



**UNIVERSITÀ DEGLI STUDI DI MILANO**  
**FACOLTÀ DI MEDICINA E CHIRURGIA**

Dipartimento di Scienze Cliniche "L. Sacco"

Scuola di Dottorato in Scienze Fisiopatologiche, Neuropsicobiologiche e  
Assistenziali del Ciclo della Vita  
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**NEW METHODS OF ANALYSIS OF TRANS-ABDOMINALLY  
ACQUIRED FETAL ELECTROCARDIOGRAM**

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Dottoranda  
Tamara Stampalija  
Matricola R09133

Tutor:  
Chiar.mo Prof. Enrico M. Ferrazzi

Coordinatore del Dottorato:  
Chiar.mo Prof. Roberto L. Weinstein

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## Abstract

### NEW METHODS OF ANALYSIS OF TRANS-ABDOMINALLY ACQUIRED FETAL ELECTROCARDIOGRAM

#### **Background.**

Our group was one of the first to apply Phase Rectified Signal Averaging (PRSA) analysis in obstetric field by analyzing fetal ECG acquired non-invasively by trans-abdominal ECG. PRSA is a new method that emphasizes quasi-periodic oscillations masked by unrelated non-stationary elements in the signal. It is robust to the non-stationarities, noise and artifacts, and, thus, this method is superior to standard methods of heart rate (HR) evaluation such as power spectral analysis. The PRSA series can be employed to quantify the “average acceleration capacity” (AAC) and “average deceleration capacity” (ADC) of the signal. When applied to HR, AAC and ADC represent an indirect integrated quantification of the activities of the sympathetic and parasympathetic autonomic systems.

In a previous study, we explored the correlation between AAC and ADC and acid-base status in an in-vivo pregnant sheep model at term exposed to acute hypoxia (progressive umbilical cord occlusions)[*Submitted for publication*]. We also evaluated specific PRSA parameters (in particular the T value that determines the frequencies over which AAC and ADC are computed). In conclusion, our study showed the evidence of autonomic nervous system (ANS) activation in sheep fetus exposed to acute hypoxia through a computation of ACC and ADC of FHR, and proved a correlation with acid-base status biomarkers (T 2÷6 best enhanced differences among different grades of hypoxic severity, and the correlation between AAC/ADC and acid-base biomarkers was highest in that interval).

Based on our preliminary results we have hypothesized that AAC and ADC might reflect more closely the homeostasis of the ANS than short-term variation (STV), and thus might be used in the monitoring of the fetal wellbeing in fetuses with intrauterine growth restriction (IUGR). Indeed, IUGR fetuses are exposed to chronic nutrient deprivation and hypoxemia that can alter ANS regulation of cardio-vascular system, and, thus, affect FHR variability.

#### **Aim of the study**

The aim of the study was to investigate AAC and ADC computed by PRSA analysis on fetal ECG obtained by trans-abdominal ECG in preterm IUGR (<34 weeks) fetuses, and to explore the influence of different T values on AAC and ADC computation in relation to IUGR. Relationship of AAC and ADC with STV and Doppler velocimetry parameters of utero-placental and fetoplacental districts has been also evaluated.

#### **Material and methods**

This is a prospective longitudinal single center case-control study. Preterm IUGR (<34 weeks), n=22, were recruited at admission and monitored on daily bases until delivery by trans-abdominal fetal ECG (Monica AN24, Monica Healthcare, UK). The results of fetal ECG were blinded to the management clinicians and midwives. Computerized CTG, Doppler velocimetry evaluation of utero-placental-fetal districts, and timing of delivery were performed according to standard hospital protocol. A control group was made of uncomplicated pregnancies matched for gestational age recruited at low risk antenatal clinic (n=37).

Fetal ECG was analyzed off-line by PRSA method, and AAC and ADC were computed for T values in a range 1÷50. Differences between IUGR and healthy fetuses matched for gestational age were evaluated. In order to account for gestational age influence on ANS maturation, we divided the cohort in two sub-groups: 26-<30 weeks, and ≥30-<34 weeks, respectively. Correlation with Doppler velocimetry parameters was evaluated.

### ***Principal findings of the study***

1) In the 26-<30 weeks group, except for T=1, the AAC and ADC were significantly lower in IUGR fetuses than in matched for gestational age healthy fetuses (for T values 2÷50); 2) In the ≥30-<34 weeks group, except for T 1÷3, the AAC and ADC were significantly lower in IUGR fetuses than in matched for gestational age healthy fetuses (for T values 4÷50); 3) The amplitude of the difference was higher for very early IUGR group (26-<30 weeks) than in early IUGR group (≥30-<34 weeks); 4) STV computed on fetal ECG R-R intervals from trans-abdominal ECG was lower in IUGR fetuses than in matched for gestational age healthy fetuses in 26-<30 weeks group; 5) There were no differences in STV in ≥30-<34 weeks group; 6) There was no correlation between AAC/ADC and uterine arteries mean PI; 7) When evaluating ADC correlation with other districts, there was: a significant negative correlation with umbilical artery PI, a significant positive correlation with middle cerebral artery PI, and significant positive correlation with cerebro/placental ratio, respectively; the same was true for AAC but in opposite direction; and 8) The correlation was found to be significant in all districts in a narrow range of T values, 1÷6; 9) When considering the Doppler velocimetry districts as categorical variables (normal, pathological), there was a clear trend of lower values of AAC and ADC as Doppler parameters deteriorated, but this difference did not reach statistical significance.

### ***Conclusions***

In conclusion, our study showed the evidences of an ANS “functional reduction” in IUGR fetuses through a computation of ACC and ADC of FHR in comparison to healthy matched for gestational age fetuses. This was particularly true for very early IUGR group (<30 weeks). Moreover, we proved a correlation with Doppler velocimetry parameters of fetoplacental districts in the same T interval in which we found the highest correlation between AAC and ADC with acid-base biomarkers (data from a previous study). Thus, we hypothesize that AAC and ADC might have a role in the management of IUGR fetuses and we propose a choice of parameters for the computation of PRSA. Those findings put the solid ground for future clinical studies.

# INTRODUCTION

## INTRAUTERINE GROWTH RESTRICTION

The definition of small for gestational age (SGA) is not unanimous, and, thus, the analysis of the literature and scientific data are not easily interpretable. SGA is defined by the World Health Organization as a newborn whose birth weight is less than 2,500 grams at term [*World Health Organization*]. This definition is useful for underdeveloped areas of the world, where the exact gestational age is often unknown, and small and premature newborns are not assisted due to restricted availability of the resources.

Starting from the 1970s, in wealthy western countries, the term SGA has been introduced to define the newborn's birth weight as below the 10<sup>th</sup> percentile, based on the local birth weight charts and in relation to the gestational age at birth [*Battaglia 1967*]. According to this definition, a SGA fetus could be a growth restricted fetus, or small because of chromosomal abnormality or congenital infection, or simply constitutionally small. Birth weight below the 5<sup>th</sup> or 3<sup>rd</sup> percentile is occasionally adopted in order to increase the probability of recognizing a true growth restriction [*Marconi 2008*]. Thus, the usefulness of SGA definition can be recognized in the identification of classes of subjects, but not clinically to classify a single case.

Intrauterine fetal growth restriction (IUGR) is a pathological condition that refers to a fetus that fails to reach his/her genetically predetermined growth potential. It is associated with increased perinatal mortality and morbidity due to higher risk of intra-uterine fetal demise (IUFD), intra-partum morbidity, and increased rate of iatrogenic prematurity (<34 weeks of gestation) [*Bernstein 2002*]. Besides the short-term complications such as polycythemia, hypoglycemia, hypothermia, and respiratory difficulties due to induced prematurity [*Hernandez-Andrade 2013*], IUGR newborns have a higher risk of long-term complications such as developmental delay, and behavioral dysfunctions [*Villar 1990*]. Moreover, an increasing number of reports suggest the causative link between IUGR and metabolic syndrome in adulthood [*Barker 2005*].

Normal development and functional integrity of the placenta are essential prerequisites for appropriate fetal growth. Trophoblast is a metabolically active tissue that uptakes, modifies and transfers nutrients, produces hormones, exchanges gases, and eliminates waste substances. A complete transformation of maternal spiral arteries following

the trophoblast invasion is crucial for the physiological development and functioning of the placenta. In pregnancies complicated by IUGR of placental origin, this mechanism is inadequate [Burton 2009], and leads to placental insufficiency with consequent functional deficit of variable degree and severity. The histo-pathological findings of the placenta include: altered cytotrophoblast proliferation, trophoblast apoptosis, villous necrosis with the subsequent fibrin deposition, syncytial knots, increased villous maturation, thickening of trophoblastic basement membrane, and multiple placental infarctions [Benirschke 2006]. The proportion of these lesions is inversely correlated to the proportion of functional placenta, and can determine decreased delivery of oxygen and nutritional substances to the fetus, and increased resistance to placental blood flow in maternal [Ferrazzi 1999] and fetal compartment [Karsdorp 1994].

Dysfunctional placental exchange can precede abnormal umbilical artery Doppler velocimetry for a long period of time. Thus, nutrient deprivation of the fetus is a chronic process that is initiated far before fetoplacental abnormalities that can be identified by Doppler velocimetry [Rigano 2001]. Progressively, there will be a preferential shunting of the blood towards the vital organs (brain, heart and adrenals), with consequent hypo-perfusion of the remaining districts and deprivation of abdominal adipose deposits [Godfrey 2012]. Indeed, fetus with a growth restriction is characterized by a preferential venous blood flow from umbilical vein towards the right atrium [Bellotti 2004], and this process, together with nutritional deprivation, is at the basis of fetal biometrical asymmetry.

### *Diagnosis of IUGR*

Accurate determination of gestational age is a prerequisite for the diagnosis of IUGR. Certain last menstrual period and first trimester ultrasound remain the gold standard for pregnancy dating. When these parameters are not available ultrasound at 19–20 gestational weeks can provide sufficient approximation [Rossavik 1989]. Nevertheless, it is the ultrasound biometry assessment that is crucial for an accurate diagnosis of IUGR. Pregnancies at risk of IUGR should be considered for longitudinal ultrasound monitoring beyond the routine screening at 20 weeks of gestation [March 2012]. The following conditions increase the risk for IUGR: 1) previous preeclampsia of placental origin or IUGR [Conserva 2012]; 2) abnormal uterine artery Doppler velocimetry at 20 weeks of gestation [Cnossen 2008]; 3) congenital or acquired thrombophilia [Scholten 2013]; 4) hypertension of placental origin [Melchiorre 2012]; and 5) decreased symphysis-fundal height measurement [Robert Peter 2012].

Measurements of fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) allow for the comparison of individual growth and local reference values or customized growth charts [Figueras 2009]. Beside the comparison with standard growth curves and an auxologic assessment of head to abdomen proportion, these measurements allow the calculation of estimated fetal weight (EFW). The most commonly used formulas are those suggested by Shepard et al. [Shepard 1982] and Hadlock et al. [Hadlock 1985]. Based on these formulas, IUGR is defined as an EFW below 10<sup>th</sup>, 5<sup>th</sup> or 3<sup>rd</sup> percentile of local reference values and gestational age. EFW is of great importance when preterm delivery is expected, given the impact of birth weight on neonatal short-term and long-term outcomes. Nevertheless, taken alone, EFW below the 10<sup>th</sup> percentile has two major limitations: the biological and the statistical one. From the biological point of view, it has been suggested that a prematurely delivered newborn often does not reach its full growth potential; in fact, the prematurity is closely related to infective, inflammatory or hypoxic damage that can involve the placenta [Baker 2008]. Consequently, the growth of a prematurely born fetus might be influenced by these factors. Therefore, birth weight references from 26 to 36 weeks of gestation, which include prematurely born neonates, might not reflect the true growth potential of individual fetuses. The second limitation is that, by using the definition of the 10<sup>th</sup> percentile, 10% of normal fetuses will be defined as SGA. This definition includes heterogeneous fetal conditions, among which a majority will be represented by constitutionally small, but healthy, newborns. Those fetuses do not require intensive surveillance and anticipated delivery.

Beside the absolute EFW measure, the evaluation of AC decline over time is helpful to differentiate IUGR from SGA. Indeed, neonates with delayed growth of AC have worse neonatal outcome [Roth 1999]. Ratios derived from biometric measurements, such as HC/AC ratio, are more informative while reflecting the proportion of somatic to visceral growth. This measurement provides integrated information regarding liver and adipose tissue growth early, as from second trimester of pregnancy [Padoan 2004]. By taking into account the HC/AC ratio, two distinct patterns of IUGR have been described [Campbell 1977]. The first one, or symmetrical IUGR, is characterized by proportionally small development of the fetal body (normal HC/AC). This condition is more frequently associated with the factors that act at the early stages, such as congenital infections and aneuploidy. The second type, or asymmetrical IUGR, is characterized by preserved growth of HC (adequate for gestational age) in contrast to AC that shows growth retardation (increased HC/AC ratio), and is typically associated with placental insufficiency [Al Riyami 2011]. In fact, a newborn with a birth weight

within normal ranges could still have suffered from intrauterine growth restriction, observed by a decline in AC of more than 40 percentiles [*Marconi 2008*].

### *IUGR of placental origin*

Doppler velocimetry of the uterine arteries provides important information on the origin of the IUGR [*Ferrazzi 2011; Cnossen 2008*]. In case of IUGR that originates from placental insufficiency, the uterine arteries will be characterized by high blood flow resistance in the placenta, as assessed by high values of Pulsatility Index (PI) and Resistant Index (RI). The high impedance correlates with the uterine arteries blood flow volume, which in IUGR is reduced two to three-folds per unit fetal weight when compared to normally grown fetuses [*Ferrazzi 1999*].

Mathematical model of the umbilical-placental circulation showed that PI of the umbilical artery increases with the vascular disease of the placenta [*Thompson 1990*]. Fifty to sixty percent of the terminal arterial vessels must be obliterated before the increase in umbilical artery PI can be observed. However, this process does not occur uniformly. Initially the raise of umbilical artery PI values is slow, but beyond a certain cutoff (80–90%), the blood flow in umbilical arteries deteriorates sharply [*Thompson 1990*]. At present, umbilical artery PI evaluation constitutes the most valuable vessel for the assessment of IUGR severity [*Karsdrop 1994*]. Abnormal Doppler velocimetry of the umbilical artery is a consistent proxy of severe placental pathology [*Burton 2009*]. The natural history of these fetuses, without adequate monitoring and medical intervention, is intrauterine death frequently associated with the occurrence of maternal hypertensive disorder.

Once the diagnosis of IUGR has been established, the main issue is the right timing of delivery. Several studies confirmed worse neonatal outcome of early IUGR with absent or reversed end diastolic flow (ARED) in umbilical artery than in appropriate for gestational age (AGA) newborns that were delivered prematurely, such as: lower Bayley motor development index, and lower Kaufman mental score at 2 years of age, mental retardation [*Vossbeck 2001*], and higher cognitive impairment at early school age, especially in boys [*Morsing 2011*]. Similarly, later in childhood, survivors with ARED in umbilical artery had higher incidence of neurodevelopmental sequelae [*Valcamanico 2004; Wienerroither 2001; Schreuder 2002*]. Yet, one of the most important decision-making factors is the gestational age at delivery, which constitutes in large series the main predictor of post-partum outcome also in the presence of IUGR [*Baschat 2007; Shand 2009*]. The impact of decision-making criteria adopted for delivery might be derived from two studies. In the GRIT study, a randomized controlled trial, the effect

of delivering early vs. delaying the birth was compared in cases in which there was a clinical uncertainty (thus, subjective), and when umbilical artery PI showed absent or reverse end diastolic flow. The percentages of neonatal deaths were 8 % and 4 % at 32 weeks of gestation for an average weight varying from 1,200 gr to 1,400 gr, respectively [Thorton 2004]. In the TRUFFLE study, conversely the timing of delivery was based on longitudinal monitoring of ductus venosus (DV) and computerized fetal heart rate (FHR) monitoring [Lees 2013]. In the cohort of 509 IUGR newborns, the composite results showed that the percentage of neonatal deaths was 4%, for an average weight of 980 gr at 31 weeks of gestation. The lower percentage of neonatal deaths than the one of the GRIT study was achieved in fetuses delivered almost one week earlier and 300 grams less. Thus, the decision-making policy is extremely complex, particularly before 32 weeks of gestation, while challenged by the fact that besides growth restriction, there is an issue of prematurity.

#### *Monitoring of the fetal wellbeing in IUGR fetuses*

Biophysical monitoring of the fetal wellbeing is based on: 1) evaluation of the fetal growth (although it cannot detect short-term changes in wellbeing); 2) amniotic fluid evaluation; 3) Doppler interrogation of fetal districts; 4) fetal heart rate variability; and 5) fetal biophysical profile.

**Amniotic Fluid** estimation is based on a semi-quantitative assessment obtained by summing the four major vertical pouches of amniotic fluid (amniotic fluid index, AFI) [Chauhan 2008]. The reduction of amniotic fluid is a biophysical evidence of reduced fluid turnover in the fetus, and mostly a decrease in fetal kidney function. Oligohydramnios *per se* does not constitute the decision making tool for the timing of delivery. In severe early IUGR, it is a marker for more frequent Doppler velocimetry evaluation and FHR monitoring [Baschat 2006].

**Umbilical Artery Doppler Velocimetry** interrogation of IUGR is based on indices of severity. Umbilical artery PI above the 95<sup>th</sup> percentile requires examination twice per week; in the presence of AEDF the examination should be performed every other day [TRUFFLE Trial]. Monitoring of IUGR with umbilical artery Doppler velocimetry showed a reduction in mortality (OR 0.71; 95 % CI 0.52–0.98), hospital admission, induction of labor, and delivery by cesarean section [Alfirevic 2010; Stampalija 2010]. When hypertensive disorders emerge as a consequence of severe placental damage the vicious circle of high blood pressure and coagulation disorders often require a strict daily fetal monitoring.



