrasound records. *Am J Obstet Gynecol* 1981; **141**(1): 43-52.

22.   Street P, Dawes GS, Moulden M, Redman CW. Short-term variation in abnormal antenatal fetal heart rate records. *Am J Obstet Gynecol* 1991; **165**(3): 515-23.

23.   Pardey J, Moulden M, Redman CW. A computer system for the numerical analysis of nonstress tests. *Am J Obstet Gynecol* 2002; **186**(5): 1095-103.

24.   Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983; **148**(3): 839-43.

25.   Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011; **12**: 77.

26.   Lees C, Marlow N, Arabin B, et al. Perinatal morbidity and mortality in early-onset fetal growth restriction: cohort outcomes of the trial of randomized umbilical and fetal flow in Europe (TRUFFLE). *Ultrasound Obstet Gynecol* 2013; **42**(4): 400-8.

27.   Giussani DA, Spencer JA, Moore PJ, Bennet L, Hanson MA. Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol* 1993; **461**: 431-49.

28.   Giussani DA. The fetal brain sparing response to hypoxia: physiological mechanisms. *J Physiol* 2015.

29.   Fletcher AJ, Gardner DS, Edwards CM, Fowden AL, Giussani DA. Development of the ovine fetal cardiovascular defense to hypoxemia towards full term. *Am J Physiol Heart Circ Physiol* 2006; **291**(6): H3023-34.

30.   Booth LC, Malpas SC, Barrett CJ, Guild SJ, Gunn AJ, Bennet L. Renal sympathetic nerve activity during asphyxia in fetal sheep. *Am J Physiol Regul Integr Comp Physiol* 2012; **303**(1): R30-8.

31.   Reuss ML, Parer JT, Harris JL, Krueger TR. Hemodynamic effects of alpha-adrenergic blockade during hypoxia in fetal sheep. *Am J Obstet Gynecol* 1982; **142**(4): 410-5.

32.   Lewis AB, Donovan M, Platzker AC. Cardiovascular responses to autonomic blockade in hypoxemic fetal lambs. *Biol Neonate* 1980; **37**(5-6): 233-42.

33.   Kitanaka T, Alonso JG, Gilbert RD, Siu BL, Clemons GK, Longo LD. Fetal responses to long-term hypoxemia in sheep. *Am J Physiol* 1989; **256**(6 Pt 2): R1348-54.

34.   Allison BJ BK, Niu Y, Kane AD, Herrera EA, Thakor AS , Botting KJ, Cross CM,, Itani N SK, Beck C, Giussani DA. Fetal in vivo continuous cardiovascular function during chronic hypoxia. *J Physiol* 2015.

35.   Murotsuki J, Bocking AD, Gagnon R. Fetal heart rate patterns in growth-restricted fetal sheep induced by chronic fetal placental embolization. *Am J Obstet Gynecol* 1997; **176**(2): 282-90.

36.   Shaw CJ AB, Brew AJ, Lees C, Giussani DA. . Disruption of ontogenic changes in fetal heart rate variability (FHRV) in late gestation chronically hypoxic fetal sheep. . *Reprod Sci* 2015; **22**(1): 350A.

37.   Schneider U, Schleussner E, Fiedler A, et al. Fetal heart rate variability reveals differential dynamics in the intrauterine development of the sympathetic and parasympathetic branches of the autonomic nervous system. *Physiol Meas* 2009; **30**(2): 215-26.

38.   Ohta T, Okamura K, Kimura Y, et al. Alteration in the low-frequency domain in power spectral analysis of fetal heart beat fluctuations. *Fetal Diagn Ther* 1999; **14**(2): 92-7.

39.   Varvarigou AA. Intrauterine growth restriction as a potential risk factor for disease onset in adulthood. *J Pediatr Endocrinol Metab* 2010; **23**(3): 215-24.

40.   Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993; **341**(8850): 938-41.

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

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

	ΔAAC	ΔADC	ΔSTV	AAC[1]	ADC[1]	STV[1]
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)

Δ = 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

Figure legend

Title: Longitudinal progression of phase-rectified signal averaging and computerized cardiotocography indices. Figure 1 a-c: Longitudinal changes of average acceleration capacity (AAC) (a), average deceleration capacity (ADC) (b) and short term variation (STV) (c) during the 5 days prior to delivery or intrauterine death.