Fetal reactivity assessment during intrapartum stress by analysis of the fetal ECG signal

Sofia Blad

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UNIVERSITY OF GOTHENBURG

Perinatal Center
Department of Physiology
Institute of Neuroscience and Physiology
The Sahlgrenska Academy at University of Gothenburg
Sweden

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Abstract

Fetal responses to the stress of labour and delivery are constituted by a combination of changes in neuronal, hormonal and organ based reactions. The aim of electronic fetal monitoring is to identify fetuses at risk of hypoxia during birth, thus enabling timely intervention to avoid an adverse outcome. Visual assessment of fetal heart rate (FHR) patterns is associated with substantial variation in interpretation and there is data to demonstrate the benefits of computer support decision tools. Therefore, the aims of this project were to validate computer-based methods with enhanced data analysis to monitor fetal reactivity, using alterations in RR intervals and ST waveform of the fetal ECG as signs of autonomic nervous system and myocardial metabolic reactivity changes associated with intrapartum stress. A new mathematical model was used for quantifying FHR variability (FHRV). A polynomial function was applied to a sequence of real RR data, producing an RR trend. The difference between the RR trend and the actual beat-to-beat interval at every heartbeat was calculated and a Residual value was obtained. The closer to zero the lesser the FHRV was. In the thesis, the parameters were set to allow for baseline FHR shifts. These Residual features were then tested for their ability to identify four index cases with loss of reactivity in connection with adverse outcome. The parameter settings required to identify the index cases were then tested for accuracy in a large EU database of > 7800 deliveries. The analysis showed that 2.3% of these deliveries revealed non-reactive FHR features associated with an increased risk of neonatal care. Only one of 59 cases with metabolic acidosis showed consistently reduced FHRV. In a subsequent case-controlled study of spontaneous vaginal deliveries we demonstrated that active pushing was associated with a FHRV rise in 100% of deliveries with metabolic acidosis as compared to 89% of the cases without metabolic acidosis. Metabolic acidosis was also associated with a significantly more pronounced rise in FHRV and in cases with more severe acidosis the rise was followed by a decrease in FHRV. A combined FHRV and T/QRS rise occurred in 88% of the metabolic acidosis cases as compared to 5% of controls ($p<0.001$). The FHRV and ST parameters were also validated experimentally in an animal model of intrauterine inflammation in fetal lambs. These data showed that baseline FHRV increased with increasing maturity, while inflammation caused fetal demise particularly in preterm fetal lambs, which was associated with an increase in FHRV in connection with ST waveform depression and negative T waves. In summary, settings obtained in index cases with loss of reactivity and adverse outcome indicated increased risk of neonatal care, but could not be used to identify fetuses with metabolic acidosis *per se*. Instead, the initial pattern of reaction to develop metabolic acidosis in normal vaginal delivery was a substantial increase in FHRV followed by a decrease as the acidosis progressed. The Residual method may in the future help to identify fetuses at risk and provide additional support in decisions to intervene.

Keywords: FHR variability, fetal ECG, Asphyxia, Intrauterine infection, Residual method, STAN

List of Original Papers

This thesis is based on the following papers, published or in manuscript, which will be referred to by their Roman numerals:

I. Blad S, Larsson D, Outram N, and Rosén KG. **Assessment of fetal reactivity biopatterns during labour by fetal ECG analysis.** *International Joint Conference on Neural Networks*, 2009. p347 – 352.

II. Blad S, Outram N, Larsson D, Norén H, Sävman K, Mallard C and Rosén KG. **Assessment of fetal heart rate variability and reactivity during labour - a novel approach.** *In manuscript.*

III. Blad S, Outram N, Larsson D, Norén H, Sävman K, Mallard C and Rosén KG. **Alterations in fetal heart rate variability as a measure of fetal reactivity in connection with metabolic acidosis and spontaneous vaginal delivery.** *In manuscript.*

IV. Blad S, Welin A-K, Kjellmer I, Rosén KG and Mallard C. **ECG and heart rate variability changes in preterm and near-term fetal lamb following LPS exposure.** *Reproductive Sciences*, 2008 Jul; 15(6): 572-83.

List of Abbreviations

ANS	autonomic nervous system
BD	base deficit
BD_{ecf}	base deficit in extra-cellular fluid
BE	base excess
bpm	beats per minute
CP	cerebral palsy
CS	caesarean section
CTG	cardiotocography
DS	decision support
FBS	fetal blood sample
ECG	electrocardiogram
FECG	fetal electrocardiogram
HR	heart rate
FHR	fetal heart rate
FHRV	fetal heart rate variability
HF	high frequency
HIE	hypoxic ischemic encephalopathy
LF	low frequency
LPS	lipopolysaccharide
NVD	normal vaginal delivery
MAP	mean arterial pressure
MinMax	lowest highest value
PSA	power spectrum analysis
ResPct	95^{th} percentile over 20 minutes running Residual data
REvent	reactivity event
STAN	fetal monitor with ST analysis
95^{th} Pct	95^{th} percentile over 2 minutes running Residual data

Introduction

The birth process is a complex mission. The fetus must adapt to a new environment and establish itself as an air-breathing individual with its own nutritional supply and pattern of reactions. Before becoming an independent individual, most fetuses have to use some adaptive/defence mechanisms to cope with the challenge of being born. Fetal physiology is a complex area as two individuals are linked together and influence each other's well-being at the same time as fetal regulatory functions, such as the autonomic nervous system (ANS) are developing.

Active management of labour requires access to as much detailed information as possible regarding the ability of the fetus to respond to changes in the environment, i.e. the reactivity of the individual fetus and its bioprofile. The bioprofile constitutes the physiological responses of the fetus and pattern of reactions on both cell and organ function level. In modern obstetric care different methods are used to monitor the fetus during labour. The aim with all monitoring techniques is to obtain information about the status of the fetus and its ability to oxygenate the tissues, maintain organ function and prevent intrapartum asphyxia. If left unattended, the process of insufficient oxygen delivery to the fetus will lead to anaerobic metabolism with metabolic acidosis and eventually organ failure that may result in hypoxic-ischemic brain injury or death. This process is generally referred to as birth asphyxia. In a recently published study by Himmelmann *et al*, it was reported that 1.4 of 1000 term babies were born with cerebral palsy (CP) and of those 13% had an acute intrapartum hypoxic event severe enough to cause CP (Himmelmann *et al* 2010). There is a need for strict definitions of intrapartum hypoxia sufficiently severe to cause CP (MacLennan 1999). The presence of metabolic acidosis at birth is of specific importance as it directly links neonatal encephalopathy and subsequent CP to insufficient intrauterine oxygen delivery. Recent clinical outcome data has shown a substantially reduced risk (>90%) of metabolic acidosis in term fetuses and a lower incidence of cases with hypoxia-associated severe brain damage, when cardiotocography (CTG) was combined with ST analysis and fetal blood sampling (FBS) (Norén and Carlsson 2010). Much of this achievement is related to the ability of the staff to assess the fetal bioprofile continuously and to respond according to clinical guidelines. However, these methods could still be improved and more research is needed to further strengthen the methodology and increase its applicability by providing additional user support to enhance the identification of adverse reactions.

General Background

Intrapartum fetal monitoring is a great challenge with strict limitations on what information may be obtained and the development of new techniques of intrapartum fetal monitoring is continuing. Many of these techniques have focused on monitoring fetal heart rate (FHR). The wooden stethoscope invented by Dr Pinard in 1876, which allows the detection of fetal heart sounds and thereby FHR, was the first fetal monitoring method developed and is still in clinical use. Electronic FHR monitoring, such as CTG was developed in the 1960s. CTG measures FHR in relation to uterine contractions. An ultrasonic sensor, which detects motion of the fetal heart, is strapped to the abdominal wall of the mother. A second, pressure-sensitive contraction transducer, a tocodynamometer, measures the tension of the maternal abdominal wall, which is an indirect measure of the intrauterine pressure. CTG has further been developed by applying internal detectors, where a spiral-shaped electrode is attached to the fetal scalp and uterine contractions are measured by an intrauterine pressure sensor. Internal measurements are generally considered to be more precise but require onset of labour with ruptured membranes. The CTG method allows continual remote surveillance, however CTG curves have to be visually interpreted by the midwife or obstetrician.

There were great hopes that the CTG technique would lead to marked improvements in care during birth with appropriate intervention for oxygen deficiency. In particular it was believed that this technique would result in fewer babies put at risk of brain damage from oxygen deficiency during birth. This has not been the case. Instead, operative deliveries are overly used for what is, often wrongly perceived as threatening oxygen deficiency (Martin 1998). This frequently means unnecessary intervention with normal labour with an increased health risk for the mother and child as well as increased health expenditure. Studies have shown that there are large variations in visual FHR interpretation from CTG tracings (Donker *et al* 1993, Bernardes *et al* 1997, Ayres-de-Campos *et al* 1999, Costa-Santos *et al* 2005, Palomäki *et al* 2006). Therefore, different automatic assessment techniques of the FHR have also been developed with the aim to reduce the variability in visual interpretation. However, these methods have not been tested to the extent of proven efficiency regarding improved fetal outcome (Alfirevic *et al* 2006). Further methods for fetal monitoring will be introduced below in section “Fetal monitoring”.

Fetal physiology during delivery

Fetal cardiovascular adaptations to stress

Fetal responses to the stress of labour and delivery is constituted by a combination of changes in neuronal, hormonal and organ based reactions (Lagercrantz *et al* 1986). All of these patterns of reactions indicate the level of fetal reactivity. The fetal cardiovascular system and the myocardium in particular has become one of the clinically most relevant sources of information (Rosén *et al* 2004). Ideally, the capture of such changes requires continuous information and an important source available for such an analysis during labour is the fetal electrocardiogram (FECG) (Figure 1).

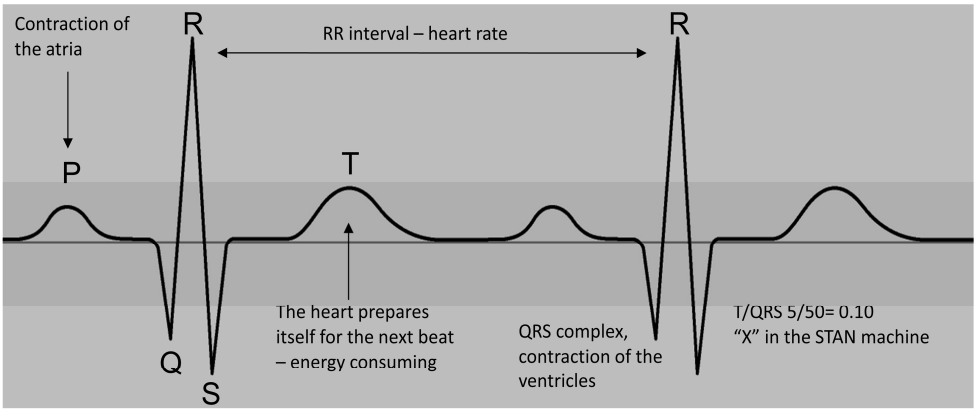

Figure 1. This picture shows the different parts of the FECG. The P-wave corresponds to the contraction of the atria, the QRS complex corresponds to the contraction of the ventricles. The T-wave is when the heart prepares itself for the next beat, an event that requires energy. The shape of the ST interval is dependent on the sequence of ventricular repolarization and by the metabolism of the myocardium. A way of quantifying the height of the T-wave is to calculate the ratio between the T wave and QRS amplitudes as recorded by the STAN device. The RR interval is used to calculate the beat-to-beat variation and heart rate (reprinted with permission from Neoventa Medical, Mölndal, Sweden).