**Middle Cerebral Artery.** The fetal response to chronic hypoxia is a redistribution of blood circulation, shifting the blood from visceral compartments to vital organs such as brain, heart and adrenal glands. This adaptation is called brain-sparing effect. Middle cerebral artery is the most easily accessible cerebral vessel, and thus the redistribution of the blood flow at the level of the brain can be documented by increased diastolic flow and decreased PI in middle cerebral artery [Arbeille 1995]. These changes usually occur in the presence of increased umbilical artery PI, and precede the comparison of AEDF [Mari 1996]. Cerebral-placental ratio (the ratio between the middle cerebral artery and umbilical artery) can identify placental-umbilical deterioration prior to the appearance of Doppler velocimetry alterations in umbilical artery and middle cerebral artery taken singularly [Gramellini 1992]. While brain-sparing effect is generally thought to be protective, in reality, there is an association between reduced Doppler velocimetry indices in middle cerebral artery and low levels of pO<sub>2</sub> and high levels of pCO<sub>2</sub> [Karsdrop 1994]. Nevertheless, in case of extreme prematurity, where gestational age is a crucial predictor of neonatal outcome [Shand 2009], and the knowledge that most likely it is the acidemia rather than hypoxemia that causes irreversible developmental consequences [Soothill 1992], brain vasodilatation does not constitute per se the indication for delivery [Ferrazzi 2002; Bilardo 2004]. In cases of severe IUGR, inutero monitoring should continue favoring fetal maturity, until extreme changes in umbilical artery, such as reverse end diastolic flow, or alteration in ductus venosus, occur. At this point, the late cardio-vascular manifestations become more likely [Ferrazzi 2002]. In case of advanced stages of fetal hypoxia due to the loss of the compensatory mechanism, brain vascular resistance might increase as a consequence of cerebral tissue edema, and middle cerebral artery PI values apparently return to normal values.

**Venous District Doppler** interrogation is of great importance. The analysis of the flow wave in ductus venosus allows indirect measurement of the quantity of the blood that ductus venosus deviates directly to the right atrium, thus depriving the liver of blood enriched by oxygen and nutrients [Bellotti 2004]. Decreased or reversed *a* wave is the reflection of the dilatation of the ductus venosus [Bellotti 1998; Ebbing 2009]. Ultimately, the pulsatile pattern in umbilical vein is determined by the presence of increased central venous pressure [Hellevik 2009]. According to three prospective non-randomized trials, the dilatation of ductus venosus, as assessed by the PI of the waveform sampled at the inlet of the vessel, proved to be the best predictor of poor neonatal outcome in severe IUGR [Baschat 2007; Ferrazzi 2002; Bilardo 2004]. The clinical advantage of assessing ductus venosus dilatation and abnormal PI is its

occurrence a few days before the appearance of abnormal computerized cardiotocography (CTG).

**Fetal Heart Rate.** Standard CTG or non-stress test (NST) is a monitoring tool widely used for the surveillance of high-risk pregnancies. This method provides information regarding the fetal wellbeing throughout the evaluation of the FHR frequency base line, its variability, and periodical changes. A reactive CTG reflects an adequate oxygenation of the central nervous system (CNS). Nevertheless, due to visual interpretation, the important limitation of this method is a significant intra-operator and inter-operator variability. In most European countries, the limitation of the visual analysis of FHR has been widely overcome by adopting a computerized FHR analysis, which is capable of measuring the short-term variation (STV) of FHR variability. Moreover, in premature fetuses the autonomous nervous system (ANS) is immature making the interpretation of CTG complex. In these cases, and especially in IUGR, the computerized CTG (cCTG) can be helpful. Thus, the advantages of cCTG are the reduction of the inconsistencies in visual interpretation of the CTG, and, more importantly, the ability to provide measurements of the homeostasis of sympathetic and parasympathetic nervous autonomous system throughout the assessment of the short-term and long-term variability. These measurements revealed to be more indicative of the fetal acid-base status [*Ribbert 1991*].

**Fetal Biophysical Profile.** The fetal motility, which is part of the biophysical profile, has been adopted in the USA as a monitoring tool. This direct fetal assessment derives from earlier studies on fetal behavioral states. FHR response to movement shows coherent patterns after 32 weeks of gestation, thus making possible to correlate HR patterns to behavioral states. As a matter of fact, fetal movements should be reflected in a healthy fetus by accelerations in the FHR recordings. This is the reason why visual analysis of FHR after 32 weeks of gestation might exploit the presence or absence of small and large accelerations to assess fetal wellbeing. The fetal biophysical profile sums up the same positive parameters as observed by real time ultrasound and FHR recording. Fetal motility is usually not affected by increased placental resistance, the reason why the fetal biophysical profile might be useful tool for monitoring early IUGR in the presence of severe umbilical artery abnormalities. Indeed, the global fetal activity will start to decline in the presence of severe hypoxemia/acidemia [*Ribbert 1991*], and thus fetal biophysical profile, will result affected late in the cascade of the fetal deterioration [*Baschat 2006*].

## STANDARD METHODS OF FETAL HEART RATE MONITORING

### *Cardiotocography, CTG*

CTG was introduced in the early '70. It represents at the moment the most widely used method of fetal surveillance outside and during labor. It consists in a simultaneous recording of FHR by Doppler ultrasound, and uterine contractions by external tocomanometer.

There are 4 principal parameters that have to be taken into account when interpreting a CTG tracing:

1. **FHR Baseline:** it represents the mean FHR over a period of at least 10 minutes in an absence of uterine contraction or periodic variations (accelerations/decelerations) for at least 2 consecutive minutes [ACOG 2005]. For a term fetus the baseline should be between 110-160 beats per minute (bpm). When the baseline is below 110 bpm, or above 160 bpm there is a condition of bradichardia and tachycardia, respectively. The control of the FHR baseline is given by the ANS: the parasympathetic activation (vagal activation) gives a fast decrease of the baseline, while the activation of the sympathetic branch results in a release of the stress hormones resulting ultimately in a increase of the baseline.
2. **Baseline variability:** it is given by the fetal cardiac frequency variations, which, in a healthy fetus it is not stable and modifies continuously to external and internal stimulus. The variability is comprised between 5-25 bpm. Similarly to baseline, the FHR variability indicates a good function of ANS and the modulation capacity of the cardio-regulatory centres. Thus, the variability is an index of the reactivity and reflects the wellbeing and the adaptation capacity of the fetus. It is the expression of the integrity of the cerebral cortex, of the mesencephalon, vagal system, and cardiac conduction system [Valensise 2009]. Reduced FHR variability (<5pm) has several meanings: it could represent a period of fetal sleep, or, more rarely, could represent a fetal compromise caused by ANS depression such in case of hypoxia, metabolic acidosis, infections, or other causes. On the other hand, increased variability (>25 bpm) is always an expression of a pathological condition and needs further investigation [Chandrabaran 2008].
3. **The presence/absence of the accelerations:** the acceleration is defined as an increase of the FHR (peak in less than 30 seconds) from the baseline of at least 15 bpm and of length of  $\geq 15$  seconds, but less than 2 minutes [ACOG 2005]. Acceleration that last >2 minutes, but <10 minutes is defined prolonged; in case the length is >10

minutes than it is considered as a modification of the baseline. The accelerations reflect the integrity of the nervous system from the moment that it is associated with fetal movements. According to some Authors, the presence of accelerations allows to exclude the presence of acidosis [Beard 1971]. The accelerations are physiologically absent during the fetal rest and sleep. The absence of accelerations should alert for pathological conditions such as hypoxia, infections or cerebral haemorrhage [Chandrahara 2008].

4. **The presence/absence of the decelerations:** the deceleration is defined as transitory reduction in the FHR of at least  $\geq 15$  bpm and length  $\geq 15$  seconds [ACOG 2005]. There are two types of decelerations:

- a. *Uniform deceleration*, which has a gradual insurgence and ending (nadir in  $\geq 30$  seconds), with a profile similar to inverted uterine contraction. It is identical, without variations, to other decelerations. In respect to uterine contractions, those decelerations can be further classified in *early* or *late* decelerations. The early decelerations show a specular profile to uterine contraction. In other words they start before the peak of the uterine contraction, and the nadir of the deceleration occurs almost in correspondence of the peak of the contraction. Ultimately, the deceleration ends at the termination of the contraction. Those decelerations are caused by the vagal reflection due to the compression of the fetal head, and do not have a pathological significance from the moment that they are not associated to hypoxia or acidosis [Valensise 2009]. Nevertheless, the presence of the early uniform decelerations at the beginning of the labour have to be interpreted with caution, while the compression of the fetal head is not typical for that stage [Chandrahara 2008]. At the contrary, the *late* decelerations start after the peak of uterine contraction and return to the baseline at least 20 seconds after the end of the contraction. Usually, the late decelerations indicate an insufficiency of the utero-placental blood flow in fetuses with altered compensatory condition, or when the contraction is of excessive length with consequent transitory hypoxia and chemoreceptor activation. The most significant decelerations in predicting a compromised fetus are the late decelerations associated to reduced/absent variability. This type of deceleration reflects a myocardial depression consequent to a marked hypoxia. The severity of the acidosis is usually correlated to the depth of the decelerations, except for some pre-agonic fetuses in which the decelerations

become progressively less profound due to further worsening of the myocardial depression and loss of fetal capacity to react to the stimuli.

- b. *Variable decelerations* represent decelerations that differ between them both in profile and relationship with the uterine contractions. They are caused by: 1) vagal stimulation due to the umbilical cord compression; 2) placental hypoperfusion due to vena cava or abdominal aorta compression; or 3) fetal head compression [Valensise 2009]. Those decelerations are classified in *typical* or *atypical*, according to the length or entity of the reduction of FHR. A variable deceleration is defined typical if the length is <30 seconds independently of the nadir, or if the FHR is >80bpm independently of the length, or if the FHR nadir is between 70-80 bpm and the length is ≤60 seconds, in the context of CTG with normal baseline and variability. On the contrary, a variable deceleration is considered atypical or severe if nadir of FHR is ≥70 bpm and/or the length is ≥60 seconds [ACOG 2005].

**Visual analysis of CTG.** Based on 4 parameters described in a previous paragraph, a CTG tracing can be defined as: reassuring, non-reassuring or abnormal (*Table 1*). When interpreting a CTG, other than its intrinsic characteristics, it is of crucial importance to take into consideration other factors that can have some influence: the presence or absence of labor, stage of labor, maternal conditions, fetal growth, risk factors, and others. Based on the characteristics of the FHR, a CTG tracing can be classified as normal, suspected, or pathological (*Table 2*).

**Advantages and disadvantages of CTG.** The fetal electronic monitoring was introduced in the clinical practice in the 70-80ies [Gibb 1992] aiming to facilitate the identification of fetal hypoxia during the labor, and, thus, to allow timely intervention. Ultimately, the goal was to reduce perinatal mortality and cerebral palsy. Unfortunately, those objectives remained unachieved [Clinical Guideline C 2011, NICE]. Exception made for the neonatal seizures, the introduction of CTG in clinical practice did not influence significantly neonatal outcome in terms of metabolic acidosis, hypoxic encephalopathy or cerebral palsy [Alfirevic 2007]. This is particularly true for women with low risk pregnancies [Alfirevic 2007]. On the other hand, by introducing CTG the rate of operative deliveries increased significantly [Clinical Guidelines 55; Alfirevic 2007] with consequent increased risk for maternal complications (such as infections, hemorrhage, bladder and/or other organs damage, risks associated to anaesthesia, thrombo-embolic complications, increased complications, and mortality in subsequent pregnancies).

<b>CTG</b>	<b>Baseline (bpm)</b>	<b>Variability (bpm)</b>	<b>Decelerations</b>	<b>Accelerations</b>
REASSURING	110-160	≥5	Absent	Present
NON-REASSURING	100-109 161-180	<5 for 40-90 min	- Early decelerations - Variable decelerations - Single prolonged deceleration >3 min	The absence of accelerations with otherwise normal CTG is of uncertain significance
ABNORMAL	<100 >180 Sinusoidal pattern for >10 min	<5 for ≥90 min	- Atypical variable decelerations - Late decelerations - Single prolonged deceleration >3 min	

**Table 1.** Classification of the FHR parameters according to the National Institute of Health and Clinical Excellence (NICE). Bpm-beats per minute; min-minutes

<b>Category</b>	<b>Definition</b>
NORMAL	A tracing that has all 4 parameters within reassuring category
SUSPECTED	A tracing that has 1 parameter in a non-reassuring category and other 3 parameters in reassuring category
PATHOLOGICAL	A tracing that has 2 or more parameters in a non-reassuring category or one parameter in a pathological category

**Table 2.** Classification of CTG according to the National Institute of Health and Clinical Excellence (NICE).

The increase in the rate of operative deliveries can be explained by the high sensitivity, but poor specificity of CTG, resulting in a consistent number of false positive cases and low positive predictive value of the method. Indeed, the vast majority of “alterations” of CTG are not associated with metabolic acidosis. Thus, CTG is not a diagnostic test, but a screening test. Nevertheless, the high sensitivity (85-90%) of this method supports the most important value of monitoring by CTG: its high negative predictive value. The latter is of high clinical impact while in case of reassuring CTG the fetal compromise is unlikely.

CTG is mainly a qualitative assessment, making feasible a quantitative evaluation only in computerized CTG (cCTG). Thus, the main limit of CTG is represented by high inter- and intra-observer variability. The inter-observer variability varies from 25-40%, while intra-observer variability from 15-27% [Devane 2005]. There is the highest accordance for “normal” CTG tracings, while the lowest for “suspected” or “abnormal” ones [Ayres de Campos 1999; Blix 2003]. The parameter that brings the lowest accordance is the interpretation of the baseline variability [Devane 2005; Lotgering 1982; Trimbo 1978].

### *Short term variability and computerized CTG*

From the parameters described previously, the baseline variability is considered one of the most important for the prediction of fetal wellbeing. A healthy fetus presents periods of high variability in correspondence of fetal movements or REM phases of sleep [Schneider 1992], that alternates with periods of low variability that correspond to fetal quiet. The FHR variability is regulated by ANS, which on other hand, is under regulation of central structures. Lost of FHR variability is strictly correlated to the hypoxic stress of the superior central structures while the control of the FHR is determined by the level of central oxygenation [Valensise 2009].

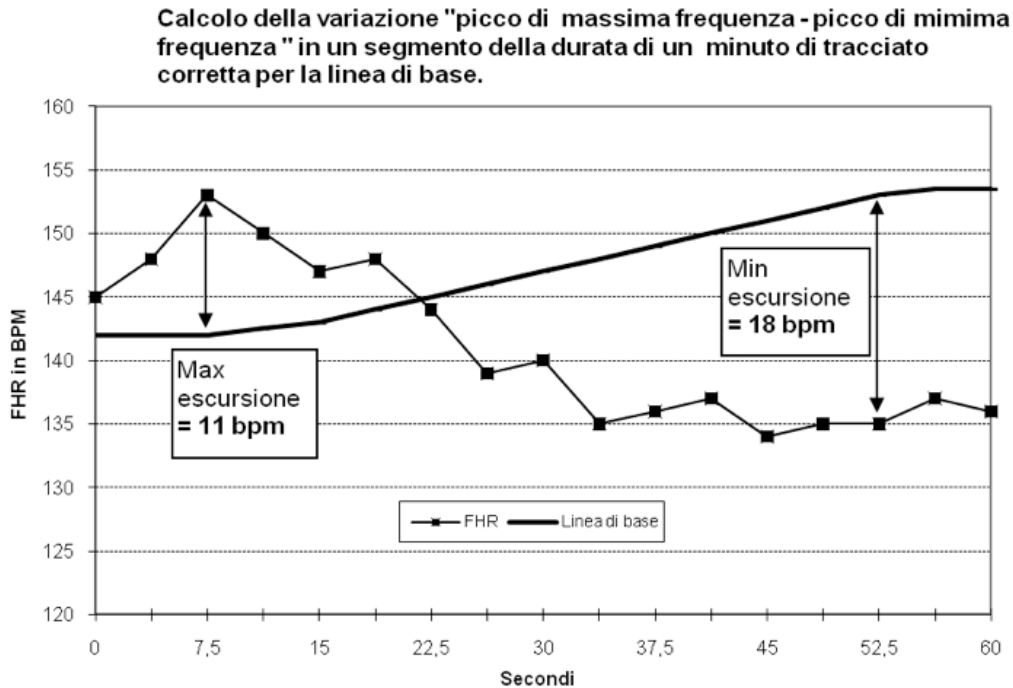
The FHR variability can be considered in short or long term, respectively. The long-term variability represents the global aspect of the FHR in time, and it is given by the variation from peak to peak of FHR in 1 minute interval. Based on long-term variability the CTG tracing can be classified in:

- Absent variability;
- Reduced variability ( $\leq 5$  bpm);
- Normal variability (6-25 bpm);
- Increased variability ( $> 25$  bpm).

The short-term variability represents changes of FHR on beat-to-beat basis. Standard CTG uses Doppler ultrasound and auto-correlation method to represent FHR. In other words it represents the mean of the 4 consecutive cardiac beats. Thus, the standard CTG is not able to evaluate the time differences from one beat to the next one (*short term variability*, STV). In order to evaluate beat-to-beat changes and the differences from consecutive R-R intervals a computerized methods with electronic elaboration has to be adopted [Valensise 2009].

For the analysis of the macro-fluctuations, or long-term variability, the CTG is divided in intervals of one minute each. Each interval that contains decelerations, or part of

deceleration, loss of the signal or artefacts is excluded from the analysis. For each one-minute segment intervals from minimal to maximal HR peak are calculated (*Figure 1*).



**Figure 1.** Representation of short-term variation calculation. FHR-fetal heart rate; bpm-beats per minute.

The cut-off value of peak-to-peak variability is considered an interval of 32ms. Low variability is considered when the value of peak-to-peak variability is <30 ms for at least 5-6 consecutive minutes. At the contrary, a high variability is considered if the peak-to-peak variability is >32 ms for at least 5-6 consecutive minutes. This classification in segments of high or low variability allows to discriminate more accurately healthy foetuses from those that are potentially compromised.

Short-term variability is measured by calculating the interval  $t$  between two cardiac cycles. The presence of STV is the most important indicator of fetal homeostasis. It reflects the “integrity” of the ANS and cardio-circulatory system [Valensise 2009]. STV cannot be calculated visually, and a computerized algorithm is needed. Each minute of CTG tracing is subdivided into 16 segments of 3.75 seconds each. Next, the FHR is calculated for each segment and is expressed as an interval between the beats in ms. Finally, the differences between the FHR of consecutive segments are calculated and a mean over a period of one minute is calculated representing STV in ms. The evaluation of the STV is important while its



reduction, or absence, has been correlated to the development of metabolic acidosis. The evaluation of STV in a non-reactive tracing (e.g. without episodes of long term variability) correlates, independently from the baseline, with the fetal metabolic acidosis and increases the risk of intrauterine demise [Dawes 1992; Street 1991].

Table 3 shows the correlation between the STV values and the probability of metabolic acidosis or intrauterine demise. Of note that 2.5 ms represents an important cut-off: below that value the risk of intrauterine demise is around 72%.

STV (ms)	Probability of developing metabolic acidosis or intrauterine death
>4.00	0
3.5-4.0	8%
3.0-3.5	29%
2.5-3.00	33%
<2.5	72%

**Table 3.** Values of short-term variability (STV) and risk of metabolic acidosis or intrauterine fetal demise.

Based on cCTG and STV Dawes and Redman defined the criteria of normality for CTG:

- There has to be at least one episode of high variability (>10pc for gestational age);
- There has to be no decelerations that cause the lost of FHR >20 beats, or >100 beats if the tracing lasts more than 30 minutes;
- The baseline has to be between 116-160bpm for more than 30 minutes of monitoring;
- There has to be at least 3 accelerations and one fetal movement;
- There has to be no sinusoidal profile;
- The STV has to be  $\geq 3$ ms;
- There has to be at least 1 acceleration (>10 pc for gestational age).

These criteria are used only in women not in labor.

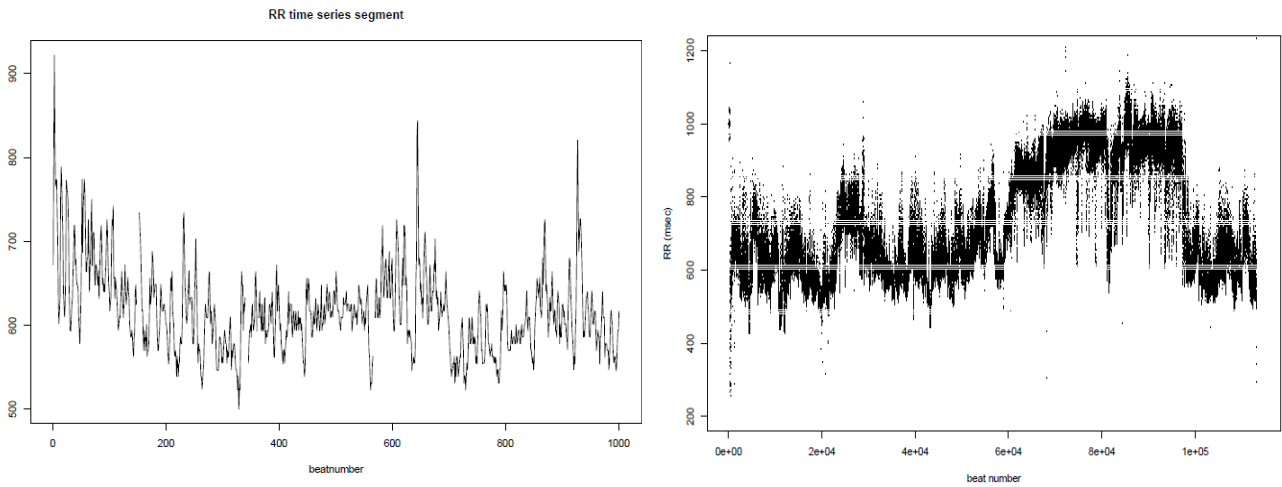
### *Spectral analysis of fetal heart rate*

Spectral analysis is a method that identifies frequencies of periodic components of a signal. Through spectral analysis a periodogram is obtained that allows the identification of the specific cyclic components within the signal. In other words, the spectral analysis highlights the frequencies, and thus the periodicities, that are the most important within the signal. The periodogram measures the intensity of those frequencies, and estimates the contribution of each periodicity within a series. Thus, the spectral analysis is a method that can be used to detect and quantify the changes in HR objectively [Akselrod 1981; Siira 2007].

According to the Fourier theory, each periodic signal is made from a series of sinusoidal waves. The Fourier transformation decomposes the original signal in a sum of periodic functions of different frequencies, and generates a spectrum of frequencies. The amplitude of each frequency component, which can be visualized on the histogram of logarithmic intensity distribution, indicates the contribution of that specific frequency to the original signal. Thus, by applying the Fourier transformation it is possible to decompose and re-compose a generic signal in a sum of infinite sinusoids with different frequencies, amplitudes and phases. This is called amplitude spectrum, or phase spectrum. In other words, the Fourier transform is a mathematical method that transforms data from quantity (FHR) varying over time to amplitude (spectral power) varying over a range of frequencies, and it performs the conversion of a function in the time domain to frequency domain. When the Fourier transformation is applied to the harmonic signal, the lowest frequency obtained is called fundamental harmonic frequency and it has the highest influence in a final re-composition of the signal. The other frequencies are multiples of the fundamental frequency and are called secondary harmonic frequencies.

As it concerns the HR analysis, the spectral analysis uses the fast Fourier transform algorithm and decomposes sequential R-R interval series into a sum of sinusoids of different amplitudes and frequencies [Van Ravenswaaij 1993]. As already discussed, HR is not stable, and it is characterized by beat-to-beat variability caused by multiple stimuli on the atrial sinus through ANS. Those stimuli, such as respiration, baroreceptor and chemoreceptor inputs, and others, are characterized by different frequency domain which interact in a complex way to determine the HR. Starting from the 80ies a computerized analysis of the temporal series

extracted from the ECG tracing over 24 hours took place (e.g. Holter). With this method short segments of a R-R series can be isolated and plotted in order to give a tachogram (*Figure 2*).



**Figure 2.** *Left panel: An example of the tachogram; Right panel: An example of the tachogram 24 hours period.*

Considering the influence on FHR variability by the sympathetic and parasympathetic nervous system, the power spectrum reflects the magnitude (power) present at different frequency ranges. The impulses from the parasympathetic nervous system are conveyed much faster than impulses from the sympathetic nervous system [Akselrod 1981]. Thus, sympathetic modulations of the FHR leads to slow oscillations while parasympathetic modulations leads both to slow and fast oscillations. As a result, both sympathetic and parasympathetic modulation components are situated in the low-frequency (LF) range, while only parasympathetic modulation is located in high-frequency (HF) range [Akselrod 1981]. The spectral analysis has the potential to evaluate the oscillations present in beat-to-beat FHR, and, thus, to monitor the ANS modulation.

The R-R series (intervals) can have qualitative and quantitative differences in relation to physiologic and pathologic conditions. By applying the power spectral density analysis to FHR variability, at rest, 3 principal spectral components can be recognized: 1) high frequency (HF) between 0.15 to 0.4 Hz, synchronized with the respiration, and determined by the parasympathetic modulation; 2) low frequency (LF) from 0.04 to 0.15 Hz, determined by the baroreceptors, and composed of sympathetic and parasympathetic component of ANS; and 3) very low frequency (VLF)  $>0.04$  Hz, determined by the renin-angiotensin-aldosterone system, and correlated to the cyclic fluctuation of the vasomotor tonus associated to the thermo-

regulation [Chung 2001]. Nevertheless, the definition of frequency bands vary between the studies and takes into account the physiological range of FHR and respiratory movements rate.

The relationship between sympathetic and parasympathetic component in the regulation of the FHR is measured by LF/HF ration [Cerutti 1989]. Thus, by applying the power spectral density analysis to beat-to-beat fluctuation of the FHR makes feasible, in a non-invasive manner, to evaluate the state of ANS. The same method can be applied to CTG in order to evaluate FHR [Cerutti 1989]. The application of power spectral density analysis to CTG at 26 weeks of gestation has identified a peak in LF range, around 0.1-0.04 Hz [Ferrazzi 1989]. At 36 weeks of gestation, beyond LF peak, another HF component can be found (0.8-0.13 Hz). The HF component can be identified during the respiratory movements of the fetus, but not during the fetal rest. Thus, it can be concluded that this component corresponds to the maturation of the parasympathetic component of ANS. Some Authors showed an increase in LF at the end of the second trimester, and its decrease at the end of the third trimester [David 2007]. Van Laar et al. compared term and post-term fetuses, and found that during the periods of fetal rest in post-term fetuses HF component was significantly higher than in term fetuses, while LF component and LF/HF ratio were significantly lower [Vaan Laar 2009]. Thus, it seems that LF decreases along with the gestational age, reflecting a diminished regulation of FHR by the sympathetic component that gives place to an increased modulation from the parasympathetic branch of ANS. This suggests a later maturation of the parasympathetic component than the sympathetic component.

This method has been also used for the analysis of CTG in labor [Chung 2001] or to ECG obtained by STAN system [Van Laar 2009].

Several studies found that spectral power in the LF and HF ranges decreases in the presence of fetal hypoxemia or acidemia [Siira BJOG 2005; Suzuki 2001; Ohta 1999; Rantonen 2001]. These studies used absolute values of LF and HF power spectrum, and assumed that the decrease in LF and HF spectrum was the result of immaturity or impairment of the fetal ANS. Nevertheless, while the changes in total power influences LF and HF in the same direction, the normalized values of LH and HF power seem more suitable for fetal monitoring. Normalized LF (LFn) and normalized HF (HF<sub>n</sub>) power are computed by dividing LF and HF power respectively by total power. This allows to detect also relative changes and balanced behavior of the two branches of the ANS.

**Limits of power spectral analysis.** In order for power spectral information to be reliable the Fourier transform requires sample data to be equidistant. In fact, the Nyquist

criterion states that in order to obtain reliable spectral information, the signal has to be sampled at least twice the frequency of the highest frequency of interest. The rapidly fluctuating fetal ANS results in non-stationarity of FHR signal, and, thus in R-R intervals that are by definition not equidistant.

Many biologic and physiologic phenomena present themselves with a periodicity determined by intrinsic and extrinsic mechanisms of regulation, which interacts between them in a complex way, and determines interruptions and change in their periodicity [Bauer 2006]. The heart rate, oscillation of the membrane potential, neuronal signal, synthesis and secretion of the hormones, circadian rhythm are just some of biological periodic phenomena [Tyson 2002]. Nevertheless, the non-stationarity of these signal represent major issue for their analysis, while the interruptions of the signal determine a de-synchronization of the oscillations. Consequently, a periodic signal becomes quasi-periodic, while made of segments of different periodicities [Bauer 2006]. Thus, techniques such as power spectral analysis, that compare the signal to a “perfect” sinusoid at a certain frequency, do not consider the “phase re-programming” of the control system induced by the perturbations, and, consequently, cannot be used for the analysis of non-stationary signals. In fact, if the signal is made from a series of small segments at different periodicities with a specific frequency, some of those segments will be in phase with the analyzed sinusoid, but the vast majority will not be in-phase, and, thus, will be excluded from the analysis.

Other limitation of the power spectral analysis is that it is not robust to the background noise and artifacts, limiting its application to ECG or CTG analysis.

## PHASE-RECTIFIED SIGNAL AVERAGING (PRSA) ANALYSIS

The determination of the quasi-periodicities of HR is of high clinical impact while they reflect the regulation mechanisms of ANS on the heart [Bauer 2006]. The phase-rectified signal averaging (PRSA) is a new mathematical algorithm capable to identify periodic and quasi-periodic patterns of a complex biological signal, and it is superior to the power spectral analysis in identifying periodic components. Moreover, this method is able to analyze separately the cyclic fluctuations of the cardiac frequency in relation to the average acceleration and deceleration capacity of the heart. This opened a new insight to the ANS regulation of HR [Bauer 2006]. PRSA analysis provides an estimate of the autonomic regulation of the HR even when phase de-synchronizations due to abrupt changes in the system, miss-detected beats and signal losses are present. Ultimately, the aim of PRSA analysis is to comprise the long-lasting monitoring signal in a shorter sequence that can be interpreted visually. This method was introduced in adult cardiology, and showed the supremacy of average deceleration capacity, as a prognostic indicator in patients with myocardial ischemia over standard parameters [Bauer 2006].

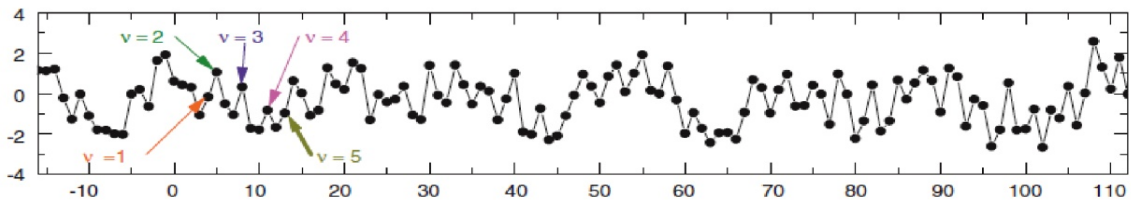
Moreover, PRSA analysis allows quantification of the *coherence time* and *coherence length* of each quasi-periodicity present in the signal. The coherence (coherence phase) is the characteristic of a wave to maintain a certain relationship with itself during propagation. When considering the waves usually we refer to perfectly periodic wave. In reality, each wave, after certain propagation (or certain time), is subjected to deviation from its perfect sinusoidal pathway. Those changes are mathematically describable as a deviation from the wave phase. When the relation with the phase is lost, due to propagation deviations, than the wave has lost its coherence time. Thus, the coherence time can be defined as a mean time interval in which the wave oscillates in a predictable way (in a perfectly sinusoidal way). During this time interval the wave will perform a certain number of oscillations before changing the phase. The result of coherence time and the propagation velocity is called coherence length.

## Computation of PRSA

The computation of PRSA can be summarized in 4 consecutive steps:

- 1. DEFINITION OF THE ANCHOR POINTS.** The R-R intervals are extracted from the ECG tracing in order to build the tachogram that will show the behavior of the R-R intervals over time. Secondly, the anchor points are selected and are represented by the R-R intervals (*Figure 3*). The selection of anchor points is based on the characteristics of the signal. In order to analyze the acceleration capacity of the heart the anchor point will be made from the R-R intervals shorter than the preceding ones, e.g. segments in which the HR is accelerating. Similarly, in order to compute the deceleration capacity, the anchor points will be represented by the R-R intervals that are longer than the preceding ones.

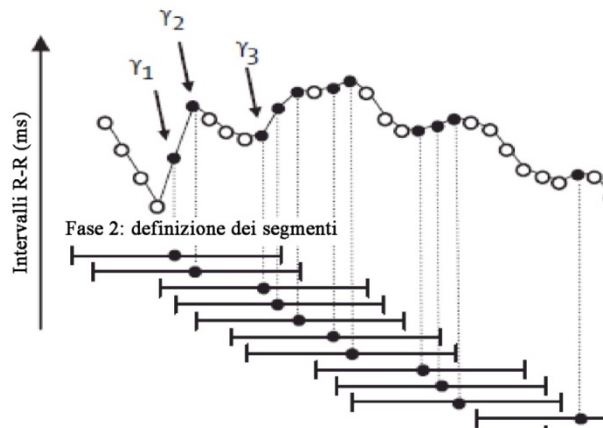
Other criteria of selection can be applied in order to eliminate possible artifacts. Sudden important increase or decrease of HR constitute an artifact, and thus has to be excluded from the selection of anchor points. For this reason, the R-R intervals that exceed 5% from the mean are excluded from the analysis. Typically, around half of the points of the temporal series are selected as anchor points [*Bauer 2006*].



**Figure 3:** Selection from the tachogram of the R-R intervals of increasing length (Increase Events) as anchor points ( $v_1, v_2, \dots$ ) [*Bauer 2006*].

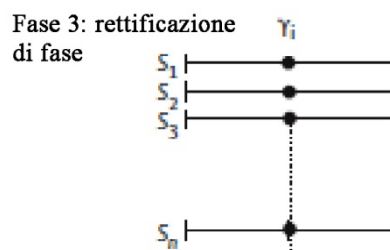
- 2. DEFINITION OF THE SEGMENTS.** Around the anchor points the segments of the signal are selected. All segments will be of same length that is chosen based on coherence length (*Figure 4*). The latter constitutes the length of the segments of periodicities in which the signal can be considered sinusoidal (e.g. periodical), and is given by the non-stationary nature of the periodicity [*Bauer 2006*]. Moreover, the length of the segments is chosen based on the lowest oscillation frequency that wants to be detected. In other words, small segments of signal, that surround the anchor points, are analyzed in order to evaluate the signal oscillation in the proximity, before and after, of the anchor point.

The lower the oscillation frequency the longer the length of the segments, and *viceversa*. Finally, longer segment than the expected coherence length is chosen. Some segments will overlap from the moment that many anchor points are close to each other.



**Figure 4.** Definition of the segments starting from the anchor points ( $Y_1, Y_2, \dots$ ).

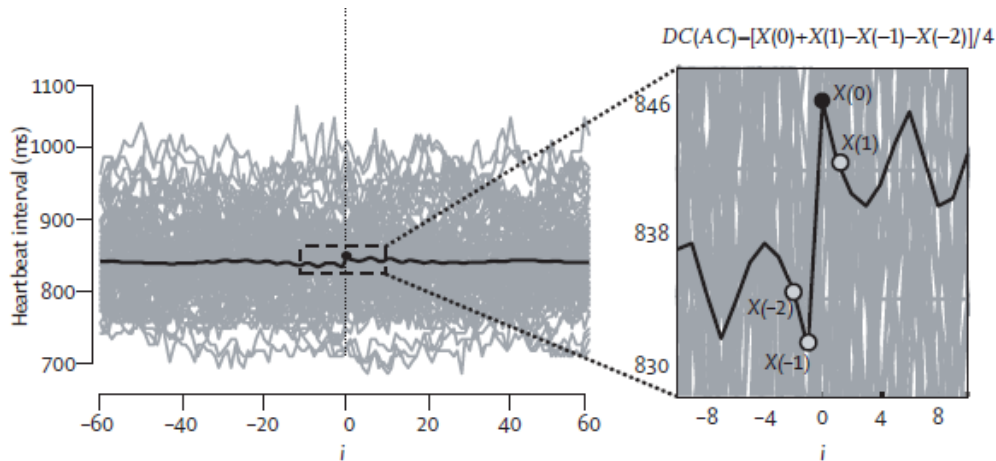
3. **PHASE-RECTIFICATION.** The third step is given by the rectification of the signal: all selected segments are aligned and overlapped around a single referral point that corresponds to all anchor points (*Figure 5*). In this phase, rectification of the signal, the components that do not have a synchronized phase with the anchor points will be eliminated: consequently, non-stationary events, the artifacts and the background noise will be excluded from the analysis. Only those events that present a fixed phase relation with the anchor points, and thus represent periodicities and quasi-periodicities, will be considered for the analysis.