ST analysis with an increase in T wave amplitude provides direct information on the ability of the myocardium to react with an increase in contractility and to maintain cardiac output despite the depressant effect of hypoxia *per se* (Hökegård *et al* 1981). Largely, this increase in functional reactivity is activated by an adrenaline surge, beta-adrenoceptor activation and anaerobic glycogenolysis to provide the supply of ATP and energy required (Rosén *et al* 1984). Once this important defence is utilized the cardiovascular system fails with a rapid drop in blood pressure and lack of cerebral responses (Rosén *et al* 1985). As illustrated in Figure 2, the Frank-

Starling relationship is affected by the response to stress with increased T wave during hypoxia with adrenaline surge. However, the immature fetal heart muscle is limited in its ability to increase contractility compared to the adult heart muscle, which could negatively affect the Frank-Starling relationship.

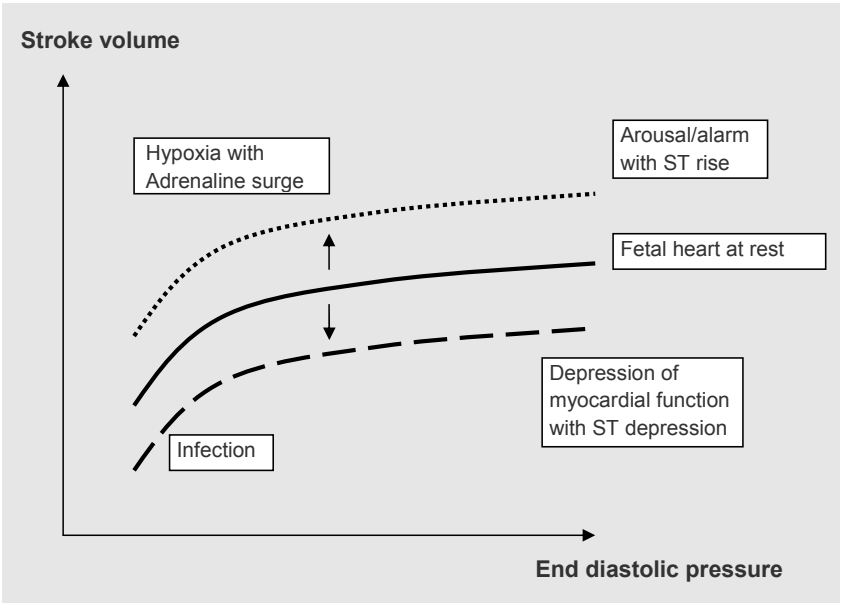

Figure 2. Patterns of changes in the Frank-Starling relationship indicating alterations in myocardial performance in connection with alterations in ST waveforms (reprinted with permission from Rosén KG).

During labour at term, the FHR is under the influence of numerous factors, summarized in Figure 3. The ANS regulates the fetal response to the dynamic environment through sympathetic and parasympathetic activation and tachycardia or bradycardia occurs as clinical signs of ANS alterations.

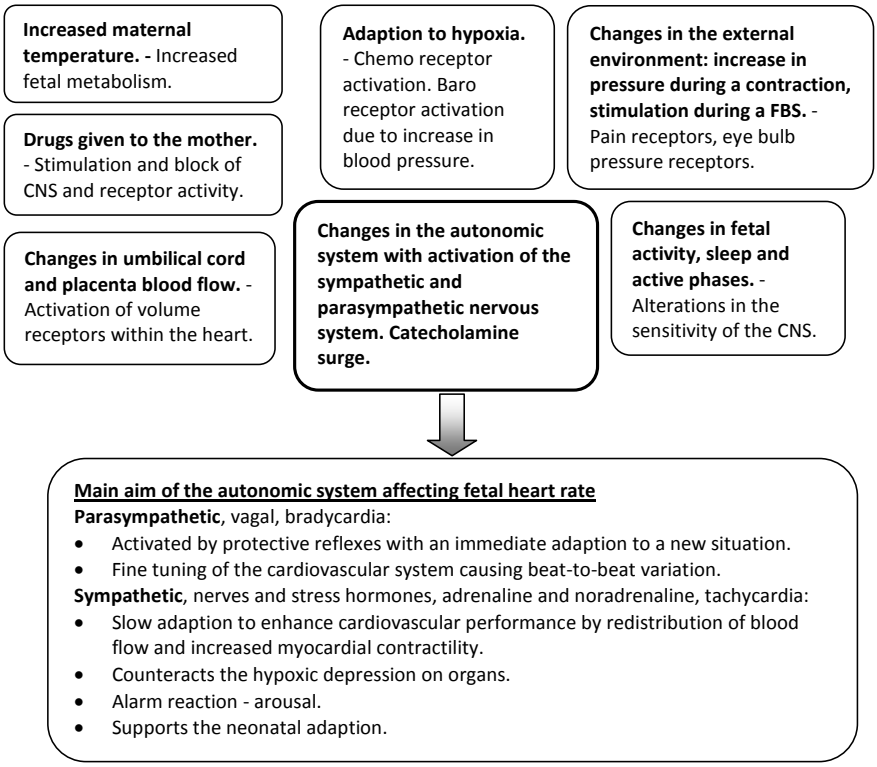

Figure 3. This scheme illustrates different factors that influence the autonomic nervous system and in what way they affect the FHR. Increased maternal temperature, drugs, contractions, external stimuli, hypoxia and normal changes in fetal activity can all be present during delivery and influence the FHR.

Fetal maturity may influence some of these reactions especially as the parasympathetic autonomic nervous system is known to mature later than the sympathetic. Despite its incomplete development, the ANS in human fetuses at term is capable of eliciting a strong response to severe stress (Dawes *et al* 1959, van Laar *et al* 2010). Judging from the level of catecholamine release in response to fetal distress (Lagercrantz *et al* 1977), hypoxemia is the predominant factor that may activate the autonomic nervous system, which subsequently modulates the detailed sequence of heartbeats (Dalton *et al* 1977, van Ravenswaau-Arts *et al* 1993, Yu *et al* 1998, Siira *et al* 2005, van Laar *et al* 2010). The role of the ANS in providing detailed control of the cardiovascular system is well-established (Mott 1982). In a situation of hypoxia there are two patterns of reactions: down regulation of organ activity/hibernation or an arousal/alarm reaction. Both the process of hibernation and the opposite, the alarm reaction, provides us with a possibility of classifying the fetal state.

The process of hibernation may occur well in advance of the fetus reaching a "preterminal" state. A marker of decreased reactivity would serve to indicate reduction in the fetal capacity to actively respond eventually causing a deteriorating fetal state. Clinically, uterine hyper-stimulation in labour has been shown to cause decreased fetal oxygen saturation and decreased FHR variability (FHRV) (Simpson *et al* 2008). Furthermore, the detection of an alarm response with marked increase in FHRV could also be important. Although functional and an important part of fetal response to hypoxia, extensive alarm reactions carry the risk of less control of given resources (stored glycogen, buffering capacity) and would thereby reduce the time during which a functional defence can be maintained. Thus it seems relevant to identify such situations as well.

Beta-adrenoreceptors and hypoxia

Hypoxia in itself exerts a depressant effect on myocardial function. The fetus as a whole is dependent on the ability of the heart to perform despite this depressant influence. Beta adrenoceptor activation becomes a key to prevent a reduction in myocardial performance. From the studies on the influence of exogenous β-mimetics (Dagbjartsson *et al* 1989) it appears as if the beta-receptor population may increase its sensitivity to beta-receptor activation during mild and moderate hypoxia. Furthermore, beta blockade curtails the fetal response, reducing the ability to preserve intact cerebral function during acute hypoxia in the term fetal lamb (Dagbjartsson *et al* 1987). The graph (Figure 4) indicates the relationship between the degree of oxygen deficiency, activation of fetal defence systems and the impact of beta-adrenoceptor activation and blockade. The sensitivity of these receptors will increase with hypoxia and externally given beta-mimetic drugs, such as terbutaline, may cause a metabolic overreaction with rapid utilization of glycogen stores with decreased ability to handle hypoxia as may also be noted in case of exogenous beta adrenoceptor blockade. These observations indicate the importance of a balanced fetal response to hypoxia.

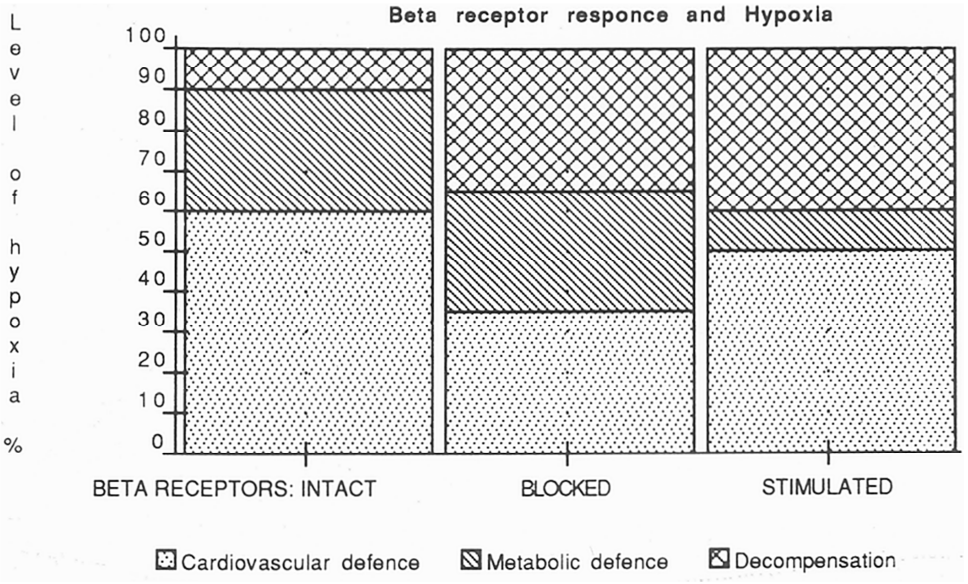

Figure 4. The graph illustrates the relationship between the level of hypoxia and the activation of the fetal defence system. The different bars display the pattern of response when the beta receptors are intact, blocked or stimulated. Data from studies by Dagbjartsson *et al* (Dagbjartsson *et al* 1987 and 1989). Reprinted with permission from Neoventa Medical, Mölndal, Sweden.

Beta adrenoceptor activation is an essential component in the fetal defence against oxygen deficiency and the surge of catecholamine is the hallmark of fetal reactivity in response to hypoxia. Hepatic glycogen stores have accumulated during gestation and serve as a source of glucose, to be used predominantly during hypoxia by the fetal brain (Shelley 1961). Glycogen has also accumulated in the skeletal and cardiac muscles and the myocardial stores are responsible for the superior ability of the fetal heart to maintain its activity during anoxia (Dawes *et al* 1959). To meet the requirement of an increased accessibility of energy substrate during labour, the fetus adapts through enhanced hepatic glycogenolysis and lipolysis (Hägnevik *et al* 1984). Thus, metabolic acidosis may be regarded as a functional response indicating metabolic reactivity. This can explain the positive correlation between elevated catecholamine and lactate levels under normal vaginal deliveries (Nordström *et al* 1996). In cases of complicated pregnancies and deliveries, the correlation is even stronger, suggesting stress-induced catecholamine liberation in parallel with a switch to increased anaerobic metabolism (Nordström *et al* 1998).