**Figure 5.** Phase rectification of the segments ( $S_1, S_2, \dots$ ) around the referral point ( $Y_i$ ).

4. **SIGNAL AVERAGING.** During this step events, related to the anchor points in periodicities or quasi-periodicities, are averaged (*Figure 6, Left panel*).

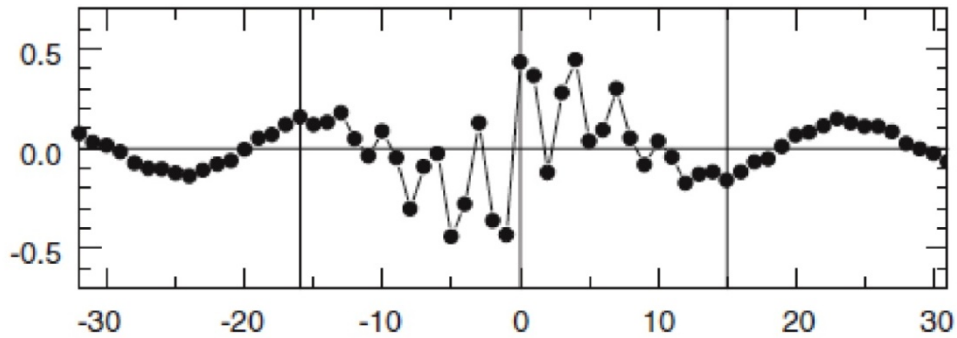




**Figure 6:** Right panel, signal averaging. Left panel, an example of the PRSA curve of average deceleration capacity.

After the signal averaging the PRSA curve is obtained. It represents the average deceleration capacity, and is easily interpretable. The central deflexion represents the average cardiac capacity to decelerate the HR from one beat to another (*Figure 6, Right panel*). The X-axis represents the coherence time, and the point  $X_0$  represents the mean of all anchor points. The points  $X_{(-1)}$  e  $X_{(1)}$  represent the mean of the segments that precede and follow the anchor points. Distribution of the intervals that precede and follow the anchor points is represented on the left and right, respectively. The Y-axis represents the interval from one beat to another, and is expressed in milliseconds.

When analyzing the figure below it can be observed that the signal is distributed around the point 0, and follows two main oscillation frequencies: 1) the fast one, that corresponds to the central part of the graph; and 2) the slow one, that corresponds to the peripheral part of the graph, respectively (*Figure 7*). In this manner it is possible to quantify the periodicity of the frequency oscillation of the heart, and to eliminate the non-stationary components, artifacts and noise. Ultimately, it is possible to calculate the average acceleration (AAC) and deceleration (ADC) capacity of the heart. The ability to separate the variations of HR due to acceleration or deceleration events represent one of the biggest advantages of the PRSA method over the standard models of HR analysis.



**Figure 7.** Average acceleration capacity represented by PRSA curve.

### *The advantages of PRSA analysis*

In summary, the advantages of PRSA analysis can be summarized as follows:

- It identifies the periodic and quasi-periodic components of complex signals;
- It discriminates between the average acceleration and deceleration capacity of the heart;
- It is robust to the non-stationary signal, to background noise and artifacts;
- It comprises long recordings in a short sequence, keeping the contribution of all (quasi-) periodicities;
- It allows quantification of the coherence time and the coherence length of each period of quasi-periodicities.

## Our experience with PRSA analysis, the state of the art and the aim of the study

Our group was one of the first to apply Phase Rectified Signal Averaging (PRSA) analysis in obstetric field. We have analyzed fetal ECG, acquired non-invasively by trans-abdominal ECG, with PRSA method. In a cohort of 80 women with an uneventful pregnancy monitored at term we found that the intra-individual average acceleration capacity (AAC) is significantly higher in the periods of high variability than in those of low variability [Stampalija 2011, Appendix]. Moreover, when analyzing fetuses according to the gestational age (39, 40, and 41 weeks of gestation) we found a progressive increase both of AAC and average deceleration capacity (ADC) [Stampalija 2011, Appendix]. This finding confirmed that autonomous nervous system (ANS) progressively matures as the pregnancy progresses (at term). Based on these studies we have hypothesized that AAC and ADC might reflect more closely the homeostasis of the ANS than short-term variation (STV), and thus might be used in the monitoring of the fetal wellbeing. Moreover, we have applied PRSA analysis to maternal ECG in hypertensive disorders and found a significant activation of ANS in women with hypertensive disorder of placental origin [Casati 2014, Appendix]. We also described for the first time the application of Bivariate Phase Rectified Signal Averaging (BPRSA) analysis in obstetrics [Bauer 2012, Appendix]. This method represents a powerful tool for the analysis of inter-relationships between two or more synchronously recorded signals generated by complex biological systems. We adopted BPRSA analysis to evaluate the coupling between fetal heart rate (FHR) and uterine contractions, and we found that there is a coupling between FHR periodicities and uterine contraction [Casati 2012, Appendix; Manuscript in peer-review process]. Moreover, we proved that BPRSA analysis is superior to standard methods in evaluating coupling between FHR and uterine contractions.

Next we wanted to verify the correlation between AAC and ADC and acid-base status, in order to explore the potential of AAC and/or ADC to be applied in identification of fetal hypoxemia or metabolic acidemia. In a study, entitled “*Acceleration and Deceleration Capacity in an In-vivo Sheep Model*”, we applied PRSA on a direct fetal-ECG in a fetal in-vivo near term pregnant sheep model [Submitted for publication]. We evaluated AAC and ADC in different phases of umbilical cord occlusion. This study represents the first evaluation of ACC and ADC under various hypoxic conditions in an in-vivo animal model. Moreover, specific PRSA parameters were evaluated (in particular the T value that determines the frequencies over which AAC and ADC are computed). We found that: 1) The absolute values of AAC and ADC have a growing trend as the hypoxic insult augments; 2) AAC/ADC correlate to acid-base

status biomarkers collected at different stages of hypoxia; 3) The value of  $T \approx 6$  best enhances differences among protocol phases (this was particularly true for severe cord occlusion), and the correlation between AAC/ADC and biomarkers is highest in that interval; 4) There is a difference in ADC (or ACC) calculated during entire fetal R-R series and stable fetal R-R series, respectively; 5) The time reversal symmetry is missing between AAC and ADC in a strict  $T$  value interval.

In conclusion, our study showed the evidence of ANS activation in sheep fetus exposed to acute hypoxia through a computation of ACC and ADC of FHR, and proved a correlation with acid-base status biomarkers. We evaluated the impact of  $T$  parameter and FHR on PRSA computation, and we proposed a choice of parameters for the computation of the PRSA. Those findings put the ground for future clinical studies.

All abstracts of our previous studies on PRSA can be found in Appendix.

### *The rationale*

Small for gestational age (SGA) fetuses represent a heterogeneous group, made of constitutionally small fetuses, small due to chromosomal, genetic or infectious disorders. Nevertheless, the most common cause of SGA, especially those <34 weeks of gestation, is inadequate placentation that determines a failure of the fetus to reach his/her genetically predetermined growth potential, and, thus, represents a pathological condition termed intrauterine fetal growth restriction (IUGR). IUGR is one of the most common causes of perinatal mortality and morbidity: it puts the fetus at risk of hypoxemia, impaired neurodevelopment, intrauterine fetal demise and other short and long-term complications [Bernstein 2002; Karsdorp 1994]. Moreover, it is becoming increasingly clear that harmful insults during intrauterine life can cause permanent changes in physiological metabolism of a newborn, increasing the risk of metabolic disease in adult life [Barker 2005]. Indeed, substrate and energy deprivation modify fetal metabolism through an epigenetic pathway with possible life-long impacts [Banister 2011]. The decision to distance the fetus from the hostile environment, by anticipating the delivery, has to be balanced with the risk of prematurity and its consequences such as respiratory distress, intra-ventricular hemorrhage, periventricular leucomalacia and others. The optimal method to predict fetal compromise is still unknown, and criteria for the timing of delivery are not standardized. Thus the management of IUGR fetus is still controversial and represents one of the biggest challenges for obstetricians. In those cases a clinician has to balance between: 1) the decision to prolong the pregnancy, thus

gaining in fetal maturity, but exposing the fetus to intrauterine hypoxia/acidemia up to, in extreme cases, to intrauterine demise; and 2) the decision to deliver in order to distance the fetus from the hostile intrauterine environment, but risking the consequences of severe fetal prematurity.

Assessment of FHR variability is a mirror of the functional state of ANS. The chronic nutrient deprivation and hypoxemia that characterize IUGR, on long term scale and depending on the severity, can alter ANS regulation of cardio-vascular system, and, thus, affect FHR variability. Standard methods of fetal wellbeing monitoring are not able to differentiate the influence of sympathetic from parasympathetic component on FHR. Other methods, such as power spectral analysis, can differentiate the two branches of ANS, but their application is limited due to the complexity of the FHR signal, characterized by non-stationarity, noise and artifacts. PRSA method synchronizes the phase of all quasi-periodic components of a complex biological signal (i.e. FHR), overcoming the non-stationarity, noise and artifacts. Moreover, it makes feasible the separation of the acceleration and deceleration capacity of the HR, giving an approximate distinction of the separate effects of the sympathetic and parasympathetic components of ANS.

### *The aim of the study*

The aim of the study was to investigate the AAC and ADC computed by PRSA analysis on fetal ECG obtained by trans-abdominal ECG in preterm IUGR (<34 weeks) fetuses, and to explore the influence of different T values on AAC and ADC computation in relation to IUGR. Relationship of AAC and ADC with STV and Doppler velocimetry parameters of utero-placental and feto-placental districts has been also evaluated.

## Material and methods

### *Study design and population*

This is a single-center prospective longitudinal case-control (1:2) study conducted at the Department of Obstetrics and Gynecology, Children's Hospital Vittore Buzzi, University of Milano, Italy. The cases were pregnancies complicated by fetal IUGR diagnosed before 34 weeks of gestation. The control group was given by uncomplicated pregnancies matched for gestational age at recruitment. For the purposes of this study IUGR were divided in two groups, based on gestational age at recording: 1) 26 -<30 weeks of gestation; and 2)  $\geq$ 30-<34 weeks of gestation. This choice was made in order to have more homogeneous groups, and to minimize the influence of gestational age on FHR. Indeed, FHR undergoes physiological changes during gestation, and, thus, FHR deriving from different gestational periods might present intrinsic differences. Multiple pregnancies, fetuses with chromosomal or structural anomalies were excluded.

Pregnancies with IUGR were recruited at the time of admission or during their stay at the high-risk ward. At the admission a detailed ultrasound anatomy survey was performed together to the Doppler velocimetry evaluation of utero-placental-fetal districts. The mother and the fetus were monitored as per standard clinical protocol, according to the severity of the growth restriction, Doppler velocimetry findings of umbilical artery, middle cerebral artery, ductus venosus, and computerized CTG. The same was true for the timing of delivery decision. As part of this study monitoring by trans-abdominal ECG was performed. After obtaining the signed informed consent patients were monitored by trans-abdominal ECG on daily basis until delivery. In cases of skin irritation the monitoring was interrupted until skin recovery or patients consent to continue with monitoring. For the purposes of the present study the closest available trans-abdominal ECG monitoring to the delivery was used. If multiple Doppler velocimetry measurements were performed, the closest to the trans-abdominal ECG recording were used.

Women with an uncomplicated pregnancy were recruited at the low-risk antenatal clinic, and followed until delivery. Single trans-abdominal ECG monitoring was performed.

All women provided written informed consent prior to the monitoring by trans-abdominal ECG. The study was approved by the Ethic Committee of ICP-Istituti Clinici di Perfezionamento, Milano, Italy.

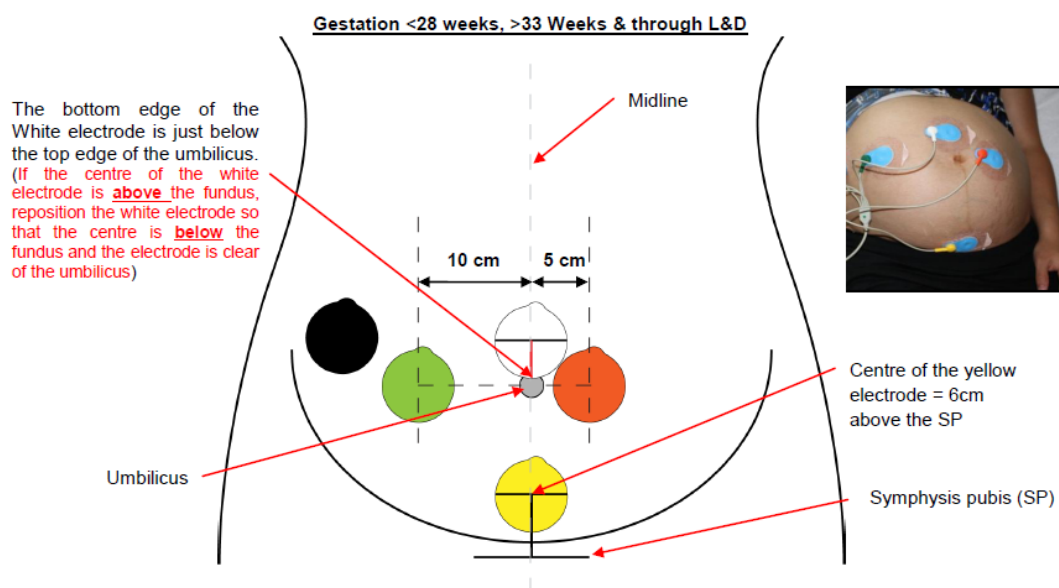
### Clinical definition

The definition of IUGR was based on ultrasound parameters, and was defined as the abdominal circumference  $\leq 5^{\circ}$  percentile for gestational age, or a decrease of abdominal circumference  $>40^{\circ}$  percentiles in two consecutive ultrasound measurements, at least two weeks apart.

Patients were considered to have an uncomplicated pregnancy if they did not experience any obstetrical, medical, or surgical complications of the pregnancy, and delivered a normal term ( $\geq 37$  weeks of gestation) neonate whose birth weight was between 10<sup>th</sup> and 90<sup>th</sup> percentile for gestational age without complications (Montecatini reference for fetal growth).

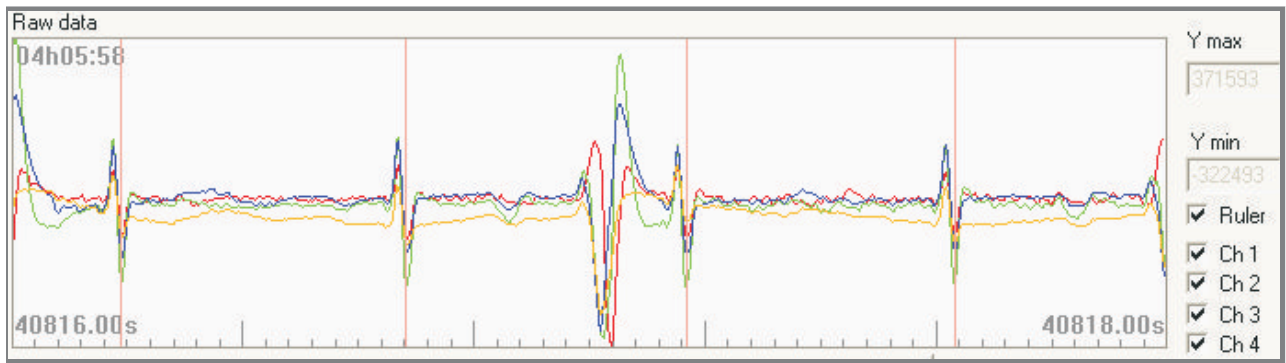
### Trans-abdominal ECG

FHR recordings were performed with the AN24 fetal ECG monitor (Monica Healthcare, Nottingham, UK).



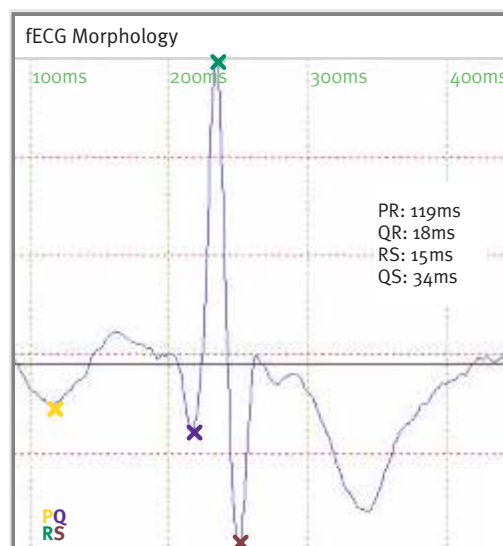
**Figure 8.** An example of electrode position for trans-abdominal fetal ECG.

The electro-physiological signal contains the maternal ECG, fetal ECG and electro-histogram, and is recorded with a sample frequency of 900 Hz using 5 disposable electrodes placed on the maternal abdomen in a standardized manner (Figures 8 and 9).



**Figure 9.** Maternal and fetal ECG complexes obtained from 4 channels.

The methodology used for fetal ECG signal extraction and analysis was described in detail by Pieri et al [Pieri 2001]. Fetal ECG complexes were used to calculate R-R pulse intervals with an accuracy of approximately 1ms (Figure 10). STV (in ms) was calculated from the derived R-R intervals according to the analysis described by Dawes et al [Dawes 1991].



**Figure 10.** An example of fetal QRS complex used to extract fetal R-R intervals.

### Doppler velocimetry

Pulse-wave and color Doppler ultrasound examination of the uterine arteries, umbilical arteries, middle cerebral artery, and ductus venosus were performed in pregnancies with IUGR. Uterine artery Doppler velocimetry was defined as abnormal if the mean (the average of right and left) pulsatility index (PI) was above the 95<sup>th</sup> percentile for gestational age (using the reference ranges proposed by Gomez et al.) [Gomez 2008]. Umbilical artery Doppler velocimetry was defined as abnormal if either the PI was above the 95<sup>th</sup> percentile for gestational age (using the reference ranges proposed by Arduini and Rizzo) [Arduini 1990]



or in the presence of abnormal waveforms (absent or reversed end-diastolic velocities, ARED)[*Trudinger 1991*]. Cerebro-placental ratio was calculated as follows: PI CMA/PI Um, and the value <1 was considered abnormal.