Fetal acid-base

A central component in any attempt to assess quality of care is the availability of biomarkers of potentially adverse outcome. The choice of a marker has to be done with care. It has to be possible to obtain the information routinely, preferably during the perinatal period; it should have a high specificity and have a "reasonable" prevalence and it should be directly linked to neonatal morbidity and subsequent adverse outcomes. Hypoxic-ischemic encephalopathy (HIE) is the most reliable clinical indicator of increased risk for neurological sequels after impaired oxygen delivery during birth. Similarity, early amplitude integrated electroencephalography and magnetic resonance imaging at 5-10 days after birth, are readily used to predict outcome in an increasingly exact manner. The evaluations are, however, not available immediately after birth and significant HIE as well as extensive brain damage are rare conditions. To evaluate intrapartum hypoxia a more immediately available instrument is thus needed. Umbilical cord acid-base analysis has become part of routine care and serves as a quality measure as well as to provide risk assessment for evaluating monitoring systems and obstetric management. This practice is important because umbilical cord blood gas analysis may assist with clinical management and excludes the diagnosis of birth asphyxia in approximately 80% of depressed newborns at term (Thorp *et al* 1999). Excessive metabolic acidosis (pH <7.0 and base deficit (BD) ≥ 12) is also a key factor in the chain of events that has been suggested to identify those adverse outcomes that could indeed be explained by intrapartum events (MacLennan 1999). Umbilical cord blood gas analysis could thus serve as a clinical marker for intrapartum restricted oxygen delivery and an increased risk of adverse outcome, in spite the fact that most fetuses suffering from hypoxia and presenting with metabolic acidosis will be perfectly healthy (Goodwin *et al* 1992).

The process of obtaining and analyzing cord samples for acid base data analysis is a relevant issue. The procedure has been reviewed and standards have been set for the correct use of algorithms to calculate BD as well as to verify what vessel the sample is obtained from (Westgate and Rosén 1994, Westgate *et al* 1994). The pH value denotes the log value of the hydrogen ion concentration $[H^+]$. $[H^+]$ is calculated as 10^{9-pH} nmol/L.

The cut-off value of 7.0 for defining severe acidemia is not universally agreed upon, and some researchers have reported cases of hypoxic-ischemic injury in neonates with a cord pH above this value (Yudkin *et al* 1995, Korst *et al* 1999, Pasternak 1998). A umbilical cord pH value <7.05 identifies fetuses adjusting to hypoxia and is associated with neonatal complications, but a statistically significant increase in the incidence of serious neonatal morbidity is not seen until the umbilical cord artery pH level is <7.00 (Gilstrap *et al* 1989, Goldaber *et al* 1991).

Graham *et al* reviewed the scientific literature in order to examine the role of intrapartum hypoxic injury in causing neonatal encephalopathy in non-anomalous term infants in developed countries (Graham *et al* 2008). The combined data of seven studies found an incidence for umbilical cord pH <7.0 of 3.7 (range: 2.9-8.3) per 1000 term live births. The incidence of neonatal neurologic morbidity and mortality for term infants born with cord pH < 7.0 was 23.1%.

The cord pH is, however, dependent on the immediate gas exchange as well as the presence of anaerobic metabolism. A short term interruption in gas exchange will result in an isolated increase in pCO_2 and lowered pH. The BD in the extracellular fluid (BD_{ecf}) in the umbilical artery and vein, on the other hand, serves as a marker of the metabolic part of a low pH and in the clinical situation as a marker for the duration of hypoxia (Westgate and Rosén 1994, Low 2004). A high BD_{ecf} in the cord artery combined with a normal value in the cord vein would indicate a short lasting hypoxic process. On the contrary, when high levels are reached in both umbilical artery and vein, the underlying process of hypoxia lasted long enough for equilibrium to have been reached in both vessels. The risk of complications, such as neurological damage, increases when tissue oxygen levels are sufficiently impaired to cause metabolic acidosis (indicating insufficient oxygen delivery), with the cut-off level of BD ≥ 12 mmol/L (Goodwin *et al* 1992, Low *et al* 1997, Andres *et al* 1999). A complete blood gas analysis thus provides important information on the type of acidemia (respiratory/mixed *versus* metabolic) and the duration of the event (acute *versus* semi acute).

Gestational age

Compared to the infant born at term, the preterm infant is at increased risk of mortality and morbidity including neurological disorders such as cerebral palsy. It is debated to what extent the preterm fetus is able to demonstrate similar cardiovascular responses to intrauterine hypoxia-ischemia as the more mature fetus. The sympathetic nervous system is effective as early as mid-gestation, while the parasympathetic nervous system matures much later in pregnancy. The parasympathetic nervous system begins to exert typical reflex responses at term and reaches adult levels only after birth (Assali *et al* 1977). Studies have shown that the midgestation fetal sheep has the capacity to react to umbilical cord occlusion (Bennet *et al* 1999, Welin *et al* 2005). Bennet *et al* showed that the preterm fetus responded, similarly to the near-term fetus, with marked cardiovascular centralisation during umbilical cord occlusion. Recently Welin *et al* (Welin *et al* 2005) demonstrated the capability of the midgestation fetal sheep to respond with a significant increase in the amplitude of the ST wave form together with an augmentation of blood pressure, which then subsides as the occlusion continues. The appearance of negative ST segment appears to signify significant cardiac dysfunction. The characteristic progression of ST waveform

changes in response to umbilical cord occlusion in midgestation fetal sheep suggest that monitoring the ST waveform may contribute clinically important information also in the preterm individual.

Infections

Several studies have found an association between intrauterine inflammation, preterm rupture of the membranes and preterm delivery (Romero *et al* 1988, Goldenberg *et al* 2000, Jacobsson 2004, Shim *et al* 2004). The preterm newborn (<37 gestational weeks) accounts for more than 50% of perinatal morbidity and 75% of perinatal mortality, although only 5-10% of the newborns are born preterm. Epidemiological evidence has shown that intrauterine infection also contributes to the development of brain injury, such as cerebral palsy, in the newborn (Nelson and Willoughby 2000, Dammann and Leviton 2000, Wu 2002). Furthermore, experimental studies in fetal sheep show that administration of endotoxins, such as lipopolysaccarides (LPS) from gram-negative bacteria, result in very similar brain damage as found in preterm infants (Mallard *et al* 2003). Additionally, both clinical and experimental data have demonstrated that perinatal inflammation might interact with secondary insults to make the infant more vulnerable for further stresses (Badawi *et al* 1998, Eklind *et al* 2001, Wang *et al* 2007).

Today, very little is known about the FHR and FECG responses to infection. Griffin *et al* (Griffin *et al* 2001) have shown that abnormal heart rate characteristics, including reduced variability and transient decelerations, can be identified in the early course of neonatal sepsis. It is currently not known how intrauterine infection may affect the FECG ST waveform or FHRV and how fetal maturation may affect these reactions to infection.

Fetal monitoring

Cardiotocography

Interpretation of CTG tracings is generally performed by health professionals in charge of labour (midwives or obstetricians), but this has a well-demonstrated poor reproducibility due to both inter- and intra-observer variations (Donker *et al* 1993, Bernardes *et al* 1997, Ayres-de-Campos *et al* 1999, Costa-Santos *et al* 2005, Palomäki *et al* 2006). Computer analysis of CTG tracings emerged as a way of overcoming this problem and programs with this specific objective have been developed over the last three decades. In spite of widespread interest and acclaimed need, computer analysis of CTGs still plays a limited role in the clinical setting, especially in intrapartum care. There are many possible explanations for this. Firstly, there is still no consensus on which CTG parameters are best associated with fetal oxygenation, so different criteria are used by the few systems that have been developed for intrapartum analysis. Secondly, the

complexity of intrapartum FHR behaviour constitutes a major challenge to the development of classification algorithms. The FHR can be classified into three categories: reassuring, non-reassuring Grade 1 and non-reassuring Grade 2 (Figure 5) where the last category is considered to be most predictive of fetal acidemia (King and Parer 2000). Finally, this is a research area in which the clinical effectiveness of any methodology is extremely difficult to demonstrate, as has been shown by several randomised controlled trials that did evaluate the intrapartum CTG (Grant 1989, Vintzileos *et al* 1995, Thacker *et al* 2000,). More information about the fetal condition during birth is needed to further strengthen the obstetric healthcare and decrease the unnecessary interventions due to misinterpreted fetal distress reactions.

Reactivity is a key parameter but difficult to measure when assessing fetal heart rate			
FHR Classification	Baseline heart rate	Variability /Reactivity	Decelerations
Reassuring	110-150 bpm	6-25 bpm Accelerations present	Early decelerations Variable decelerations with a duration of <60 s and depth <60 beats
Non-reassuring Grade 1	Bradvcardia: Rate < 110 bpm (without accelerations) Episodes > 2 min duration regardless of reactivity or variability Tachycardia: Rate 150-170 bpm and minimal variability Rate > 170 bpm	≤ 5 bpm for > 40 min ≥ 25 bpm for > 40 min Accelerations absent	Variable decelerations with a duration of > 60 s or depth > 60 beats Repetitive late decelerations
Non-reassuring Grade 2 – Preterminal	Absent variability and reactivity regardless of other FHR patterns Sinusoidal pattern		

Figure 5. This picture presents the US STAN clinical guidelines matrix. The non-reassuring Grade 2 pattern or preterminal pattern is considered to be one of the most predictive patterns for fetal acidemia (King and Parer 2000). The lack of reactivity can be missed in stressful labour ward situations. The pattern can either be present at the onset of recording or develop over time (reprinted with permission from Neoventa Medical, Mölndal, Sweden).

Fetal monitoring techniques, adjuncts to CTG

There are several techniques for intrapartum fetal surveillance with the aim to increase the specificity and sensitivity of CTG. Fetal pulse oximetry records the oxygen saturation by applying a sensor against the fetal chin. Two randomised trials were performed in order to find out if the use of fetal pulse oximetry would reduce the level of caesarean sections (CS) without affecting the infant´s condition at birth (Garite *et al* 2000, Bloom *et al* 2006). The first trial showed a reduction in CS for non-reassuring FHR, however there was no difference in the overall CS rate between the two groups (Garite *et al* 2000). The second study did not confirm the result of reduced CS with non-reassuring FHR with the use of fetal pulse oximetry as an adjunct to CTG (Bloom *et al* 2006). Fetal complications following birth did not differ between the study groups in any of the two trials.

Fetal scalp pH is another commonly used technique that is used to add information about the fetal condition during delivery. A pH <7.20 has been chosen as a cut off value to recommend intervention. The amount of blood needed for an FBS is between 30-50 µl with a failure rate between 11-20% and an intervention time of 18 minutes (Tuffnell *et al* 2006). Lactate is a metabolite of anaerobic metabolism which has also been studied as an adjunct to the CTG. An advantage with lactate measurements is that less blood is needed than for pH analysis. Wiberg-Itzel *et al* compared the two methods and found that there was no difference in the rate of acidemia at birth between the use of pH or lactate together with CTG (Wiberg-Itzel *et al* 2008). Both pH and lactate measurements have strict limitations as they only provide discontinuous data. Furthermore, FBS procedures can be uncomfortable for the mother.

Fetal electrocardiogram (FECG) waveform analysis with automatic evaluation of the ST interval (STAN®, Neoventa, Mölndal, Sweden) is a technology that has been added to intrapartum CTG monitoring, when the latter is acquired with a scalp electrode. The method was largely developed at the University of Gothenburg and Chalmers University of Technology during the 1970s (Lilja *et al* 1985) and then commercialised during the last decade of the 20th Century. The system has been shown to decrease the incidence of newborn metabolic acidosis and hypoxic-ischemic encephalopathy (HIE) as well as operative vaginal deliveries (Neilson 2005, Amer-Wåhlin *et al* 2007). However, this methodology depends on the combined evaluation of CTG and FECG data and is therefore influenced by the poor reproducibility of visual CTG interpretation (Westerhuis *et al* 2007, Doria *et al* 2007, Westerhuis *et al* 2009). Combining computer analysis of the FHR with automatic evaluation of the ST interval has the potential to become a major development in intrapartum fetal monitoring as indicated by the recent report from Oporto (Costa *et al* 2009), showing the possibility of accurately identifying fetal distress.