The following Doppler velocimetry groups according to the assessed district were considered:

- Doppler velocimetry of the uterine arteries (mean PI):
  - o Normal finding (mean PI  $\leq$  95<sup>th</sup> percentile);
  - o Increased resistance (mean PI > 95<sup>th</sup> percentile);
- Doppler velocimetry of the umbilical artery:
  - o Normal finding (PI  $\leq$  95<sup>th</sup> percentile);
  - o Increased resistance (PI > 95<sup>th</sup> percentile);
  - o Absent or reversed end-diastolic flow;
- Doppler velocimetry of middle cerebral artery:
  - o Normal finding (PI  $\geq$  5<sup>th</sup> percentile);
  - o Decreased resistance (PI < 5<sup>th</sup> percentile);
- Doppler of ductus venosus:
  - o Normal finding (PI  $\leq$  95<sup>th</sup> percentile);
  - o Increased resistance (PI > 95<sup>th</sup> percentile);
  - o Reversed a wave.

### *Details of PRSA analysis*

PRSA analysis provides an estimate of the autonomic regulation of the HR even when phase de-synchronizations due to abrupt changes in the system, miss-detected beats and signal losses are present [*Bauer 2006*]. Briefly, a set of anchor points is determined on the fetal R-R series: each time point  $t$  that satisfies the following criterion is inserted into the anchor points' list (i.e. deceleration):

$$\frac{1}{T} \sum_{i=0}^{T-1} fRR(t+i) > \frac{1}{T} \sum_{i=1}^T fRR(t-i)$$

A window of length  $2L$  is centered on each anchor point (the anchor point is at position  $L+1$ ). Then, the windows are aligned and averaged, obtaining the PRSA series. Finally, the PRSA series is used to compute the ADC with:

$$\text{ADC (or AAC)} = \frac{1}{2s} \sum_{i=1}^s \text{PRSA}(L + i) - \frac{1}{2s} \sum_{i=0}^{s-1} \text{PRSA}(L - i)$$

It is worth noting that this expression is substantially equivalent to a wavelet transform (Haar wavelet) of the PRSA series, evaluated at scale  $s$  and location  $L+1$ .

Three parameters,  $T$ ,  $s$  and  $L$ , need to be specified.  $T$  sets the number of points of the low-pass moving average filter employed before the detection of anchor points. It is an upper frequency limit for the periodicities that can lead to the selection of anchor points by PRSA (*i.e.* the 3 dB pass-band of the filter ends approximately at  $f \approx 0.603/(T \overline{fRR})$  Hz, where  $\overline{fRR}$  is the average fetal RR interval in seconds).  $L(\geq T)$  determines the extension of the PRSA series. In principle it needs to be larger than the period of the slowest oscillation that one wants to collect with PRSA; however when computing AAC or ADC it suffices to be as large as  $T$ . Finally, the scale  $s$  selects the oscillations in the PRSA series which most affect AAC and ADC. Approximately, using a Haar wavelet, a scale  $s$  corresponds to the frequency  $f \approx 0.371/(s \overline{fRR})$  Hz. In here, the scale  $s$  is taken to be equal to  $T$ . This is not mandatory (*i.e.* in Bauer et al.  $T=1$  and  $s=2$  were found to be optimal for prediction of mortality after myocardial infarction in adults) but shared with two previous studies employing PRSA on fetal R-R series [Huhn 2001; Lobmaier 2012]. Using  $s=T$  we avoided the need of optimizing a further parameter. We leave this effort to further studies, even if in our preliminary experiments no significant improvements were noticed (unreported results).

AAC is computed with an identical procedure, but after employing a different criterion for selecting the anchor points:

$$\frac{1}{T} \sum_{i=0}^{T-1} fRR(t + i) < \frac{1}{T} \sum_{i=1}^T fRR(t - i)$$

### Statistical analysis

A Kolmogorov-Smirnov or Shapiro-Wilk test and visual plot inspection were used to assess normality of arithmetic data distributions. A Kruskal-Wallis with post-hoc Mann-Whitney U test was used to compare variables among and between groups. A non-parametric test was a priori chosen while the group numbers were small, and in order to account for the out-layers. In case of non-uniformly distributed data uniform distribution was obtained by

logarithmic transformation. Comparison of proportions was performed using Chi-square or Fisher's exact test, as appropriate. Correlation between two continuous variables was determined using Spearman's rank correlation.

Analyses were performed with SPSS, version 21 (IBM Corp, Armonk, NY).

## RESULTS

### *Demographic and clinical characteristics of the study population*

Sixty-six patients were recruited: 22 pregnancies with IUGR and 44 uncomplicated pregnancies. Seven women from the control group had some complication (cholestasis, preterm labor, polyhydramnios), and thus were excluded from the final analysis. Demographic and clinical characteristics of pregnancies with IUGR and uncomplicated pregnancies according to gestational age group are represented in *Table 4*. The proportion of nulliparous women was greater among women with IUGR than in those with uncomplicated pregnancy in the 26-<30 weeks of gestation group. No differences were observed by age, BMI, and gestational age at monitoring.

	Control 26-<30 wks (n=11)	IUGR 26-<30 wks (n=7)	<i>p</i>	Control ≥30-<34 wks (n=26)	IUGR ≥30-<34 wks (n=15)	<i>p</i>
Age (years)	32 (27-35)	36 (35-40)	0.06	33 (29-36)	32 (30-44)	0.82
Nulliparous	5 (54%)	7 (100%)	0.04	20 (76.9%)	11 (73.3%)	0.80
BMI (kg/m <sup>2</sup> )	21.8 (18.3-27.3)	22.0 (19.6-33.0)	0.6	20.7 (19.1-22.9)	24.2 (21.1-25.0)	0.06
GA at monitoring (wks)	27.4 (26.0-29.1)	27.2 (26.4-29.5)	0.6	31.8 (30.9-32.6)	32.2 (31.0-33.1)	0.6

**Table 4.** Demographic and clinical characteristics of the study population. Values are expressed as median (interquartile range) or number (percent). Control refers to uncomplicated pregnancies. Wks: gestational weeks; GA: gestational age.

The clinical characteristics of pregnancies with IUGR are presented in *Table 5*. The gestational age at delivery, birth weight and number of perinatal complications were higher in very early IUGR group (26-<30 weeks of gestation) than in early IUGR group (≥30-<34 weeks of gestation). As per study design, all uncomplicated pregnancies delivered at term [median gestational age at delivery 26-<30 weeks group 40.0 weeks (IQR 38.1-40.5), and ≥30-<34 weeks group 39.5 weeks (IQR 39.2-40.1), respectively], neonates with birth weight appropriate for gestational age [median birth weight 26-<30 weeks group 3750 grams (IQR 3470-3925), and ≥30-<34 weeks group 3563 grams (IQR 3177-3669), respectively].

Parameter	IUGR 26-<30 wks (n=7)	IUGR ≥30-<34 wks (n=15)
GA at admission (wks)	26.5 (25.4-27.6)	31.3 (30.0-32.3)
GA at delivery (wks)	27.3 (26.6-31.1)	33.0 (31.2-34.6)
Birth weight (grams)	750 (707-1064)	1320 (1175-1820)
pH	7.26 (7.24-7.34)	7.32 (7.28-7.33)
Base excess	-3.55 (-6.10 - -1.48)	-3.10 (-8.90 - -1.40)
NICU admission	7 (100%)	15 (100%)
Neonatal death	1	0
Perinatal complications	6 (86%)	9 (60%)

**Table 5.** Clinical characteristics and neonatal outcome of pregnancies with IUGR. The values are expressed as medians (interquartile range) or number (percentage). Wks: gestational weeks; GA: gestational age; NICU: neonatal intensive care unit. Perinatal complications: intraventricular hemorrhage, periventricular leucomalacia, necrotizing enterocolitis, respiratory distress syndrome, bronco-pulmonary dysplasia.

STV calculated on fetal R-R intervals from trans-abdominal ECG was significantly lower in IUGR fetuses than in healthy fetuses in the 26-<30 weeks group (Table 6). The same was not true for ≥30-<34 weeks group (Table 6).

	Control 26-<30 wks (n=11)	IUGR 26-<30 wks (n=7)	p	Control ≥30-<34 wks (n=26)	IUGR ≥30-<34 wks (n=15)	p
STV (ms)	10.7 (9.9-11.9)	6.0 (5.1-9.0)	0.01	10.3 (8.9-13.2)	9.9 (7.5-10.7)	0.72

**Table 6.** Short-term variation (STV) calculated on fetal ECG R-R intervals. The values are expressed as medians (interquartile range). Wks: gestational weeks; ms: milliseconds.

### PRSA analysis

The ADC and AAC computed for T values in the range 1÷10, and thereafter for increasing values of 5 (T15, T20, T25, T30, T35, T40, T45, and T50), are represented in the Tables 7 and 8, respectively.

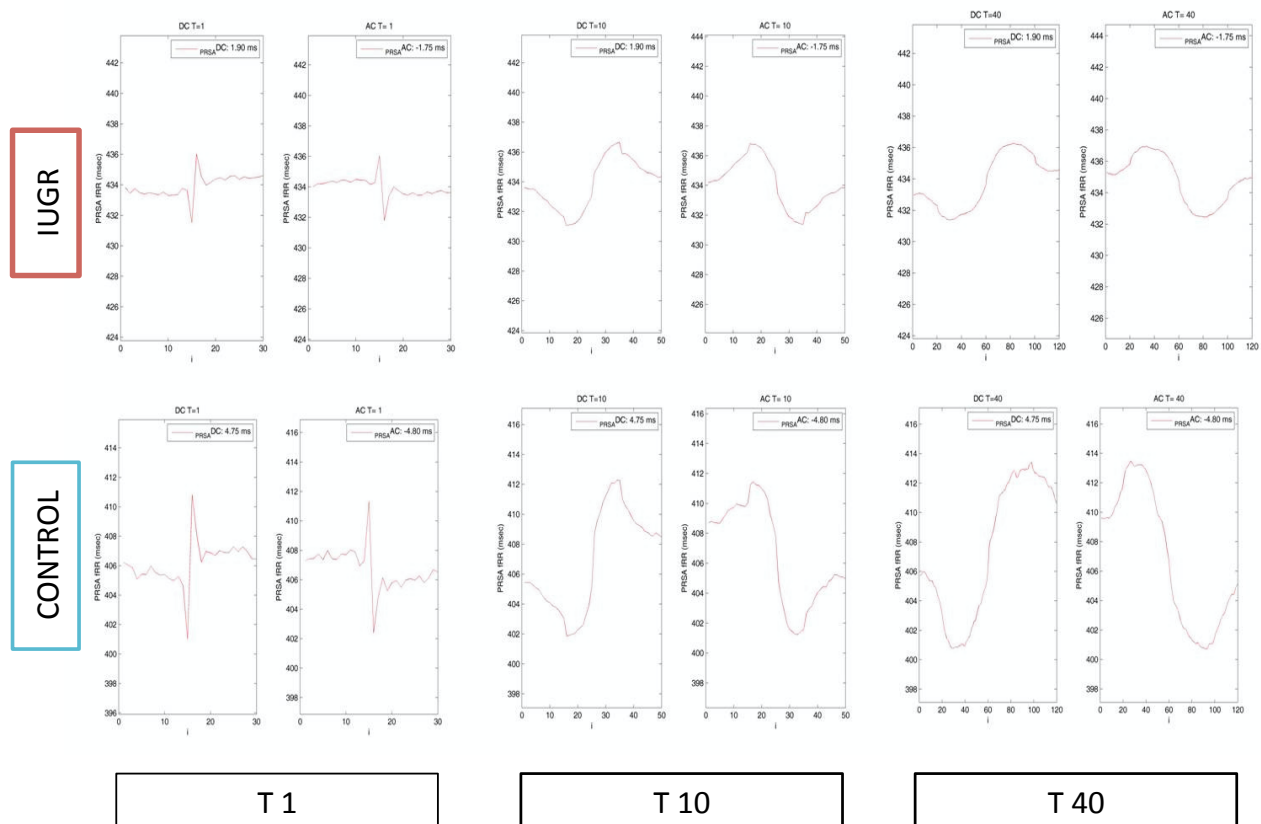
ADC	Control	IUGR	<i>p</i>	Control	IUGR	<i>p</i>
	26-<30 wks (n=11)	26-<30 wks (n=7)		≥30-<34 wks (n=26)	≥30-<34 wks (n=15)	
T1	4.64 (4.00-5.19)	2.27 (2.18-5.22)	0.063	4.46 (4.02-5.17)	4.42 (3.99-5.23)	0.978
T2	4.41 (3.36-4.86)	1.85 (1.76-4.08)	0.010	4.29 (3.48-5.29)	4.18 (3.40-4.60)	0.343
T3	4.24 (3.05-4.71)	1.76 (1.59-3.47)	0.006	4.33 (3.49-5.37)	3.55 (3.17-4.34)	0.79
T4	3.93 (3.29-4.60)	1.87 (1.53-3.12)	0.003	4.51 (3.45-5.28)	3.29 (3.14-4.08)	0.013
T5	3.88 (3.38-4.73)	1.95 (1.57-3.03)	0.002	4.75 (3.69-5.33)	3.24 (2.92-3.97)	0.004
T6	3.82 (3.51-4.60)	1.89 (1.61-2.96)	0.001	4.92 (3.68-5.24)	3.22 (2.97-4.02)	0.001
T7	3.74 (3.56-4.49)	1.90 (1.69-2.82)	0.001	4.90 (3.71-5.36)	3.33 (3.04-4.04)	<0.0001
T8	3.74 (3.62-4.44)	1.96 (1.74-2.78)	0.001	4.92 (3.75-5.34)	3.42 (3.06-4.06)	<0.0001
T9	3.73 (3.54-4.49)	2.02 (1.76-2.72)	0.001	4.93 (3.74-5.27)	3.49 (3.08-4.09)	<0.0001
T10	3.74 (3.54-4.55)	2.07 (1.74-2.64)	0.001	4.78 (3.76-5.28)	3.42 (3.08-4.19)	<0.0001
T15	3.85 (3.38-4.27)	2.15 (1.69-2.81)	<0.0001	4.60 (3.80-5.40)	3.35 (2.81-3.89)	<0.0001
T20	3.84 (3.30-4.29)	2.31 (1.65-2.72)	0.001	4.64 (3.83-5.46)	3.19 (2.67-3.86)	<0.0001
T25	3.81 (3.28-4.29)	2.39 (1.66-2.63)	0.001	4.60 (3.85-5.49)	3.28 (2.61-3.97)	0.001
T30	3.76 (3.22-4.10)	2.32 (1.66-2.57)	0.001	4.35 (3.62-5.43)	3.40 (2.44-3.95)	0.004
T35	3.81 (3.18-4.14)	2.20 (1.65-2.52)	0.001	4.27 (3.63-4.27)	3.59 (2.29-4.07)	0.005
T40	3.94 (3.31-4.13)	2.08 (1.62-2.44)	0.001	4.22 (3.59-5.42)	3.65 (2.11-4.25)	0.013
T45	4.02 (3.36-4.36)	1.99 (1.59-2.34)	0.001	4.13 (3.50-5.63)	3.52 (2.02-4.23)	0.015
T50	4.06 (3.31-4.62)	1.93 (1.54-2.29)	0.001	4.22 (3.39-5.69)	3.37 (2.03-4.27)	0.016

**Table 7.** Average deceleration capacity (ADC) computed by PRSA for *T* values 1÷50. The values are expressed as medians (interquartile range). Wks: gestational weeks. Control refers to uncomplicated pregnancies.

AAC	Control	IUGR	<i>p</i>	Control	IUGR	<i>p</i>
	26-<30 wks (n=11)	26-<30 wks (n=7)		≥30-<34 wks (n=26)	≥30-<34 wks (n=15)	
T1	-4.48 (-5.19 -3.74)	-2.24 (-5.11 -2.05)	0.063	-4.34 (-4.93 -3.73)	-4.43 (-5.19 -3.75)	1.00
T2	-3.66 (-4.50 -3.05)	-1.69 (-3.89 -1.63)	0.033	-3.86 (-4.34 -3.12)	-3.46 (-4.00 -3.12)	0.417
T3	-3.53 (-4.36 -2.76)	-1.65 (-3.24 -1.48)	0.010	-3.84 (-4.47 -2.95)	-3.34 (-3.70 -2.86)	0.110
T4	-3.61 (-4.41 -2.61)	-1.68 (-2.86 -1.45)	0.004	-3.88 (-4.55 -3.03)	-3.16 (-3.71 -2.69)	0.035
T5	-3.51 (-4.01 -2.80)	-1.81 (-2.67 -1.49)	0.002	-4.03 (-4.87 -3.16)	-3.09 (-3.81 -2.69)	0.012
T6	-3.51 (-3.93 -2.98)	-1.92 (-2.52 -1.57)	0.001	-4.10 (-4.97 -3.24)	-3.02 (-3.71 -2.65)	0.006
T7	-3.46 (-4.06 -3.15)	-1.95 (-2.52 -1.63)	0.001	-4.21 (-4.83 -3.35)	-3.01 (-3.73 -2.55)	0.003
T8	-3.40 (-4.22 -3.28)	-1.98 (-2.48 -1.69)	0.001	-4.32 (-4.73 -3.44)	-3.00 (-3.82 -2.65)	0.002
T9	-3.41 (-4.31 -3.27)	-2.02 (-2.44 -1.77)	0.001	-4.37 (-4.63 -3.51)	-3.01 (-3.97 -2.73)	0.001
T10	-3.42 (-4.34 -3.23)	-2.05 (-2.44 -1.81)	0.001	-4.40 (-4.71 -3.58)	-3.07 (-4.02 -2.70)	0.001
T15	-3.56 (-4.30 -3.29)	-2.13 (-2.65 -1.83)	<0.0001	-4.38 (-4.99 -3.67)	-3.12 (-3.94 -2.76)	0.001
T20	-3.64 (-4.24 -3.24)	-2.35 (-2.62 -1.72)	<0.0001	-4.40 (-5.15 -3.62)	-3.09 (-3.85 -2.72)	0.002
T25	-3.71 (-4.23 -3.24)	-2.45 (-2.60 -1.67)	<0.0001	-4.63 (-5.21 -3.54)	-3.08 (-3.93 -2.57)	0.003
T30	-3.83 (-4.20 -3.33)	-2.43 (-2.50 -1.66)	0.001	-4.58 (-5.36 -3.53)	-3.20 (-3.97 -2.49)	0.004
T35	-3.78 (-4.32 -3.46)	-2.44 (-2.50 -1.67)	0.001	-4.64 (-5.45 -3.54)	-3.48 (-3.82 -2.35)	0.008
T40	-3.76 (-4.77 -3.44)	-2.46 (-2.60 -1.65)	0.001	-4.81 (-5.55 -3.52)	-3.54 (-3.87 -2.21)	0.015
T45	-3.87 (-5.08 -3.40)	-2.43 (-2.64 -1.67)	0.001	-4.83 (-5.84 -3.47)	-3.60 (-4.03 -2.17)	0.035
T50	-4.11 (-5.10 -3.55)	-2.39 (-2.73 -1.71)	0.001	-4.87 (-6.07 -3.40)	-3.61 (-4.11 -2.16)	0.042

**Table 8.** Average acceleration capacity (AAC) computed by PRSA for *T* values 1÷50. The values are expressed as medians (interquartile range). Wks: gestational weeks. Control refers to uncomplicated pregnancies.