The STAN development has shown the benefit of introducing clinical decision-making based on a combination of the understanding of pathophysiology as well as having a robust parameter for measurement and quantification of changes (Rosén *et al* 2004). This approach may be beneficial when exploring other FECG-based features for fetal surveillance in connection with the stress of being born.

Uterine contractions and rupture of membranes provide a marked change in the external fetal environment and a test of the ability of the cardiovascular system and its regulatory components to respond. During delivery mature fetuses display a variation in FHRV, which alters with activity stage (Figure 6). The instantaneously recorded RR sequence provides the beat-to-beat variations required to discriminate between situations of normal, increased or lacking reactivity. The beat-to-beat difference or alteration in the RR interval is considered normal between 6 to 25 beats per minute (bpm).

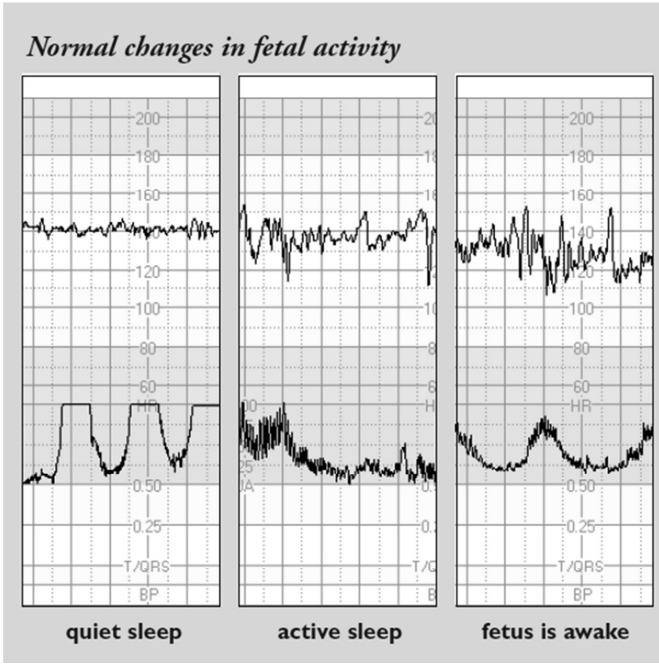

Figure 6. The picture illustrates FHRV patterns seen during quiet sleep, active sleep and when the fetus is awake. The pattern seen during quiet sleep is similar to the pattern in a hibernated fetus with down-regulation of ANS and decreased reactivity (reprinted with permission from Neoventa Medical, Mölndal, Sweden).

A decrease in FHRV may occur for different reasons. Naturally occurring changes in fetal sleep state is part of normal fetal behaviour and a reactive FHR pattern serves as a sign of active sleep and fetal well-being (Nijhuis *et al* 1982, Nijhuis *et al* 1999, Vindla *et al* 1995). The current practice is to try to and separate a normally occurring low FHRV from one serving as a sign of abnormality by considering the duration of a low FHRV pattern and the lack of accelerations in case of loss of FHRV. External manipulation such as digital manipulation tests may serve as a way to activate a fetus to discriminate between low FHRV due to normal sleep state variations or as a consequence of down-regulation/hibernation due to impending hypoxia (Skupski *et al* 2002).

FHR accelerations serve as a marker of reactivity. If they appear, they serve as a qualified marker of reactivity and normality (Royal Col Obst Gynec 2001). However, they are intermittent in nature and general clinical guidelines suggest that loss of FHRV, with no accelerations, and lasting more than 60 minutes should be regarded as an ominous pattern (Electronic FHR monitoring: Res guidelines 1997). The current understanding of FHRV has been summarized by King and Parer (King and Parer 2000) as follows:

- FHRV contains information about autonomic nervous system activity.
- FHRV is a key reflection of intact cerebral oxygenation.
- Absent FHRV can be asphyxia or non-asphyxia related.
- Normal FHR with normal baseline rate, accelerations and absence of periodic patterns is very predictive of the absence of fetal acidemia.
- Minimal FHRV with bradycardia and late uniform or complicated variable decelerations is predictive of newborn acidemia.

Experimental studies in fetal sheep have identified a pattern of an increase in FHRV followed by a decrease as hypoxia is continued (Dalton *et al* 1977, Stånge *et al* 1977). This biphasic pattern has recently been verified clinically applying power spectral analysis comparing acidotic fetuses with controls (Siira *et al* 2005). There are few studies in the literature on FHRV during delivery and intrauterine infection. Two studies have found a positive correlation between decreased or absent FHRV, in both preterm and term fetuses, and intra amniotic infections (Duff *et al* 1983, Salafia *et al* 1998). With automatic analysis of the FHRV, the parameter can more easily be included in experimental and clinical studies on both inflammation and hypoxia where the FHR is recorded. This can lead to increased knowledge about the fetus cardiovascular and ANS response to suboptimal intrauterine situations such as inflammation and hypoxia.

Computerized analysis of the FHRV

When a standard CTG recording is analysed for variability criteria, normally the time domain is used, i.e. the heart rate tracing is visually inspected and from there the "band width" is assessed (Figure 7). Ideally, this parameter should reflect the differences in consecutive RR intervals and be a measure of FHRV. However, data resolution is limited and depends on factors such as mode of heartbeat identification and speed of print-out. To visually analyse the details of different variability features over long periods of time (hours) may also be perceived as a daunting task and is likely to be a key factor behind the difficulty in obtaining consensus among experts. Furthermore, important information on the pathophysiology may not present itself due to the limitations in data acquisition and processing. Often the validation of clinically relevant parameters requires access to large sets of data for retrieval of knowledge and ultimate verification. Today, all of these aspects may be handled through computerised data acquisition through enhanced signal processing, feature extraction and validation of selected parameters.

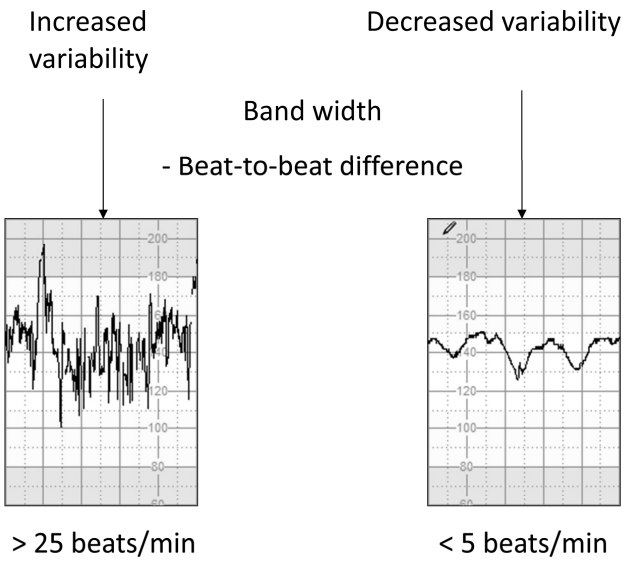

Figure 7. Two FHR tracings illustrating the variation in bandwidth, which can vary between >25 bpm to <5 bpm.

Numerous indices of FHRV have been described (Parer *et al* 1985) and a variety of automated approaches used in an attempt to increase the predictive value of FHRV. Short-term variability is relatively easy to quantify and appears to be a valuable predictive tool antenatally (Street *et al* 1991). In contrast, none of the automated methods have so far proven to be good

predictors of fetal condition during labour (Parer *et al* 1985, Spencer 1989, Dawes *et al* 1991, Pello *et al* 1991).

One way to study FHRV is to investigate the activity of the ANS. The reference method of assessing the activity of the ANS is the power spectral analysis of the FHR (Oppenheimer *et al* 1994). Power spectral analysis determines the relative energy of the cyclic fluctuations present in the heart rate signal in the frequency domain by reducing a signal to the sum of its component sine and cosine waves. In this way, spectral analysis allows superimposed periodicities to be unravelled (Sayers, 1980). The basic proposition underlying spectral analysis is that the two autonomic branches influence heart rate in a frequency-dependent way. The power spectrum of the term fetus in labour displays two broad bands of activity. There is a predominant low frequency (LF) band from 0 to approximately 0.2 Hz within which about 75% of the total spectral power is situated and a high frequency (HF) band centred at around 0.8 Hz which contains less power and is more variable in its appearance (Lewinsky *et al* 1993). The LF band can be further subdivided into very low (0-0.05 Hz) and low (0.05-0.20 Hz) bands. When the powers of the peaks are expressed as a LF/HF ratio, it provides an index of relative sympathovagal balance (Kitney 1984) with the LF band indicating changes in sympathetic tone and the HF band reflecting parasympathetic activity originating from fetal movements such as fetal breathing.

Recently, it was reported that changes in FHRV during labour (based on data originating from STAN recordings) could provide detailed information to be used for power spectrum analysis (PSA) (Siira *et al* 2005, Siira *et al* 2007, van Laar *et al* 2010). However, these methods are not ideally suited for situations with non-stationary FHR baseline, such as during delivery. In the work by Siira *et al*, this was handled by visually extracting two minute blocks of FHR and van Laar used automatic analysis from a short (64 s) window assuming that in such a short window the data have a greater likelihood of presenting a stable state. The side effect of such a short duration is the limitation in detecting very LF changes (<0.04 Hz). The non-stationary problem was further minimised by using a moving, partly overlapping, window allowing for continuous signal segments analysis. These studies also used different PSA endpoints such as LF/HF ratio or normalised LF and HF power. The latter obtained by dividing the recorded LF and HF powers with the total power. Thus, the basic choice of parameter settings is still unclear despite the common use of PSA over the last 30 years.

New approaches to assess FHRV during birth

An important development in the assessment of FHRV is the ability to detect significant changes in situations of a non-stationary heart rate, as is often seen during delivery with uterine contractions. To enable detailed assessment of beat-to-beat variations during birth, a useful

method needs to be able to reduce the impact of the large fluctuations in the heart rate that are not related to the fine-tuning of the ANS. Recent advances within this area of research have been facilitated by EU supported projects (The BioPattern project, contact no. 508803). Further, collaboration between bio- and computing engineers, physiologists and clinicians has provided us with a new tool to continuously assess beat-to-beat variations reflecting ANS reactivity. This new parameter to describe FHRV has been labelled "Residuals" as it reflects the deviation in milliseconds (ms) of the recorded beat interval from the estimated main RR trend and is not dependent on a stable baseline.

Despite limitations in analysis methods, researchers agree that hypoxemia activates the ANS, which subsequently modulates beat-to-beat heart rate (van Ravenswaau-Arts *et al* 1993). Spectral power analysis is a method that can be used to detect and quantify these changes in heart rate objectively, although only during stationary baseline recordings (Axelrod *et al* 1981, Siira *et al* 2007). Therefore, new mathematical models that are able to discriminate between different fetal reactions during the stress of labour and delivery, such as the Residual method, may be of benefit in fetal monitoring. The evaluation of such parameters is part of the present thesis.

Aims of the thesis

The overall aim of this thesis was to evaluate a new automatic assessment of FHRV by analysis of the FECG and more specifically:

- To evaluate a new mathematical model and find out whether it can be used during labour to automatically analyse the FHRV in situations of a non-stationary baseline heart rate. The aim was also to optimise parameter settings to enable the separation of FHR recordings according to criteria of lacking, low, decreasing, normal or increased reactivity.
- To validate the Residual method and determine parameter settings for reactivity features that identify a compromised fetus by using known index cases of fetal compromise and validate the outcome against a large clinical database.
- To evaluate the FHRV method and FECG during spontaneous vaginal delivery with and without cord artery metabolic acidosis.
- To study the FECG, using ST analysis and Residuals as a measure of FHRV, in lamb fetuses at different gestational ages after the induction of intrauterine inflammation by LPS exposure.