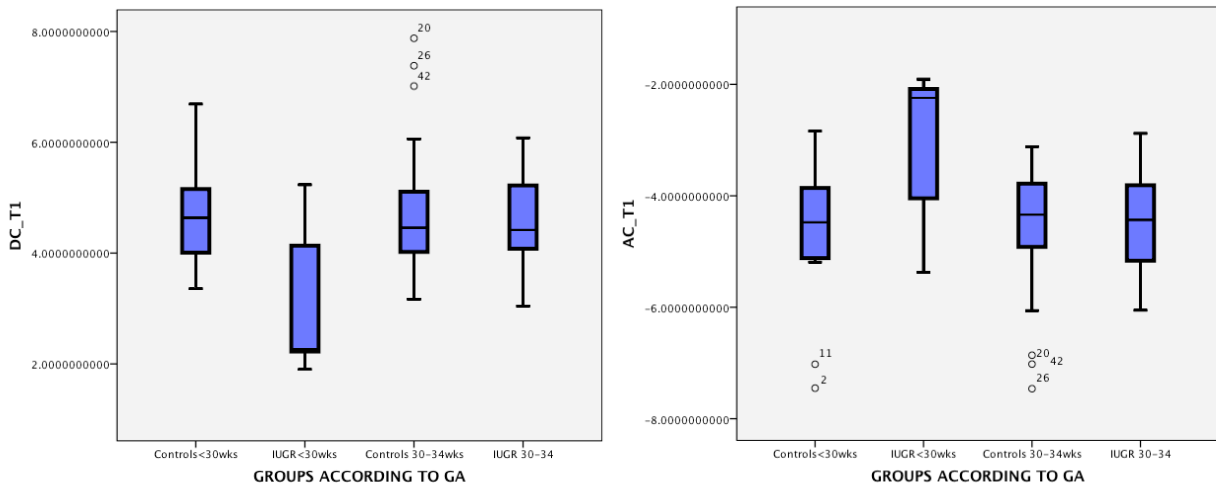
Figure 11 represents examples of ADC and AAC curves. It can be observed a reduction of the central part of the AAC and ADC curve in IUGR fetuses. The difference was higher for  $T \neq 1$ .



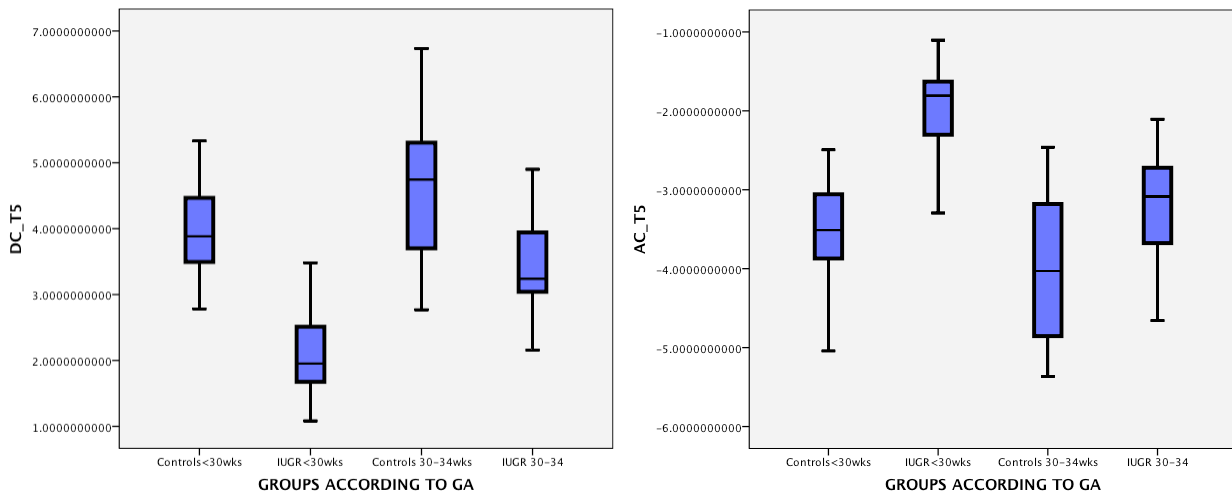
**Figure 11.** Example of ADC and AAC curves in IUGR (first line) and control (second line) fetuses, respectively. The columns represent different  $T$  values adopted for the computation of PRSA (i.e.  $T_1$ ;  $T_{10}$ ;  $T_{40}$ ). There is a reduction of the central part of the ADC and AAC curve in IUGR.

Summarizing, the ADC and AAC were significantly lower in 26-<30 weeks IUGR group than in matched for gestational age healthy fetuses for all  $T$  values ( $2 \div 50$ ) except for  $T=1$  (Tables 7 and 8; Figures 12-15). Similar results were encountered in the  $\geq 30$ -<34 weeks IUGR group, where difference was not statistically significant for  $T 1 \div 3$  (Tables 7 and 8; Figures 12-15). For all other  $T$  values ( $4 \div 50$ ), the AAC and ADC were significantly lower in IUGR fetuses than in matched for gestational age healthy fetuses. The amplitude of the difference between IUGR and control group was higher for very early IUGR group (26-<30 weeks) than in early IUGR group ( $\geq 30$ -<34 weeks), respectively (Figures 13-15).

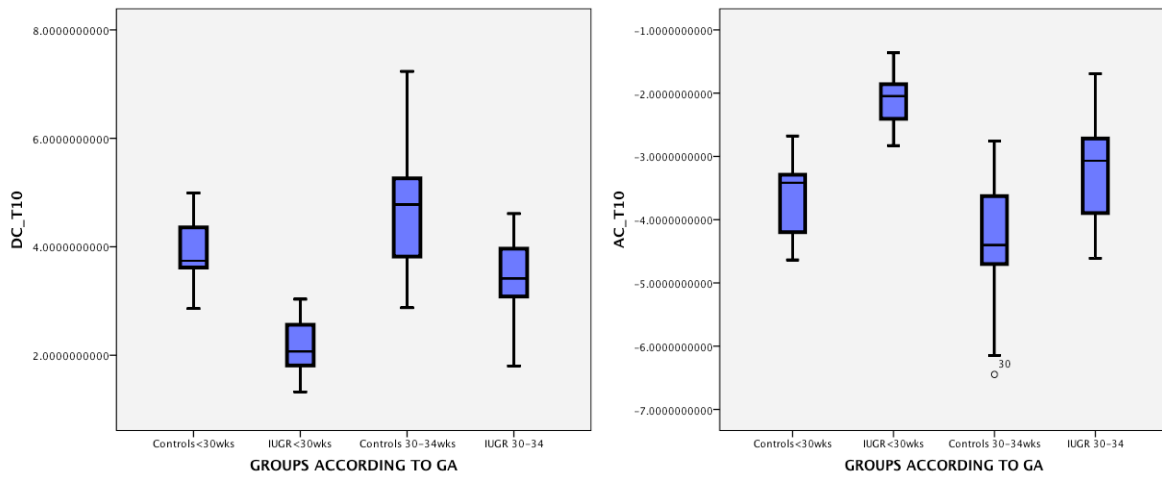




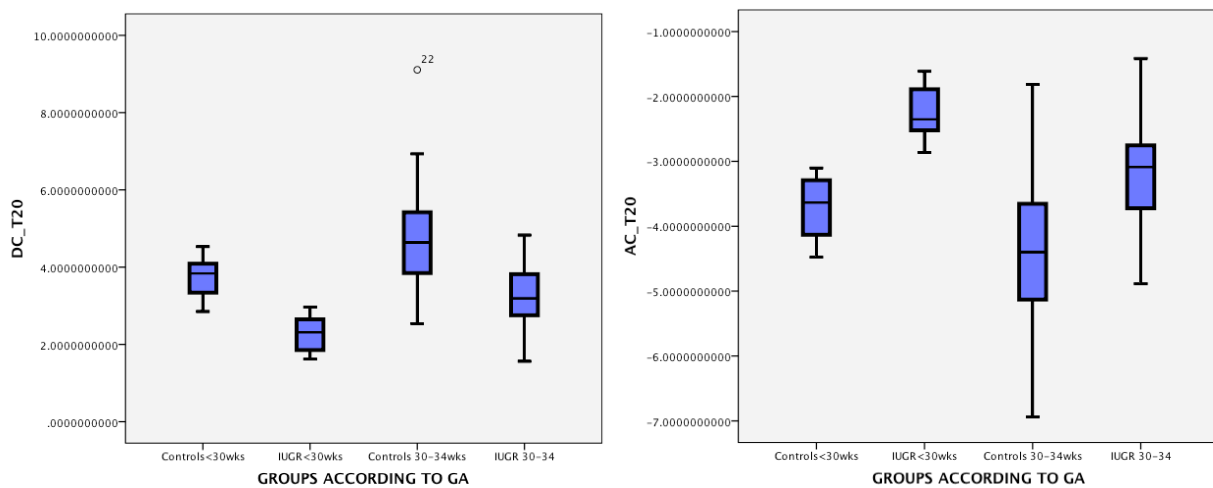
**Figure 12.** Box and whiskers representation of ADC (left panel) and AAC (right panel) for  $T=1$  by study groups. No differences were encountered between the groups ( $p>0.05$ ). Mann Whitney test was adopted.



**Figure 13.** Box and whiskers representation of ADC (left panel) and AAC (right panel) for  $T=5$  by study groups. IUGR fetuses present lower ADC and ACC than healthy fetuses (all  $p<0.05$ ). Mann Whitney test was adopted.



**Figure 14.** Box and whiskers representation of ADC (left panel) and AAC (right panel) for  $T=10$  by study groups. IUGR fetuses present lower ADC and ACC than healthy fetuses (all  $p<0.05$ ). Mann Whitney test was adopted.



**Figure 15.** Box and whiskers representation of ADC (left panel) and AAC (right panel) for  $T=20$  by study groups. IUGR fetuses present lower ADC and ACC than healthy fetuses (all  $p<0.05$ ). Mann Whitney test was adopted.

### Average acceleration and deceleration capacity and Doppler velocimetry

Doppler velocimetry characteristics of the pregnancies with IUGR are summarized in Table 9. The placental resistance and the fetal involvement were higher in very early IUGR group (26-<30 weeks) than in early IUGR group ( $\geq 30$ -<34 weeks), respectively.

Parameter	IUGR 26-<30 wks (n=7)	IUGR ≥30-<34 wks (n=15)	p
Uterine arteries mean PI	1.66 (1.45-1.74)	1.20 (0.80-1.41)	0.008
Uterine arteries mean PI>2DS for GA	6 (85.7%)	9 (60%)	
Umbilical artery PI	1.95 (1.54-3.94)	1.48 (1.17-1.62)	0.04
Umbilical artery PI >2DS for GA	2 (28.6%)	6 (40%)	
Umbilical artery, ARED	4 (57.1%)	2 (13.3%)	
Middle cerebral artery PI	0.98 (0.85-1.28)	1.65 (1.23-1.79)	0.015
Cerebro/placental ratio	0.49 (0.29-0.71)	1.08 (0.67-1.63)	0.005
Ductus venosus PI>2DS or absent/reverse a wave	4 (57.1%)	1 (6.7%)*	

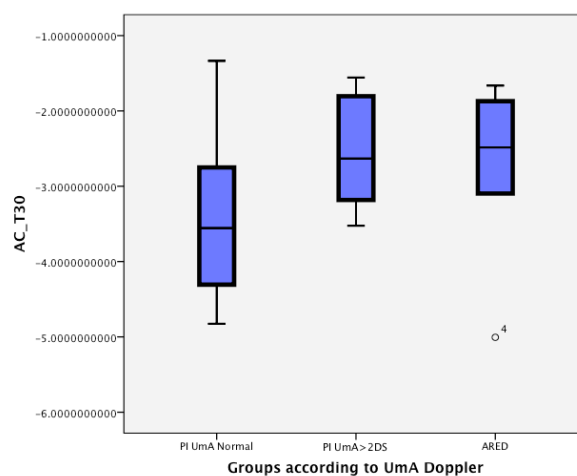
**Table 9.** Doppler velocimetry characteristics of the IUGR fetuses. The values are expressed as medians (interquartile range) or number (percentage). Wks: gestational weeks; GA: gestational age; PI: pulsatility index; DS: deviation standard; ARED: absent or reverse end diastolic flow. \* missing data n=3.

**Doppler velocimetry of uterine arteries and ACC/ADC.** There was no correlation between uterine arteries mean PI and AAC and ADC calculated for any T value in IUGR fetuses (all  $p>0.05$ , data not showed). Similarly, when considering mean uterine arteries PI as categorical variable (uterine arteries mean PI  $\leq 2DS$  and  $>2DS$  for gestational age, respectively), there were no differences in ACC and ADC for any T value (all  $p>0.05$ , Table 10).

**Doppler velocimetry of umbilical artery and ACC/ADC.** There was a significant positive correlation between umbilical artery PI and ACC for T values 1÷15 (Spearman Rho 0.43-0.521, all  $p<0.05$ ). Significant negative correlation between umbilical artery PI and ADC was observed for wider T range 1÷30 (Spearman Rho -0.43 - -0.56, all  $p<0.05$ ). For  $T\geq 20$  and  $T\geq 35$  there was no correlation between umbilical artery and AAC and ADC, respectively (all  $p>0.05$ ). When considering umbilical artery as a categorical variable (umbilical artery PI  $\leq 2DS$ , umbilical artery  $>2DS$ , and ARED, respectively), there were no differences in ACC and ADC for any T value among the groups although there was a clear decreasing trend of AAC and ADC values as Doppler velocimetry finding in umbilical artery deteriorated (all  $p>0.05$ , Figure 16). Similar results were found for middle cerebral artery and cerebro-placental ratio (data not showed).

ACC	Mean UtA PI≤2 SD (n=6)*	Mean UtA PI>2 SD (n=15)	p	ADC	Mean UtA PI≤2 SD (n=6)	Mean UtA PI>2 SD (n=15)	p
T1	-4.09 (-5.16; -3.74)	-3.93 (-5.20; -2.24)	0.6	T1	4.39 (4.12; 5.32)	3.75 (2.27; 5.23)	0.4
T5	-2.93 (-3.51; -2.73)	-2.50 (-3.81; -1.81)	0.5	T5	3.21 (3.10; 3.82)	2.87 (1.95; 3.91)	0.4
T10	-2.90 (-3.83; -2.58)	-2.74 (-3.49; -1.90)	0.5	T10	3.36 (2.83; 3.85)	3.03 (1.88; 3.42)	0.3
T15	-3.01 (-3.88; -2.57)	-2.67 (-3.34; -2.09)	0.3	T15	3.49 (2.62; 3.93)	2.81 (2.11; 3.35)	0.2
T20	-3.19 (-3.87; -2.51)	-2.72 (-3.44; -2.05)	0.4	T20	3.56 (2.58; 4.04)	2.72 (2.06; 3.19)	0.2
T25	-3.29 (-3.89; -2.58)	-2.60 (-3.48; -1.94)	0.3	T25	3.56 (2.59; 4.14)	2.63 (2.06; 3.28)	0.2
T30	-3.33 (-3.92; -2.64)	-2.50 (-3.52; -1.87)	0.4	T30	3.57 (2.59; 4.12)	2.57 (1.93; 2.57)	0.2
T40	-3.51 (-3.89; -2.77)	-2.60 (-3.84; -1.75)	0.5	T40	3.73 (2.55; 4.29)	2.40 (1.90; 3.69)	0.2
T50	-3.61 (-4.15; -2.76)	-2.48 (-3.85; -1.73)	0.4	T50	3.79 (2.39; 4.32)	2.22 (1.91; 3.72)	0.2

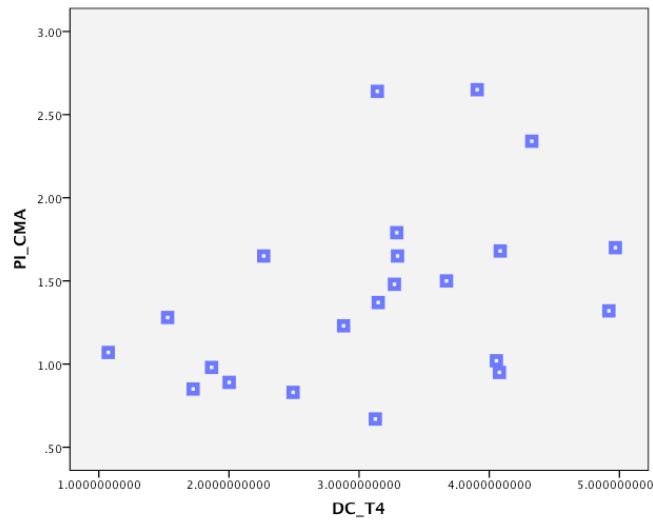
**Table 10.** Average acceleration (ACC) and average deceleration (ADC) capacity according to uterine arteries categorical finding. The values are expressed as medians (interquartile range). Not all T values are showed. UtA: uterine arteries; PI: pulsatility index; SD: standard deviation. \* 1 missing data.