Material and Methods

The thesis consists of a methodological (paper I), two clinically oriented (papers II and III) and an experimental part (paper IV). The material and methods for each study are described in detail in the articles appearing at the end of this thesis. A more general presentation with a discussion about advantages and disadvantages of each method used is presented below.

Clinical database

A clinical database was used in the first three papers. The clinical data and FECG recordings were obtained from a multicenter study as part of an EU innovation program: *Dissemination of a knowledge based system for determining appropriate intervention during labour based on a qualified analysis of the fetal electrocardiogram* (EU innovation grant, IPS-1999-00029).

The prime objective of the EU supported FECG project was to spread the STAN technology of fetal monitoring during labour. Ten academic centres across Europe were made active partners of a knowledge transfer process involving basic pathophysiology as well as the clinical application of ST analysis of the FECG with user guidelines etc. The prime goal was to test a model for the implementation of the STAN methodology.

The maternity units were equipped with STAN® S 21 units in August – September 2000 after the educational process had been ongoing during the summer months. STAN recorders were used in pregnancies longer than 36 completed weeks in which a fetal scalp electrode was applied for more detailed fetal surveillance. STAN monitoring was used in both normal and high-risk pregnancies, women with suspicious or abnormal external CTG, induced or oxytocin-enhanced labour or meconium-stained amniotic fluid.

Each unit had one research midwife responsible for the education and data collection. Apart from the STAN recording that was stored digitally, non-personalized clinical data was entered in a case record form, subsequently checked for consistency of data and entered into a standard database format, as illustrated in figure 8. The need to undertake FBS was left at the discretion of the clinician in charge and the time and pH reading was recorded. Cord acid base data was obtained by immediate double clamping of the cord and subsequent measurements of pH and PCO_2 with BD_{ecf} calculated using the Siggaard-Andersen Acid Base Chart algorithm (Siggaard-Andersen O 1971).

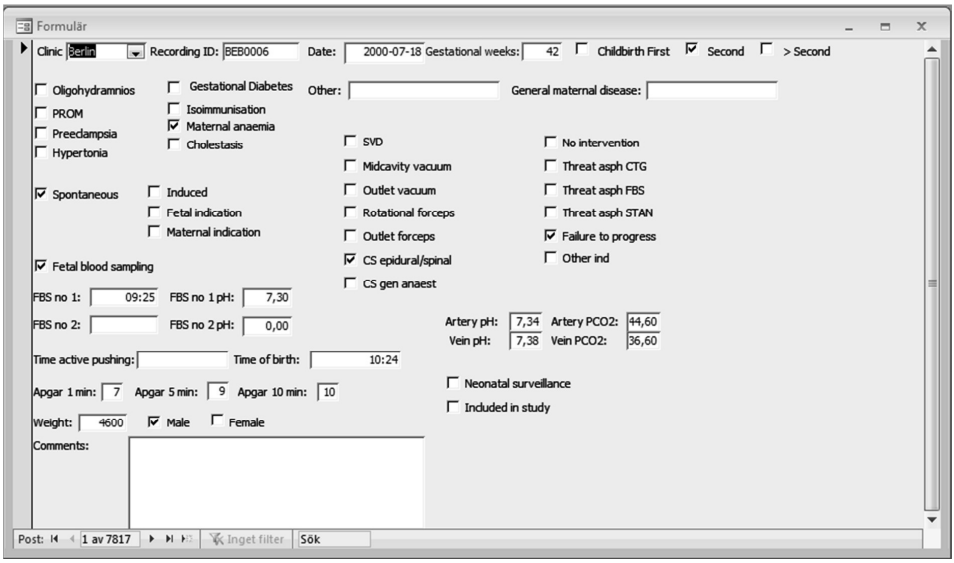

Figure 8. The figure illustrates the clinical database that was created from all paper case report forms. The recording ID corresponded to the ID created by the STAN machine. The database contains information about gestational week, parity, maternal complications, onset and outcome of labour and health status of the born baby, with Apgar, weight and acid base status in the blood obtained from the vein and artery of the umbilical cord.

Comments: In order to detect and evaluate findings that are relatively non-frequent clinical events, such as those in this type of research, a large clinical database is necessary. It is also important that the data is based on recordings from multiple centres and countries. The strength of the database is the combination of digitally stored STAN recordings and detailed information about the outcome of each delivery. Therefore algorithms can be modified and new settings can be tested repeatedly without risk to patients. To properly measure the clinical effect of a new fetal monitoring system a prospective randomised trial is needed. However, the current work aims to develop a new method for automatic analysis of FECG based on FHR data focusing on true beat-to-beat variations. Even sensitivity and specificity in indicating an adverse outcome may be partly irrelevant outcome parameters due to the need for multi-parameter data analysis and interventions made at first indication of fetal distress. The birth of a perfectly healthy baby could thus be seen as both a true and a false positive case. The identification of specific features using index cases of adverse outcome appeared as a relevant initial step. Furthermore, as such index cases originated from clinical cases, they may be regarded as shortcomings of the current method of fetal surveillance and as such provide an incentive to enhance the decision support.

RR Residual measurements

The prerequisite for the research was the need for a more robust methodology to obtain continuous information on beat-to-beat variations throughout delivery. At the same time it was thought preferential if the new method could be compared, to some degree, with the clinically used standard visual analysis of bandwidth. The general principle for FHRV measurement was as follows:

The FHR pattern was separated into two basic determinants (Figure 9):

- An estimated **main RR trend** computed from a curve fit of the RR time series using a piecewise polynomial approximation function.
- The **Residuals** obtained by subtracting the main RR trend from the original RR value at the time of each heartbeat.

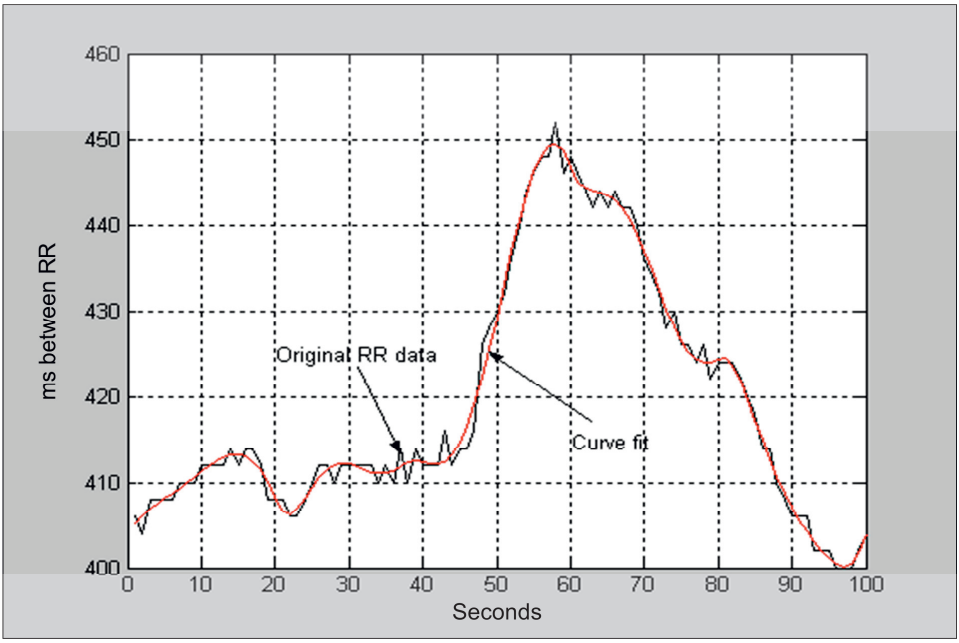

Figure 9. The picture illustrates how the new Residuals method automatically could analyse the FHRV. The black line captures 100 seconds of RR data. The red line is the main RR trend obtained from a polynomial function that was adapted to a sequence of 6 seconds real RR data. By altering the polynomial function, this trend line was made to match the original data sequence despite rapidly occurring changes in the RR trend. With low beat-to-beat difference in the FHR the difference between the red and the black line was minute, but the difference increased with increased variability. The difference between the FHR trend and the heart rate was called Residuals. These principles of RR analysis made it possible to measure FHRV even when there was a baseline shift. The graph presents data obtained in connection with a change in FHR from 145 bpm to 133 bpm.

To further illustrate the appearance of the Residuals data, Figure 10 depicts a series of recordings illustrating the distribution of Residual measurements in blocks of two minutes at situations of low (A), normal (B) and high (C) beat-to-beat variations.

From these data it was possible to quantify the Residuals function by analyzing the distribution of Residuals over a suitable period in time. As the data may not be normally distributed, a percentile measure was chosen and as the primary aim was to identify cases of low beat-to-beat variations, the 95th percentile (95th Pct) was selected.

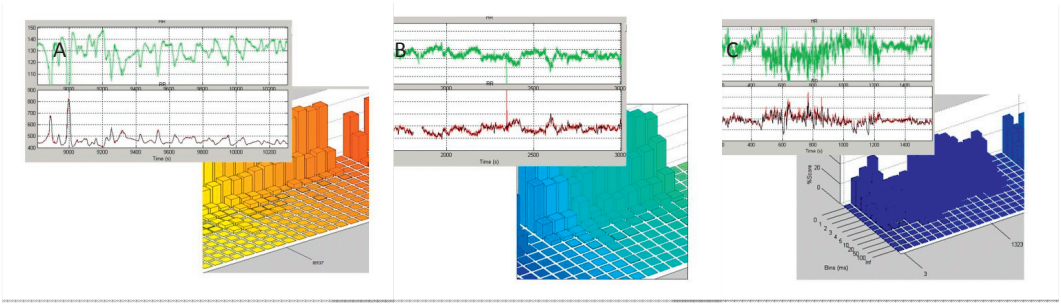

Figure 10. Three FHR sequences of low (A), normal (B) and high (C) FHRV. Each sequence contains the FHR (upper panel), the RR sequence with the main RR-trend and the histoplot of two minutes of Residuals data. The graph illustrates changes with altered distribution of Residuals with increasing FHRV (i.e. data presented in the front part of the graphs).

Comments: The invention of such a complex mathematical method as the Residual measurement might seem as an unnecessarily complicated way to quantify the FHRV. However, because of the nature of labour and the extreme cardiovascular response causing marked fluctuations in basal FHR, methods used during the "steady state" conditions ante partum are not sufficient during delivery. The possibility to continue the automatic assessment even during large fluctuations in FHR is crucial. Recent methods used to quantify FHRV have excluded situations with an unstable baseline and analysis has been made on data sequences of a more stable baseline. In the present thesis we demonstrate the distribution of Residuals over a short time sequence and by using the 95th percentile Residual value we were able to obtain information on the upper level of distribution for the selected period. In case of poor signal quality and inconsistent data, RR sequences may easily be rejected without losing core information as long as those particular periods have been identified. The STAN technology identifies those sequences and flags those as "poor data" allowing for immediate data exclusion. The main risk with the Residuals model is the calculation of the main RR trend in connection with very rapidly occurring changes in baseline FHR, which may cause a false increase in Residuals due to a lag in

the polynomial function. Furthermore, the data has to be normalised for heart rate to avoid the impact on the recorded Residuals value by the actual heart rate as such. All these aspects may be adjusted for in the settings of algorithms but it is the continuing database test that indicates the validity of the work.

Reducing the interference of unstable FHR baseline on FHRV

The results in the first paper demonstrate that when the polynomial settings were normalised (i.e. the effect of FHR baseline removed) fewer cases with good outcome were found. To correct for the effect of the baseline FHR on FHRV, Residual data was therefore normalised for a FHR of 150 bpm.

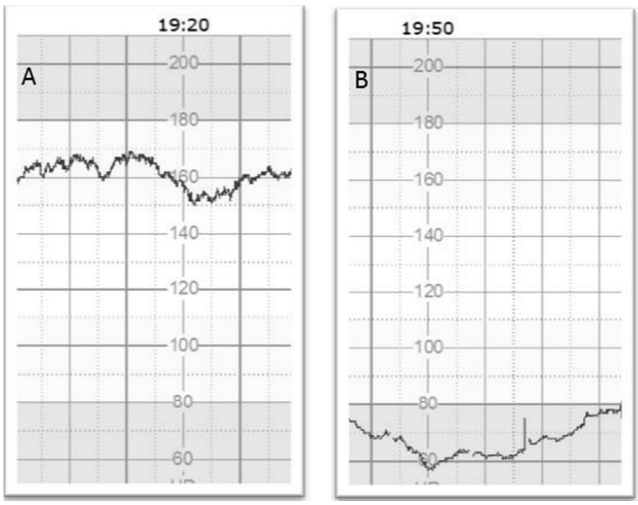

Figure 11. An illustration of a FHR recording used for the calculation of the RR difference with different baseline. The RR differences increased with decreased baseline (B). When assessing the bandwidth visually the opposite result was obtained and bandwidth appeared larger in association with higher baseline FHR (A). However, when the RR difference was recalculated to bpm during a 10 ms period, there were 5 bpm at a baseline of 160 bpm and 1 bpm at a baseline of 80 bpm.

Comment: Short-term variability, defined as the difference between one beat and the next, is difficult if not impossible to assess visually, simply because the resolution of the CTG trace does not allow it. A situation of interest is the one illustrated in Figure 11. Sequences A and B were obtained from the same FHR recording. Sequence A displays a baseline of 160 bpm and B shows a period of prolonged deceleration of 60 to 80 bmp. The average difference in RR interval for A is 1.6 ms and B is 5.5 ms, thus the beat-to-beat difference in B is more than three times bigger than in A. It is apparent from the tracings that these differences in RR would be extremely

difficult to detect by visual interpretation and may in fact provide the opposite information. An RR difference of 10 ms at a baseline FHR of 80 bpm would cause a bandwidth of 1 beat whereas it would become 5 beats at a baseline FHR of 160 bpm. Instead of normalising for 150 bpm the ratio between the RR interval and the Residuals could have been used. However, there may be an advantage to have a new parameter that relates to heart rate also quantified by using a heart rate related parameter such as milliseconds.

Residual and bandwidth

As part of the protocol for paper II, a comparison between bandwidth and Residuals was included. The bandwidth of the FHR tracings was manually calculated in the index cases by choosing two minute blocks of FHR with a stable baseline. Bandwidth was calculated from the corresponding RR data using a running 10 beat sequence and calculating the median range of these 10 beat blocks over a two minute period. The data was then compared to the 95th percentile Residual values obtained during the periods.

Comments: There is a lack of a golden standard in measuring FHRV, making it difficult to evaluate the Residual method against other techniques. The comparison with the calculated bandwidth was therefore an attempt to compare the Residuals with a clinically more recognizable method. As mentioned before, there is a large observational discrepancy when classifying FHR, even among experts, limiting the scientific value of such analyses. In the present analysis, only compromised fetuses, based on visual assessment, provided the initial input into the design of parameter settings. Thereafter, all the feature extraction process was guided by the software but verified by visual analysis. The advantage of such a model was illustrated by the identification of decreasing FHRV over two hours as a specific marker of changes in fetal reactivity patterns.

Long-term variability, defined as the oscillations around the baseline FHR, is quantified by the frequency and the amplitude of the oscillations. The frequency is difficult to assess visually and therefore variability is only described by the amplitude of the oscillations around the baseline (also called bandwidth). The unit used for variability is beats per minute (FIGO 1987). Thus, one may argue that the FHRV assessed visually may provide information that may be separate from true short-term variability assessed by a computer. There seems, however, to be reasonable similarities between the visual and the computer based analysis, but the cases included in the second paper would indicate the possibility of refining the application of FHRV by adding computerised data. A STAN monitor records the FECG during delivery with an intrauterine scalp electrode at 12 bits resolution and 500 Hz sampling rate and detects and stores R-peak intervals for further analysis. Detailed analysis of short-term variability requires accurate sampling of the R peak, ideally with a sampling rate of 1 millisecond (Dawes 1993) creating a potential limitation

of the Residuals analysis as the accuracy of the beat-to-beat RR-interval measurements is two milliseconds. However, the Residuals method uses the main RR trend as one point of measurement, that is created artificially from a linear interpolation of consecutive heart beats with subsequent subsampling, thus reducing the potential impact of a 500 Hz sampling rate to the identification of the actual R-peak.

Sequences Selected for Analysis - Index cases

The selection of sequences of STAN recordings is a fundamental part of the method development in the present thesis and required the collection of index cases that revealed the specific features that served to be identified. Four index cases were obtained as part of regular clinical use of the STAN methodology obtained between September 1998 and February 2003. The first index case is presented in Figure 12. The cases were defined as clinically complicated cases with poor neonatal outcome and with missed signs of fetal distress, i.e. a heart rate pattern identified as non-reactive (Figure 12) or with decreasing reactivity.

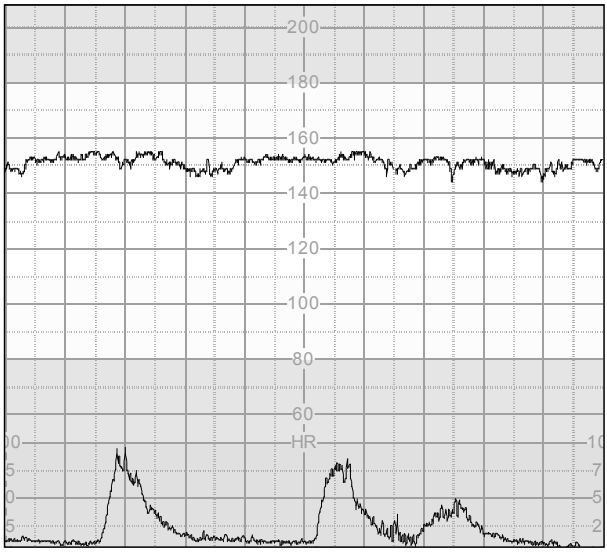

Figure 12. Illustrates the FHR pattern of the first index case resulting in perinatal death. The fetus was monitored for 1 hour and 46 minutes. The recording showed consistently low but not absent FHRV, a stable baseline with no accelerations or ST events.

Comments: The decision to base the initial feature extraction process on only four index cases can be questioned. Fortunately, situations with severely compromised fetuses with missed signs of fetal distress are very rare and therefore the number of cases collected over the years that

could be used as index cases is limited. Additional index cases could have been found, but the full database was instead used in the validation work. With current knowledge about FHR the chosen cases displayed the kind of FHR pattern that would benefit from clinical intervention. The fact that all cases were born with low Apgar score or severe complications after birth confirmed that the fetuses did not have or had lost their capability to handle the stress of labour. When comparing the thresholds from the index cases with the total EU database another 144 cases were found, but out of those only one case had an Apgar score of <5 at five minutes. In the total database an additional 38 cases were found to have an Apgar score of <5 at five minutes. None of those cases displayed a FHR pattern with low FHRV. The analysis was performed by both the Residual methodology and visual bandwidth assessment, therefore the conclusion could be drawn that there were no false negative cases in the database and the decision to base the determination of analysis parameters on index cases seemed appropriate.

Test for different polynomial settings and Reactivity Events (REvents)

Situations where there were low reactivity for a longer period of time and lost reactivity for a shorter period of time were defined as Low reactivity (LR1) and Lost reactivity (LR2) events, respectively. A data program calculated the statistical values from different polynomial functions and searched for the lowest highest value (MinMax) of a fixed period of time identified from the index cases. The program then listed all cases from the database that met the index case parameter settings of at least one event. The polynomial function which best followed the real heart rate curve during baseline shifts and had the most optimal MinMax result was chosen for detailed analysis of the index cases and the parameter settings of the REvents.

Comments: By using a data program that calculated the MinMax from different polynomial settings, the validation work of the polynomial functions became easy and very efficient. Despite the large numbers of STAN recordings, the MinMax value for one polynomial setting was achieved in a couple of hours. If this work had been done by manual calculation using Excel, it would not have reached the same high quality and taken months instead of hours

Case control study of cases with metabolic acidosis at delivery

A case control study was undertaken (Paper III) where STAN recordings obtained from 28 fetuses born with metabolic acidosis (cord artery pH <7.05 and cord artery BD_{ecf} >12.0 mmol/L) were compared with 56 control fetuses (cord artery pH >7.15). All cases had a normal vaginal delivery. Level of metabolic acidosis, Residuals and ST analysis were used to assess fetal reactivity. Every case with metabolic acidosis had two control cases obtained from the same

database. These were chosen as the cases delivered closest in time to the metabolic acidosis cases but with normal cord acid base data.

Comments: This study design was based on the previous finding that cases with metabolic acidosis did not appear to have low reactivity FHR patterns. During active pushing the fetus is reacting to the stress of labour and anaerobic metabolism would be part of such a reactivity pattern. At the same time as reactions in connection with metabolic acidosis can be identified, the outcome of the analysis could also reflect variations in fetal reactions in connection with the stress of being born vaginally. Two control cases were chosen for each case with metabolic acidosis. To choose two cases instead of one was a way of limiting the variations within the control group. Furthermore, the ability of the study to identify specific reactivity patterns as being part of the normal labour process increased.

Surgical and experimental procedure

In Paper IV, we examined the effect of in utero inflammation on FHRV in a clinically relevant, large animal model. To induce fetal inflammation, LPS (Sigma O55:B5, i.v.) was given as a single dose to the fetus. Pregnant sheep were subjected to aseptic surgery. The fetuses were instrumented with polyvinyl catheters in each brachial artery, in the left brachial vein and in the amniotic cavity. ECG electrodes were placed subcutaneously over each shoulder and one over the apex of the heart. Sham operated animals were instrumented in the same way as the LPS exposed fetuses. FECG was continuously collected using a STAN monitor. Continuous mean fetal arterial blood pressure (MAP) and amnion cavity pressure were recorded on a Grass polygraph, BIOPAC Systems (MPA150) and on a STAN monitor. Experiments were only performed in animals with at least 24h stable baseline recordings of physiological parameters (heart rate, blood pressure and blood gases).

Comments: For these experiments the sheep model was chosen because it is an internationally well-accepted preclinical model for studying fetal physiology and is well-established in our laboratory (Mallard *et al* 2003, Welin *et al* 2005, Dean *et al* 2009). Further, the relatively large size of the sheep fetus facilitates instrumentation with vascular catheters and electrodes allowing repeated blood sampling and on-line in utero recordings. The level of maturation during later stages of gestation also makes the sheep preferred over other animal models as it is possible to perform experiments on fetuses that have the same level of maturation as either preterm or term human fetuses have at birth. To experimentally induce an inflammatory process, LPS was used. LPS is a component of the cell wall of gram-negative bacteria and is a commonly used substance to induce inflammation. Still no data existed on what dosage of LPS to use to obtain a similar response in both the preterm and the near-term fetal lamb. The aim was to

achieve a non-lethal response causing fetal cardiovascular changes and a dose-response pattern had to be developed. However, we cannot be sure that a similar inflammatory response was achieved in surviving fetuses of different ages. This may be accomplished in future studies where for example plasma levels of cytokines are measured at different doses of LPS at different ages.