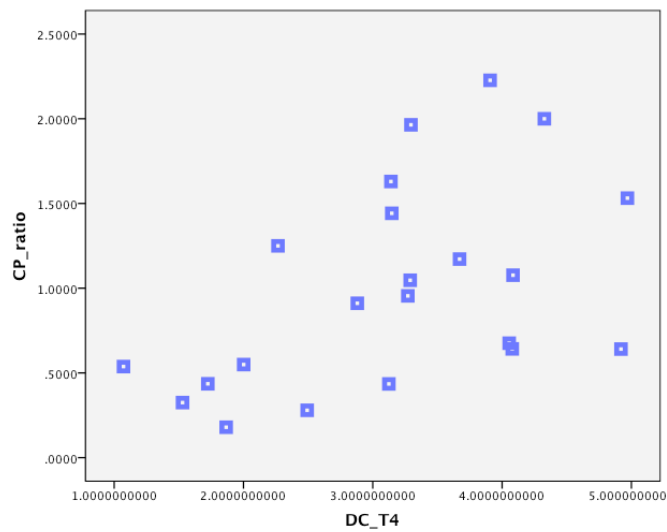
**Figure 16.** Average acceleration capacity (T=30) according to umbilical artery categorical finding. Although there is a clear decreasing trend, the difference was not statistically significant among groups ( $p > 0.05$ ). Kruskal Wallis test was adopted.

**Doppler velocimetry of middle cerebral artery and ACC/ADC.** There was a significant negative correlation between middle cerebral artery PI and AAC for T 1÷6 (Spearman Rho -0.45 - -0.48, all  $p < 0.05$ ). Similar results, but positive correlation, and in the same interval of T values (1÷6), were observed for ADC (Spearman Rho 0.44-0.50, all  $p < 0.05$ , *Figure 17*). For  $T \geq 7$  there was no correlation between middle cerebral artery PI and AAC/ADC, respectively (all  $p > 0.05$ ).

**Cerebro/placental ratio and ACC/ADC.** There was a significant negative correlation between the cerebral/placental ratio and ACC for T 1÷10 (Spearman Rho -0.46 - -0.57, all  $p < 0.05$ ); while positive correlation was found for ADC in the same interval (T 1÷10; Spearman Rho 0.48-0.59, all  $p < 0.05$ , *Figure 18*). For  $T \geq 15$  there was no correlation between cerebro/placental ratio and AAC (all  $p > 0.05$ ) or ADC (all  $p > 0.05$ ), respectively.



**Figure 17.** Correlation between average deceleration capacity (ADC; T=4) and middle cerebral artery pulsatility index (Spearman Rho 0.50,  $p=0.02$ ).



**Figure 18.** Correlation between average deceleration capacity (ADC; T=4) and cerebro/placental ratio (Spearman Rho 0.59,  $p=0.004$ ).

## DISCUSSION

### *The principal findings of the study*

1) In the 26-<30 weeks group, except for T=1, the AAC and ADC were significantly lower in IUGR fetuses than in matched for gestational age healthy fetuses (for T values 2÷50); 2) In the ≥30-<34 weeks group, except for T 1÷3, the AAC and ADC were significantly lower in IUGR fetuses than in matched for gestational age healthy fetuses (for T values 4÷50); 3) The amplitude of the difference was higher for very early IUGR group (26-<30 weeks) than in early IUGR group (≥30-<34 weeks); 4) STV computed on fetal ECG R-R intervals from trans-abdominal ECG was lower in IUGR fetuses than in matched for gestational age healthy fetuses in 26-<30 weeks group; 5) There were no differences in STV in ≥30-<34 weeks group; 6) There was no correlation between AAC/ADC and uterine arteries mean PI; 7) When evaluating ADC correlation with other districts, there was: a significant negative correlation with umbilical artery PI, a significant positive correlation with middle cerebral artery PI, and significant positive correlation with cerebro/placental ratio, respectively; the same was true for AAC but in opposite direction; and 8) The correlation was found to be significant in all districts in a narrow range of T values, 1÷6; 9) When considering the Doppler velocimetry districts as categorical variables (normal, pathological), there was a clear trend of lower values of AAC and ADC as Doppler parameters deteriorated, but this difference did not reach statistical significance.

### *The AAC and ADC are lower in IUGR fetuses than in healthy matched for gestational age fetuses*

We found that the AAC and ADC are significantly lower in IUGR fetuses than in those with an appropriate growth and matched for gestational age. This finding is partially in agreement with previous reports on PRSA analysis in IUGR fetuses [Graatsma 2012; Lobmaier 2012; Huhn 2011], while two studies found the supremacy of AAC over ADC [Lobmaier 2012; Huhn 2011]. We were not able to confirm this finding. In our analysis both AAC and ADC showed similar differences between IUGR and healthy fetuses, in agreement to the findings by Graatsma et al. [Graatsma 2012]. Curiously, the two studies that found the supremacy of ACC over ADC applied PRSA on standard CTG [Lobmaier 2012; Huhn 2011], while our study and that by Graatsma et al. applied PRSA analysis on fetal R-R intervals obtained by trans-

abdominal ECG [Graatsma 2012]. When applying PRSA on the signal obtained by standard Doppler CTG the main limitation is a low sampling frequency and the averaging of the FHR through a process of autocorrelation [Dawes 1991]. In fact, FHR obtained by Doppler does not provide the true beat-to-beat information while: 1) Doppler depicts mechanical movement of the heart, and not the electrical signal (i.e. R-R interval); and 2) standard CTG performs an averaging process of epoch-to-epoch variations. The two components of ANS operate at different frequency scales: sympathetic component in low frequency domain (0.04-0.15 Hz), while parasympathetic both in low and high frequency domain (0.15-1.0 Hz), respectively. Thus, the sampling rate is of crucial importance in order to identify high frequency bands. A possible explanation of discordant findings between the reports might be that an inadequate sampling rate adopted by CTG prevents from picking up the high frequency bands in which the vagal branch operates.

The most likely explanation of decreased ACC and ADC in IUGR fetuses is that the chronic nutritional deprivation and hypoxemia influence the function/maturation of sympathetic and parasympathetic components of ANS. This finding is in contrast to our previous results in an in-vivo term pregnant sheep model exposed to acute hypoxia (i.e. umbilical cord occlusions). In that experiment, as the hypoxic insult augmented, there was a clear increasing trend of the AAC and ADC, and this was true for a large interval of T values suggesting the activation of both sympathetic and parasympathetic component during progressive acute hypoxia. In case of appropriately grown fetus exposed to acute hypoxia the activation of ANS most likely constitutes a first line adaptive response, and results in a more pronounced FHR modulation and a larger cardio-vascular response. Indeed, in experiments on fetal lambs, acute fetal hypoxia led to increased FHR variability representing a sign of adequate fetal compensatory response [Parer 1980]. Nevertheless, if there is a prolonged acute hypoxic insult and overwhelming acidemia, parasympathetic activity will be subjected to a decreased activity [Siira 2013], causing reduced HR variability [Murotsuki 1997]. In fact, when the vagal regulation becomes inadequate some of the adaptive mechanism (such as chemoreceptor-mediated circulatory adaptation) might fail causing fetal brain damage, and ultimately fetal death. In case of chronic hypoxemia and IUGR there is a complex fetal cardio-vascular adaptation that involves hormone production, effects on fetal circulation and other phenomena. The effects of these changes on AAC and ADC are still too be elucidated.

While other studies considered a wide gestational age range (from 20 weeks of gestation to 42 weeks), we focused our study on early IUGR fetuses (<34 weeks). This choice was driven by two considerations. Firstly, there are profound physio-pathological differences



between preterm and term IUGR fetuses. Indeed, the vast majority of term IUGR fetuses, although suffer from nutrient deprivation and chronic hypoxemia, will not present Doppler velocimetry modifications in fetoplacental districts (umbilical artery, middle cerebral arteries). This fact does not preclude the possibility that AAC and/or ADC might represent a better parameter that will be able to identify those IUGR term fetuses, but, in this stage where the clear interpretation and significance of ADC and AAC in IUGR fetus is still missing, we opted to separate those two groups. Secondly, we wanted to analyze separately the very early IUGR group (26-<34 weeks) from early IUGR group ( $\geq 30$ -<34 weeks). The rationale was given by the fact that ANS, and thus FHR, are influenced by the gestational age. Sympathetic branch develops earlier in the pregnancy than the parasympathetic branch. The latter intensify its modulation later in pregnancy [Assali 1977; Signorini 2003]. In fact, our study is the first one to consider this separation, and we found that the amplitude of the difference was higher for very early IUGR group (26-<30 weeks) both for AAC and ADC. There is no clear explanation, although there might be two reasons. The first one takes into account the severity of the IUGR. Indeed, the very early IUGR group presented worse Doppler velocimetry parameters, and thus the AAC and ADC might reflect the severity of the IUGR condition. The second possible explanation is that in IUGR fetuses there is a delayed maturation of ANS.

#### *The importance of T value in PRSA computation*

It is still uncertain to which extent the PRSA method separates vagal and sympathetic modulations, and this remains still to be determined although previous studies tended to consider the ACC as an expression of sympathetic activity, and ADC of parasympathetic activity. Indeed, AAC and ADC identify different behavior of the HR during deceleration and acceleration, respectively. Nevertheless, it would be too simplistic to consider ADC only as an expression of parasympathetic modulation and ACC as an expression of sympathetic modulation. Rather, both ADC and AAC represent an integral measure of all periodic acceleration and deceleration-related oscillations that result from the interaction of sympathetic and parasympathetic component, and represent an integration of several input signals such as chemoreceptor, baroreceptor and others.

In PRSA computation the  $s=T$  parameter determines an upper frequency limit for the periodicities that mostly influence AAC and ADC [Bauer 2006]. For  $T=1$  high frequencies dominate the computation. At contrary, increasing values of  $T$  will progressively also emphasize the contribution of low frequency components.

The significance of T value has been explored in adult cardiology, and a T of 1-2 has been found as the best value [Kantelhardt 2007]. Despite there are few studies that applied PRSA method to FHR [Graatsma 2012; Lobmaier 2012; Huhn 2011], the impact of T parameter when PRSA is applied to FHR was not considered and explored. Moreover, different T values were adopted in different studies (i.e T=10 or T=40), thus making difficult to drive conclusions.

In our previous study on the in-vivo pregnant term sheep model we explored the influence of T values on PRSA computation. We found that T value in interval 2÷6 best enhances the differences between progressive cord occlusion phases (worsening hypoxia). T value in the interval 2÷6 depicts mainly high frequency bands, and thus would suggest that for higher degree of hypoxia and developing acidemia (as confirmed in the study by acid-base status), the parasympathetic component becomes predominant. These findings are in agreement with other reports that evaluated ANS response to hypoxia, but with different methodologies [Siira 2013; Frasch 2009; Durosier 2012].

When evaluating the correlation with acid-base biomarkers, the best correlation was observed for T value 2÷6. Thus, we suggested this interval for an effective computation of PRSA analysis, when detecting hypoxic/acidemic events in laboring fetuses. Although, the small dimension of the sample size and the animal model represented a major limit in our previous study, remains the fact that T value is important and has to be taken into account when interpreting PRSA results.

In the present study we observed significant differences both for AAC and ADC for all T values except for T=1 in 26-<30 weeks group, and for T 1÷3 in the ≥30-<34 group. These results would suggest that both sympathetic and parasympathetic components of ANS are affected by the nutrient restriction and hypoxemia, especially in very early IUGR group. In fact, it is well known that the earlier the manifestation of IUGR the more severe is the placental and fetal involvement due to placental insufficiency. The fact that in >30-<34 weeks group the T value becomes significant starting from T≥4 is in agreement with the previous observations that, in case of milder hypoxemia, the parasympathetic branch is probably not affected as the sympathetic branch [Gagnon 1989].

#### *AAC/ADC and short term variation*

The analysis and interpretation of FHR patterns by standard CTG is limited due to high intra and inter-observer variability and non-quantitative assessment [Figueras 2005; Pardey

2002]. The introduction of STV calculated by computerized CTG (cCTG) solved partially this problem [Dawes 1981]. Reduced STV represents one of late changes in the cascade of fetal compromise due to IUGR [Ribbert 1991; Snijders 1992; Visser 1990].

Previous studies on PRSA and IUGR found supremacy of AAC and ADC over the STV in identifying IUGR fetuses [Graatsma 2012; Lobmaier 2012; Huhn 2011]. In the present study we found lower STV in very early IUGR fetuses (26-<30 weeks), but no differences between early IUGR fetuses and healthy ones ( $\geq 30$ -<34 weeks). Thus, beside the fact that STV does not allow the separation of two branches of ANS, it appears that AAC and ADC reflect more specifically the state of ANS than STV, and thus potentially it might offer some advantages over STV. Nevertheless, in order to prove adequately this finding properly designed clinical studies are needed.

#### *AAC and ADC in relation to Doppler velocimetry of placental and fetal districts*

This is the first study that evaluated the correlation between AAC and ADC and Doppler velocimetry of utero-placental and feto-placental districts. While no correlation with uterine arteries mean PI was found, there was a significant correlation with umbilical artery PI, middle cerebral artery PI, and cerebro/placental ratio, respectively. The significance was found for all districts in a narrow range of T values, 1÷6. Interestingly, this interval overlaps that found in our previous study on pregnant sheep model exposed to acute cord occlusions. Indeed, in a T interval 2÷6 the correlation with the acid-base markers (pH, base excess and lactate) was highest. Doppler velocimetry in IUGR fetus evaluates progressive and gradual changes [Baschat 2001], and late Doppler changes correlate to fetal metabolic acidosis. The T interval 2÷6 respects high frequency changes, and thus parasympathetic component of the ANS. Thus, it would seem that the parasympathetic component of the ANS is more influenced than the sympathetic component by the Doppler modifications of the feto-placental districts. Whether AAC and ADC could predict the acid-base status in IUGR fetuses remains to be determined.

#### *Conclusions*

Power spectral analysis has been proposed as a method to quantify FHR variability. Indeed, several studies proved changes in power spectrum in relation to fetal hypoxia and/or acidemia during labor [Siira 2013; Rantonen 2001; Van Laar 2008]. As many other composite signals, FHR is generated by a non-stationary system influenced by internal and external

perturbations that alter its behavior. Moreover, the phase de-synchronizations due to abrupt changes in the system (as ventricular ectopic beats, maternal contractions, ...), miss-detected beats and signal losses determines a quasi-periodic behavior that limits the application of spectral analysis. PRSA method emphasizes quasi-periodic oscillations masked by unrelated non-stationary elements in the signal, and thus is robust to the non-stationarities, noise and artifacts. The PRSA series can be employed to quantify the “average acceleration capacity” (AAC) and “average deceleration capacity” (ADC) of the signal. When applied to HR, AAC and ADC represent an indirect integrated quantification of the activities of the sympathetic and parasympathetic autonomic systems [*Kantelhardt 2007*].

In conclusion, our study showed the evidences of an ANS “functional reduction” in IUGR fetuses through a computation of ACC and ADC of FHR, especially in very early IUGR group (<30 weeks). Moreover, we proved a correlation with Doppler velocimetry parameters of fetoplacental districts in the same T interval in which we found the highest correlation between AAC and ADC with acid-base biomarkers (data from a previous study). Thus, we hypothesize that AAC and ADC might have a role in the management of IUGR fetuses and we propose a choice of parameters for the computation of PRSA. Those findings put the solid ground for future clinical studies.

## APPENDIX

1. Presented at SGI 58th Annual Scientific Meeting, Miami 2011

### Analysis of Fetal Heart Rate by Phase-Rectified Signal Averaging (PRSA)

*Tamara Stampalija, Axel Bauer, Eleonora Rosti, Konstantinos Rizas, Cristina Mastroianni, Maria Signaroldi, and Enrico Ferrazzi.*

Department of Obstetrics and Gynaecology, Children's Hospital Vittore Buzzi, University of Milan, Milano, Italy and Department of Cardiology, University of Tuebingen, Tuebingen, Germany.

**Background:** Fetal heart rate variability (FHRV) is a window to the homeostasis of autonomous nervous system (ANS). Accurate analysis of FHRV requests R-R intervals, reason why standard CTG is inadequate. Alternative methods of analysis, such as spectral analysis of fetal ECG, cannot be applied due to the non-stationary nature of fECG. PRSA is a novel method described in adult cardiology able to cope with the non-stationary signals and to eliminate artifacts and noise from the original signal.

**Aim:** To prove the feasibility of application of PRSA analysis to trans-abdominally acquired fECG.

**Methods:** The study included 80 uneventful pregnancies from 39th to 41th gestational weeks. In all cases, 40 to 60 minutes recording of maternal and fetal ECG were performed by MonicaAN24 device and fetal R-R intervals were acquired. PRSA analysis of acceleration/deceleration capacity of the FHR was applied. PRSA analysis is based on four steps: 1. Definition of RR-intervals that are shorter/longer than the preceding RR intervals (so-called anchors); 2. Definition of segments around anchors; 3. Averaging of all segments; 4. Quantification of PRSA signal by acceleration/deceleration capacity (AC and DC).

**Results:** Figure 1 shows an example of typical acceleration related PRSA-signal of fetal heart rate. Clear oscillations in the high and low frequency range can be observed.

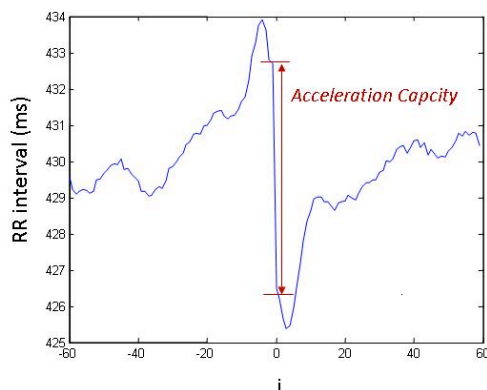


Figure 1 – Example of average acceleration capacity of fetal heart rate

**Conclusions:** We applied for the first time PRSA analysis to trans-abdominally acquired fetal ECG, resolving the problem of the non-stationarity of the signal, noise and artifacts. This method allows for a separate characterization of acceleration and deceleration related

modulations of fetal heart rate. We speculate that the acceleration capacity is more powerful predictor of fetal wellbeing in comparison to STV. Future studies are needed to prove the clinical usefulness of PRSA-based analysis of fetal heart rate variability.

*2. Presented at SGI 58th Annual Scientific Meeting, Miami 2011*

## **Relationship Between Acceleration Capacity of Fetal Heart Rate and Low-/High Variability**

*Konstantinos Rizas, Tamara Stampalija, Eleonora Rosti, Cristina Mastroianni, Maria Signaroldi, Axel Bauer, Enrico Ferrazzi.*

Department of Obstetrics and Gynaecology, Children's Hospital Vittore Buzzi, University of Milan, Milan, Italy and Department of Cardiology, University of Tuebingen, Tuebingen, Germany.

**Background:** More than 20 years ago Dawes-Redman criteria were proposed for the definition of low and high variability of fetal heart rate (FHR). While periods of low variability are physiological during the sleep state of the fetus, extremely low FHR variability and for prolonged period are sign of hypoxemia and compromised fetus.

A novel method of analysis has been proposed in adult cardiology, Phase Rectified Signal Averaging PRSA. The advantage of the method is the ability to extract periodic components out of non-stationary, noise time series.

**Aim:** To investigate the relationship between acceleration capacity of the FHR derived from PRSA analysis and periods of low and high FHR variability.

**Methods:** Women with uncomplicated pregnancy at term were monitored with Monica AN24 device. Trans-abdominal fetal and maternal ECG were acquired and subsequently analysed by PRSA analysis. Mathematical algorithm by Matlab was implemented in order to discriminate automatically periods of low and high variability by Dawes-Redman criteria. Acceleration capacity was calculated separately for periods of low and high variability in all individuals.

**Results:** 29 traces of fetal ECG with both low and high variability segments were analysed by PRSA method. Intra-individually acceleration capacity was significantly larger (more negative) in high variability segments when compared to low variability periods (mean -2.260.72 ms vs -1.090.36 ms respectively;  $p < 0.00001$ ) (Figure 1).

**Conclusions:** Our data suggest that acceleration capacity derived from PRSA analysis is able to identify low and high variability segments of FHR. Future studies should test whether acceleration capacity provides diagnostic information that is independent from that obtained by standard methods.

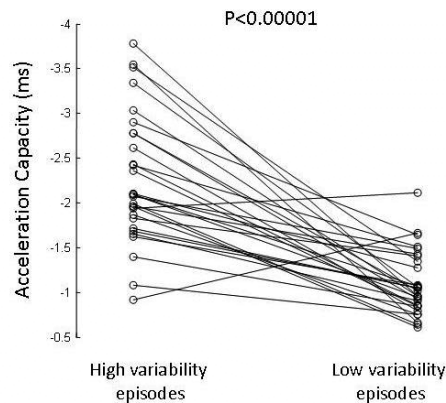


Fig 1. Intra-individual relationship between acceleration capacity and periods of low and high FHR variability.

3. Presented at SGI 59th Annual Scientific Meeting, San Diego 2012

### **Bivariate Phase-Rectified Signal Averaging (BPRSA): Description of Novel Technique**

A. Bauer, D. Casati, T. Stampalija, C. Zanardi, E. Rosti, C. Mastroianni, V. Signorelli, S. Zullino, E. Ferrazzi.

Cardiology, University of Tuebingen, Germany; Obstetrics, Childrens' Hospital Buzzi, University of Milan, Italy.

Non-stationarities are a major problem in the analysis of long recordings of complex biologic signals. Many internal/external perturbations cause interruptions of the periodic behavior and lead to phase de-synchronization of the oscillations. Phase-Rectified Signal Averaging (PRSA) showed the ability to extract *quasi*-periodic components out of non-stationary, noisy time series. Bivariate PRSA allows the analysis of the interrelations between two synchronously recorded biologic signals that -supposedly- influence each other. **Methods:** BPRSA technique analyzes the variations in one signal (target) when another signal (trigger) is in a certain phase/state. It is based on 4 steps: 1.Selection of anchor points in target signal contemporary to increase/decrease in trigger signal; 2.Definition of segments around anchors; 3.Phase-rectification and 4.Averaging of all segments. We applied BPRSA to evaluate the coupling between uterine activity (UA), as trigger signal, and fetal R-R intervals (fRR), as target signal, during labor. The signals were simultaneously recorded by transabdominal ECG (ta-ECG) (MonicaAN24).

**Results:** It was possible to apply BPRSA analysis to 104 raw data recorded by ta-ECG overcoming the problems of non-stationarity, non-linear relationship, noise and artifacts. The presence/absence of coupling between UA and FHR was assessed 'condensing' long time series in one, clear, mathematically computable (and so reproducible) graph showing (*quasi*)periodicities of the target signal coupled to the trigger signal.

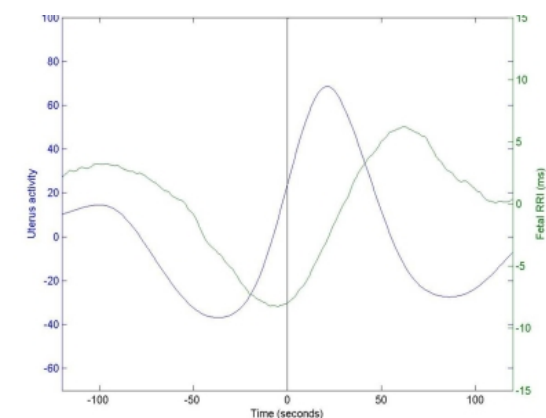


Figure 1. BPRSA: coupling between UA-trigger (blue) and fRR-target (green). The time axis allows to see how signals oscillate before and after the trigger event.

**Conclusions:** This is the first description of BPRSA application in obstetrics. BPRSA could represent a powerful tool for the analysis of inter-relationships between two or more synchronously recorded signals generated by complex biologic systems. Further studies are needed to explore the clinical usefulness of these findings.

*4. Presented at SGI 59th Annual Scientific Meeting, San Diego 2012*

### **Coupling Between Fetal Heart Rate And Uterine Activity Analyzed by Bivariate Phase-Rectified Signal Averaging (BPRSA)**

D. Casati, T. Stampalija, A. Bauer, C. Zanardi, E. Rosti, C. Mastroianni, V. Signorelli, S. Zullino, E. Ferrazzi.

Obstetrics and Gynecology, Childrens' Hospital Buzzi, University of Milan, Italy;  
Cardiology, University of Tuebingen, Germany.

Fetal heart rate (FHR) variability is the main proxy of fetal wellbeing during labor, being an indirect mirror of oxygenation and integrity of the autonomic nervous system and its ability to respond to internal and external stimuli.

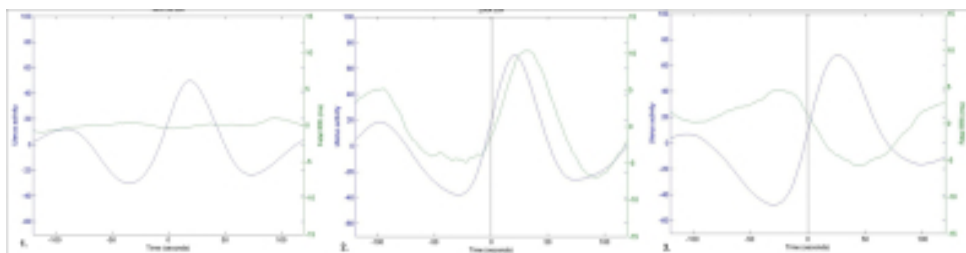
**Aim:** To evaluate the correlation (coupling) between FHR and uterine activity (UA) by Bivariate PRSA analysis, as a potential novel tool in clinical obstetrics.

**Methods:** Electrohisterogram, maternal and fetal ECG were simultaneously recorded by transabdominal ECG during the first stage of labor in women with uneventful pregnancy. BPRSA analysis (SGI 2012: Bivariate Phase-Rectified Signal Averaging (BPRSA): Description of Novel Technique) was applied to fetal RR-intervals (fRR) (target signal) in ascending phase of electrical UA (contraction) (trigger signal). Graphical representation was obtained for each recording. Different patterns of coupling were evaluated by qualitative analysis.

**Results:** 104 graphs were analyzed. Each graph compresses long time series of two related biological signals in one clear image. In 95.2% of cases there was presence of coupling with 2 different patterns: accelerative (50,5%) or decelerative (49,5%) response of FHR to UA.



Coupling could not be identified in 1.9% of cases, while 2.8% graphs were uninterpretable.



BPRSA graphs: Absence of coupling (1) between UA-trigger (blue oscillatory line), and fRR-target (green flat line); Presence of coupling (green line oscillation): decelerative (2) and accelerative (3) patterns.

Conclusions: As previously described, BPRSA analysis brings number of potential advantages over “standard” techniques. We were able to demonstrate the presence of coupling between FHR periodicity and uterine contractions identifying a cause-effect relationship between two complex biological signals. This new technique opens the door to future insights in fetal-maternal wellbeing monitoring in labor, with the need for further studies to explore the clinical usefulness.

*5. Accepted at SGI 60th Annual Scientific Meeting, Florence 2014*

### **Analysis of fetal ECG in fetal growth restriction**

*T Stampalija<sup>1</sup>, D Casati<sup>2</sup>, V Maggi<sup>2</sup>, M W Rivolta<sup>3</sup>, S Lupini<sup>2</sup>, K Rizas<sup>4</sup>, R Sassi<sup>3</sup>, A Bauer<sup>4</sup>, E Ferrazzi<sup>2</sup>*

<sup>1</sup> Prenatal Diagnosis, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy

<sup>2</sup> Dept. of Obstetrics and Neonatology, Buzzi Children’s Hospital, University of Milan, Italy

<sup>3</sup> Dipartimento di Informatica, Università degli Studi di Milano, Italy

<sup>4</sup> Innere Medizin III (Kardiologie), Eberhard-Karls-Universität Tübingen, Germany

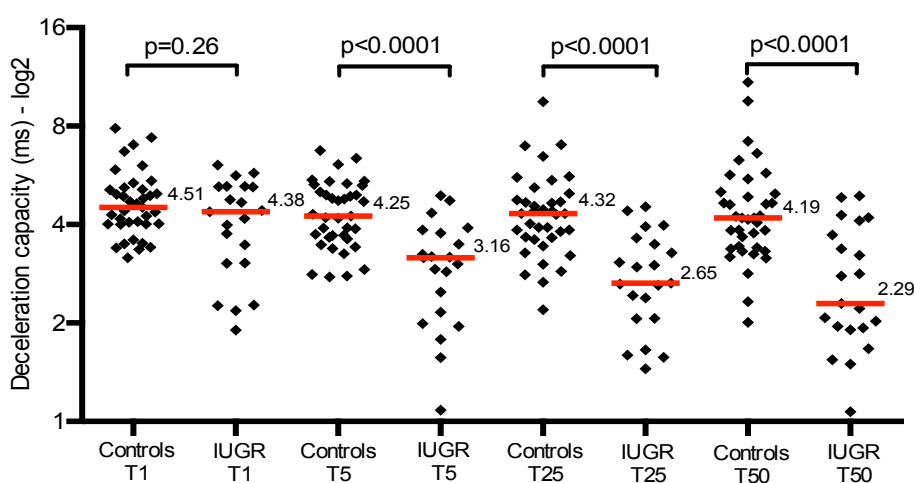
Background: Chronic intrauterine hypoxia influences fetal autonomous nervous system. Phase Rectified Signal Averaging (PRSA), a novel method of analysis of heart rate variability, is capable to calculate the average acceleration (AAC) and deceleration (ADC) capacity of the heart. PRSA depends on the parameter T which determines the periodicities that can be detected (*i.e.* the larger T the smaller the frequency of oscillations for which the method is most sensitive). The aim of the study was to explore AAC and ADC in intrauterine fetal growth restriction (IUGR) and controls.

Methods: Fetal ECG (fECG) was acquired by trans-abdominal ECG recording device (Monica AN24, UK) in 21 IUGR close to the time of delivery, and in 37 controls (defined as appropriate fetal growth and no maternal complications). All cases were recruited <34 weeks of gestation. AAC and ADC were calculated by PRSA, on inter-beats series automatically derived from fECG,

adopting different T values (from T=1 to 50). Mann-Whitney test was used to test the differences.

**Results:** There were no differences in maternal demographic characteristics and gestational age at recruitment. Except for the analysis at T=1 and 2, AAC and ADC resulted significantly lower in IUGR for all other explored T values. The magnitude of the difference was higher at increasing values of T and was essentially given by the change in IUGR, while in the control group there were no significant differences in AAC and ADC at different T values (data not shown). The figure represents the ADC in IUGR and controls at T=1, 5, 25 and 50.

**Conclusions:** Our results highlight the significant autonomic dysfunction in severe IUGR fetuses for a broad range of oscillations' periodicities (T>2). PRSA represents a novel promising tool for antepartum monitoring of fetal wellbeing.



6. Accepted at SGI 60th Annual Scientific Meeting, Florence 2014

## In vivo Evaluation of Acceleration and Deceleration Capacity of Fetal Heart Rate in Worsening Hypoxic Acidemia

MW Rivolta<sup>1</sup>, R Sassi<sup>1</sup>, D Casati<sup>2</sup>, T Stampalija<sup>3</sup>, MG Frasch<sup>4</sup>, B Richardson<sup>6</sup>, MG Ross<sup>7</sup>, K Rizas<sup>5</sup>, A Bauer<sup>5</sup>, E Ferrazzi<sup>2</sup>

<sup>1</sup>Dept. of Informatics, University of Milan, Crema, Italy

<sup>2</sup>Dept. of Obstetrics and Neonatology, Buzzi Children's Hospital, University of Milan, Italy

<sup>3</sup>Prenatal Diagnosis, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy

<sup>4</sup>Dept. of Obstetrics, CHU Ste-Justine Research Centre, Faculty of Medicine, Montreal, Canada

<sup>5</sup>Innere Medizin III (Kardiologie), Eberhard-Karls-Universität Tübingen, Germany

<sup>6</sup> Dept. of Obstetrics, Children's Health Research Institute, University of Western Ontario, London, Ontario, Canada

<sup>7</sup> Dept. of Obstetrics, LA BioMed at Harbor-UCLA Medical Center, Torrance, CA, USA

**Background.** Fetal heart variability (fHRV) reflects the homeostasis of autonomous nervous system (ANS). The conventional frequency domain analysis is inadequate for a non-stationary signal such as fHR. Phase Rectified Signal Averaging (PRSA) is a novel method able

to extract the quasi-periodic components out of non-stationary, noisy time series. The aim of the study was to determine the average acceleration and deceleration capacity (AAC and ADC), computed by PRSA method, in worsening acute hypoxic-acidemic insult as it may occur during labor.

**Methods.** In a near-term fetal sheep (n=9) repetitive umbilical cord occlusions (UCO) were induced for 1 min every 2.5 min as follows: *mild* partial UCO for 1 h; *moderate* partial UCO for 1h; and *complete* UCO x 1-2 h, until arterial pH<7.00. Arterial blood samples were collected at baseline, every 20 min during the UCO series, and at 1 h of recovery. Fetal ECG was recorded. AAC/ADC were computed from ECG for each phase of the stimulation protocol.

**Results.** AAC and ADC increased (in absolute value) across the phases of increasing UCO severity (Table).

**Conclusions.** Our findings show that during an acute hypoxic-asphyxic insult, with activation of ANS, there is an increase in AAC and ADC of fHR. These results differ from those obtained in IUGR fetuses, where decreased ACC and ADC are reported (data presented in a separate abstract). We hypothesize that the fetal metabolic reserves are differentially impacted by acute (i.e., UCO insult) versus chronic (i.e., IUGR) hypoxia, which results in a different ANS response as detected by PRSA method.

	BASELINE	MILD	MODERATE	SEVERE
ADC	3.00(2.28,4.42)	3.16(2.72,4.27)	6.19(5.07,7.19)	9.15(5.97,9.87)
AAC	-2.77(-3.66,-2.13)	-2.98(-3.94,-2.32)	-5.39(-6.77,-5.14)	-7.62(-9.97,-5.29)
pH	7.34(7.34, 7.37)	7.33(7.31, 7.34)	7.28(7.24, 7.30)	6.98(6.96, 7.06)

**Table:** Median and interquartile range of AAC/ADC ( $T=4$ ) and pH for each phase of the UCO series.

*7. Accepted at SGI 60th Annual Scientific Meeting, Florence 2014*

### **A novel methodology to analyze maternal autonomic function in pregnancy**

*Casati D<sup>a</sup>, Stampalija T<sup>b,a</sup>, Rosti E<sup>a</sup>, Lupini S<sup>a</sup>, Maggi V<sup>a</sup>, Rizas K<sup>c</sup>, Bauer A<sup>c</sup>, Ferrazzi E<sup>a</sup>.*

(a) Dept Obstetrics and Neonatology, Buzzi Children's Hospital, Biomedical and Clinical Science School of Medicine, University of Milan, Italy.

(b) Prenatal Diagnosis, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy.

(c) Innere Medizin III (Kardiologie), Eberhard-Karls-Universität Tübingen Tübingen, Germany.

**BACKGROUND:** Traditional methods to assess autonomic activity in pregnant women result in contradictory findings unable to discriminate between uncomplicated pregnancies and those complicated by hypertensive disorders. The Phase-Rectified Signal Averaging (PRSA), a novel method of heart rate variability analysis, has never been applied to maternal autonomic activity evaluation. The aim of this study was to assess the autonomic function in pregnant

women affected by intrauterine growth restriction (IUGR) and hypertensive disorders of pregnancy (HDP) by means of this new methodology.

METHODS: The study included singleton pregnancies between 25 and 40 weeks of gestation divided in following groups: 50 IUGR, 29 HDP with IUGR, and 113 controls (define as no fetal and maternal complications), respectively. Maternal and fetal ECG at admission were acquired through a trans-abdominal device (Monica AN24, UK) at a sampling frequency of 900 Herz. Each maternal R-R interval time-series was analyzed by PRSA to assess average acceleration/deceleration capacity (AAC/ADC) of maternal heart rate. Data were compared by means of non-parametric tests.

RESULTS: No significant differences were observed in demographic maternal characteristics. AAC and ADC were significantly higher in pregnancies complicated by HDP with IUGR (AAC median -8.23ms, IQR -5.23÷-9.93; ADC median 8.73ms, IQR 6.44÷10.22) than in those with IUGR alone (AAC median -5.92ms, IQR -4.72÷ -8.12; ADC median 6.85ms, IQR 5.57÷8.53;  $p=0.03$ ), and in controls (AAC median -5.84ms, IQR -4.12÷-7.23; ADC median 6.61ms, IQR 5.33÷8.01;  $p=0.001$ ). There were no differences between pregnancies with IUGR and controls ( $p=0.3$ ).

CONCLUSIONS: The maternal heart rate analysis by means of the PRSA technology showed a significant autonomic activation in pregnancies complicated by HDP with IUGR, while no differences were found between pregnancies with IUGR and controls. These data suggest that the underlying mechanism of autonomic activation is associated with endothelial dysfunction and not to placental insufficiency.

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