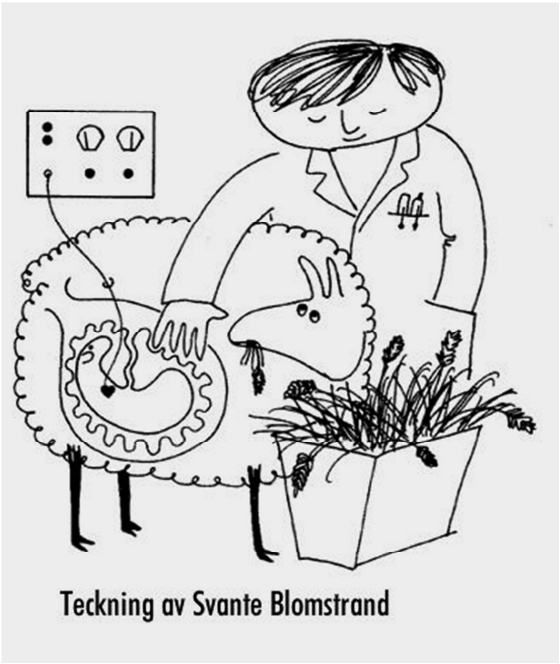

Figure 13. The drawing by Svante Blomstrand illustrates an instrumented fetal sheep (published with permission from KG Rosén).

Statistical analysis

The results were analyzed using SPSS 17.0 (IBM Corporation, New York, US), Medcalc® statistical software (version 5, Medcalc Software, Mariakerke, Belgium) or GraphPad Prism 4 (GraphPad Software, Inc, California, US). A Fisher´s exact test was used for comparing dichotomous data and Mann-Whitney U-test or Students T-test was used for comparing continuous variables. P-values <0.05 were considered significant. In the experimental study the differences over time was determined using analysis of variance (2-way ANOVA) with Bonferroni post-hoc test. Data are expressed as mean ± standard deviation, 95% confidential interval or median and range.

Comments: A parametric test was only used when a normal distribution could be tested for or assumed. The subject numbers included in analysis has a large influence on statistical tests. In the large database of more than 7800 cases, differences can be found statistical significant

although the clinical relevance of these results is limited or even irrelevant. On the contrary when using a very limited number of subjects in each group the possibility to find statistical significant differences is low. In the experimental study the number of animals included was limited due to the high cost of this study. Differences between groups should always be interpreted with the number of subjects and study design in mind and not only on the level of the p-value.

Results and Comments

Assessment of fetal reactivity biopatterns during labour by fetal ECG analysis (Paper I)

The initial study aimed to provide information on the design of a polynomial function that would allow the most accurate identification of two situations in particular; persistently low and/or non-varying RR and cases with no RR variability but rapid changes in the baseline FHR, patterns often noticed in connection with the onset of a variable deceleration. As previously illustrated (Figure 9) the principles of identifying a main RR-trend appears to be valid. In Figure 14, three levels of FHRV (low, normal and high) identified from the calculation of Residuals data for each heartbeat are illustrated.

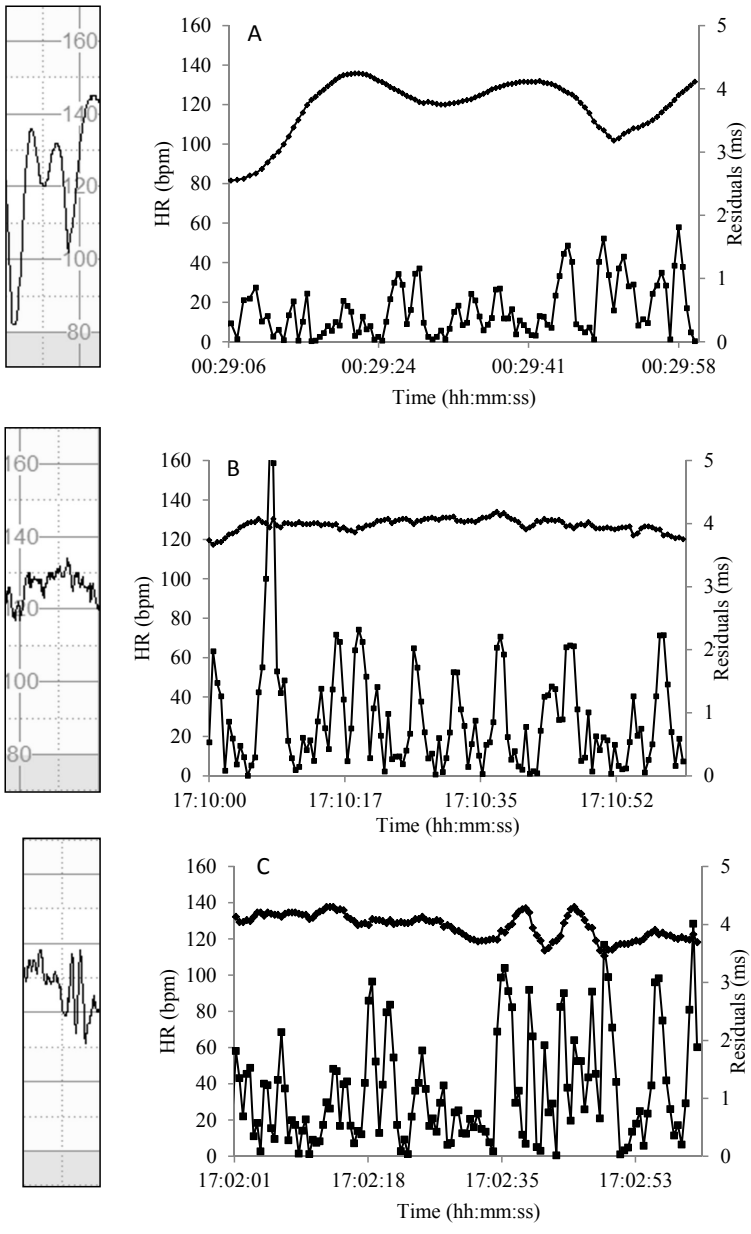

Figure 14. The graphs display a one minute sequence of FHR (above) and the corresponding Residual value (below). Each point in the FHR is one individual heart beat and each square in the Residual curve corresponds to one individual Residual value. On the left is the same minute of data as shown on the STAN monitor. Graph A shows very low FHRV, graph B shows a normal FHRV and graph C shows a high FHRV. The Residual values increase with the increased variability. The Residuals have been calculated by using the 12^{th} degree polynomial fit.

The risk when applying a polynomial function is that it would allow either too few or too many variations. When different polynomial settings were tested as expected a higher degree was found to follow the FHR curve more closely still being capable of identifying the main RR trend. The 6th degree polynomial gave an over-estimation of the Residuals in case of rapid drop in heart rate. This effect of the rapid shifts was much lower when a 12th degree polynomial fit was applied. Once a panel of algorithms behind suitable polynomials had been identified, they were assessed further by testing the extent to which corresponding RR sequences could be identified in the large EU database. We attempted to identify two settings, both identified from the index cases. The first was a situation of consistently low FHRV (LR1). Based on the index cases such a situation lasted for as long as 70 minutes. In one case (index case 1, Figure 12) with maintained but consistently low FHRV, the 6th degree polynomial appeared to provide a better separation of cases in the database compared to the 12th degree polynomial. For the second setting, a situation of very low/lacking FHRV had been identified (LR2). A 30 minutes time frame was identified as a suitable time frame during which a case should present such a low FHRV to be included and matching the index case based setting for lack of reactivity. In this case the 12th degree of polynomial was found more suitable to separate the cases in the database. Both the 6th and the 12th degree polynomials obtained the lowest MinMax when the data were normalised for a FHR of 150 bpm and a 30 second block with 200 points.

Overall, the incidence of LR1 and LR2 using appropriate polynomials was found to be 2.62% and 1.85%, respectively, indicating that these are rare situations but still in the percentage range that would make them suitable targets for automatic identification and potential decision support.

In summary, this computerized analysis indicated that with two sets of polynomials the main RR trend could be detected and two different FHR patterns with persistently low or absent variability were distinguished from other FHR patterns in the EU database.

Assessment of fetal heart rate variability and reactivity during labour - a novel approach (Paper II)

The aim of this study was to validate a novel approach to FHR beat-to-beat variance and identify reactivity events in four index cases with fetal compromise and validate the outcome against a large clinical database. Thirty control cases were selected as cases with a normal CTG at onset of the recording plus an FBS indicating a need to obtain additional information regarding fetal reactions and a cord artery pH >7.20. This part of the thesis applied the Residual model to recordings in the database and quantified changes in FHRV with specific analysis of patterns recorded during labour.

Index case 4 provided a biphasic pattern with an initial rise followed by a gradual decrease (Figure 15) as illustrated by ResPct, which is a trend analysis of Residuals. Both of these events seemed to precede the occurrence of a low reactivity scenario. The ST patterns showed the development of ST segment depression with negative T waves at max increase in ResPct. This pattern persisted for 2½ hours and was followed by an ST rise that persisted during the last hour of the recording. In this case the mother had a temperature rise of 38.8^{o}C and was treated with antibiotics. Index case 3 also had ST changes with an ST rise – the other two index cases did not present any ST changes. It was only Case 4 that showed marked changes in the reactivity patterns as the other three cases displayed a consistently low (Case 1 and 2) or slowly decreasing FHRV (Case 3).

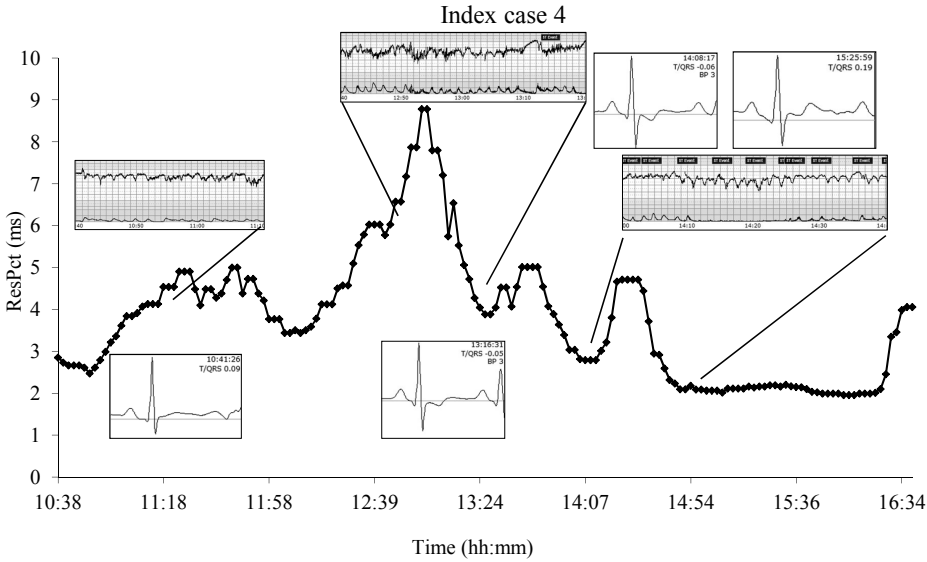

Figure 15. The graph shows the ResPct of the total recording of index case 4. The first part of the recording shows a normal FHR pattern with a normal ECG. The first biphasic ST event appears shortly after the increased FHRV. An ST rise is noticed towards the end of the recording with very low FHRV.

During low reactivity/variability sequences, the index cases showed a maximum ResPct ranging from 2.01 – 2.28 ms. Thus identifying an upper level of Residual measurement for low FHRV of <2.3 ms. The corresponding figures for the normal group was ResPct ranging from 3.0 – 23.1 ms and 7.1; 5.7-8.6 (mean; 95% CI), $p<0.05$. Eleven cases in the control group showed periods of 1 to 10 h duration where the ResPct was lowered (<3.0 ms) as was FHRV when assessed visually. During a sequence of low Residuals, all these fetuses showed episodes of a

minimum duration of 10 min where there were visual signs of increased variability (Paper II, Figure 3A and B). The ResPct showed a significant increase from 2.0 ± 0.20 to 3.0 ± 0.68 ms (mean ± S.D. $p<0.001$) in connection with these episodes. These short lasting sequences of increased variability were identified from calculating the variance of the ResPct with peaks ranging from 0.12 – 11.15 ms^2. The combination of a low value of the ResPct together with a lack of variation (variance) was thus used to identify consistently low FHRV.

Alteration in fetal reactivity in connection with fetal metabolic acidosis and spontaneous vaginal delivery (Paper III)

The first two studies formed the basis for continuous assessment of fetal reactivity focusing on decreasing or consistently low FHRV. Observations in the initial clinical data analysis were also made with regard to the lack of cases with cord artery metabolic acidosis and low reactivity. Furthermore, index case 4 presents interesting findings of a pattern of increase in FHRV as measured by ResPct followed by a gradual decrease before delivery. These observations suggest that in cases where there is an active fetal reaction, the initial response is an increase in reactivity. Furthermore, it appeared as if normal vaginal deliveries would cause an arousal reaction. The aim of the third paper was therefore to analyse the FHRV and FECG in combination with spontaneous vaginal delivery with and without cord artery metabolic acidosis.

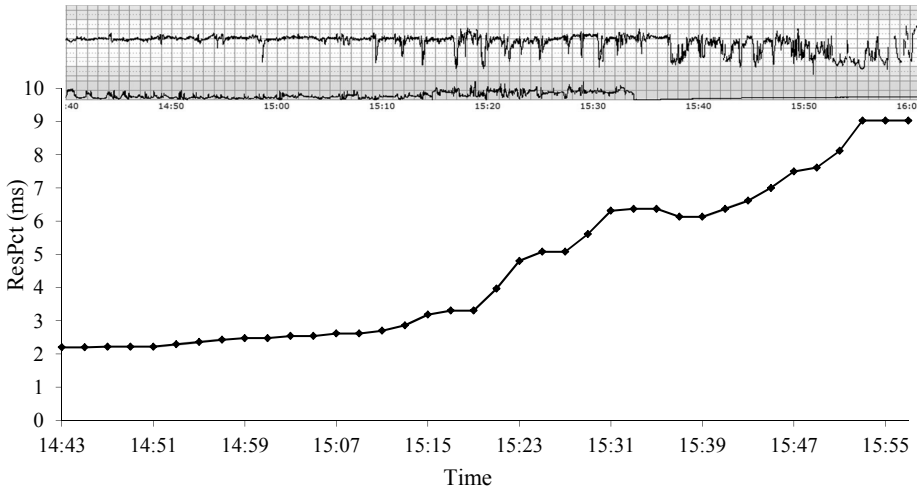

Figure 16 provides an illustration of Residual changes in one case with a normal outcome of labour, showing the variations in fetal activity with episodic as well as general increase in FHRV with progress of labour towards a normal vaginal delivery.

The FHRV and ST patterns recorded in connection with normal vaginal deliveries (NVD) were compared in cases with and without (Figure 16) cord artery metabolic acidosis. NVD per se should be regarded as a cause of significant arousal, but the research question was to find out how a normal reaction may differ from one where NVD is associated with metabolic acidosis. Figure 17 provides an illustration of a case with metabolic acidosis. In this case the FHRV increased during second stage of labour but started to decrease towards delivery. This FHRV pattern was observed in compromised fetuses, while non-compromised fetuses demonstrated an increased FHRV only. Fetuses that did not maintain an increased FHRV towards the end of labour had a higher artery BDecf (15.8 vs 13.2 mmol/L, p=0.017) and a lower Apgar score at 5 minutes (5.8 vs 7.7, p=0.019) compared to fetuses with metabolic acidosis but without this biphasic reactivity pattern.

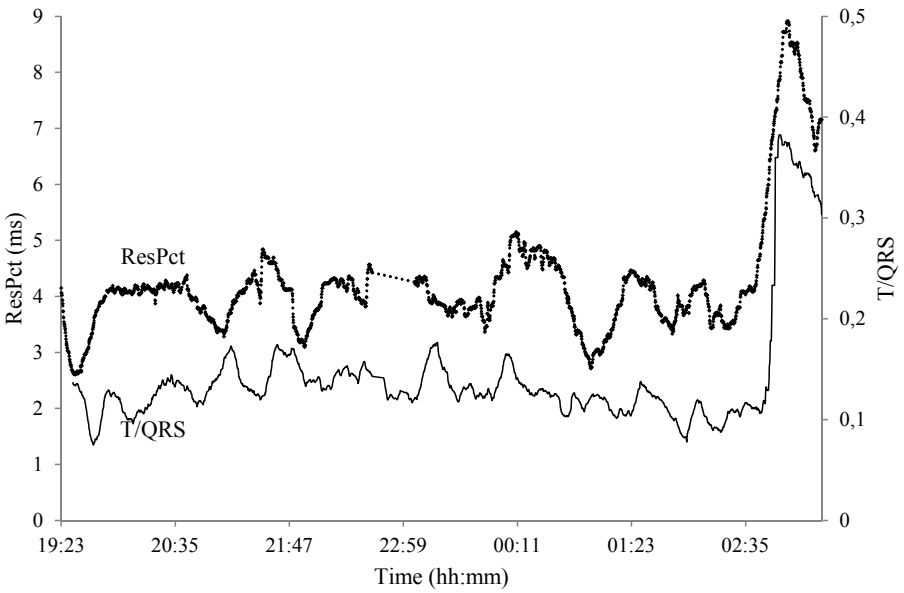

Figure 17. This metabolic acidosis case showed a marked rise from 3.6 to 8.9 ms in ResPct during 40 minutes followed by a decrease during the last 20 minutes of the recording, which finished 16 minutes before delivery. The T/QRS curve displayed a pattern similar to that of the ResPct.

In conclusion, in the majority of cases, NVD was associated with an arousal reaction associated with an increased FHRV and in some cases (5%) this arousal may border on an alarm reaction as it was associated with an ST rise. The occurrence and duration of ST changes distinguished cases with normal acid base from those with metabolic acidosis. Regarding the

FHRV, it was the rate of rise and magnitude of rise in ResPct that appeared to be most relevant. Assuming that the ResPct rise is a reflection of an increase in ANS activity triggering the cardiovascular system and cardiac performance, these data suggest that the initiating event of an arousal/alarm reaction is the increase in ANS as reflected by the ResPct. Once, the arousal has become an alarm reaction defined as an increase in both FHRV and T/QRS, it is the duration of the alarm phase that become clinically relevant.

ECG and heart rate variability changes in preterm and near-term fetal lamb following LPS exposure (Paper IV)

So far the analysis have focused on human fetuses at term and exposed to arousal reactions in connection with NVD and hypoxia. In the initial studies, observations suggested that an adverse outcome could be associated with maternal infection (index case 4). There is also significant evidence in the literature to suggest that in utero inflammation increases the risk of brain injury. The hypothesis of this study was therefore that the FHRV response would be altered in a model of in utero inflammation, that has previously been shown to result in brain injury (Mallard *et al* 2003). Lamb fetuses at 60% (preterm) or 80% (near-term) of gestation were exposed to LPS to study the effects of in utero inflammation on FHRV. We found that depending on gestational age, fetuses reacted differently. The preterm fetuses that survived the experiments reacted with an increased FHR and FHRV, a decrease in MAP and negative T/QRS compared to sham operated control preterm fetuses. In the preterm fetuses, a pattern of negative T/QRS was also observed, which transiently normalized during episodes of bradycardia. The preterm fetuses that did not survive the experiments showed similar results as the surviving fetuses, although the response was more pronounced. All term fetuses survived the LPS injection and the only response that significantly differed between the experimental and control group was an increased MAP 2 hours after the administration of LPS. The preterm fetuses had also higher baseline FHR and FHRV then term fetuses. The Residuals, on the other hand, were at baseline nearly 4 times higher in the term fetuses.

In summary, the experimental work provided information on alterations in the FECG based fetal reactivity related parameters (Residual based analysis of FHRV and ST waveform analysis) in connection with fetal exposure to LPS. A substantial difference in patterns of reactions was recorded in relation to gestational age with the near-term sheep fetuses showing no changes in any of the FECG, cardiovascular or acid-base related parameters. The preterm fetuses, on the other hand, were more profoundly affected with primary cardiovascular failure with decreasing MAP and negative T waves as the predominant findings. The increase in the Residuals parameter from a low baseline level indicates an attempt by the immature autonomic

system to respond. However, in the non-surviving group, due to end organ (myocardial) failure, this arousal reaction did not cause any functional response and the preparation deteriorated during the hours following the lethal LPS injection.

General Discussion

The series of papers in this thesis have been designed to define the applicability of a novel approach to assess FHRV continuously during labour. As part of this process, the focus has been on the assessment of fetal reactivity, i.e. the ability of the fetus to respond to stress. Stress is an integral part of being born and the aim of fetal monitoring during labour is to identify fetuses at risk of an adverse outcome by understanding how the fetus reacts to stress before it becomes compromised.

The CTG is an integral part of intrapartum care in most delivery wards and it is helpful in identifying the normally reacting fetus as well as the fetus unable to respond. The latter indicates asphyxiating conditions during labour in a small group of babies at risk of death or irreversible brain injury.

The UK 4th Confidential inquiries into Stillbirths and Deaths in infancy (1995) analysed the intrapartum deaths that were due to asphyxia in babies greater than 1500 g with no chromosomal or congenital malformation. The conclusion was that 50% of the deaths could have been avoided if an alternate care had been provided. The reasons identified for the poor outcomes were: inability to interpret CTG, failure to incorporate the clinical picture, delay in taking appropriate action and poor communication. This is not entirely surprising as the model of FHR interpretation is largely based on empirical observations of thousands of hours of recordings during human labour (Hon *et al* 1967). Large intra-and inter-observer differences in the interpretation of CTG recordings have been reported and apparently even amongst experts (Nielsen *et al* 1987, Donker *et al* 1993, Bernardes *et al* 1997).

In a recent analysis, Amer-Wåhlin and Dekker (2008) state that "CTG will always be a nonspecific method, currently dependent heavily on subjective interpretation. Thus, the health care providers (and the fetus) remain at risk for wrong/delayed action as clear-cut information is not available. Only with the addition of non-subjective information will the risk decrease".

Today, the fetal bioprofile generated during labour and delivery consists of a static assessment of FHR. The stress generated by uterine activity provides a means of testing the ability of the fetus to meet with the challenges of being born. The FHR patterns are valuable to identify normality and patterns identifying fetal reactivity (variability and accelerations) are most useful for immediate fetal surveillance. Complexity becomes vast when abnormality is assessed

due to active fetal adaptation to stress and variations in the ability to manage. Thus, each fetus has its own pattern of reactions and bioprofile to be considered. The FECG, forming the basis for FHR has been shown to be a clinically useful source of information reflecting both ANS (FHR patterns, beat-to-beat variation) and heart muscle reactivity (ST waveform changes). Automatic assessment of the ST forms the basis for the new STAN fetal surveillance methodology and additional decision support is being developed to optimize the use of the FECG and fetal reactivity measures in particular.

With the introduction of the STAN methodology, clinical experience from thousands of recordings both as part of regular clinical use and from structured research has been gained. STAN performs ST analysis automatically but requires visual assessment of the CTG (Figure 5). The CTG+ST clinical guidelines set specific standards of data interpretation which makes it possible to identify situations where data interpretation has been problematic. The assessment of variability and reactivity is one of those specific situations that would warrant additional decision support (DS).

High quality clinical data is essential when targeting DS developments. There is a need for specific software but equally important is a detailed knowledge of the pathophysiology involved as well as the clinical scenarios associated with specific reactivity patterns. The STAN development has shown the benefit of introducing clinical decision making based on detailed understanding of the pathophysiology as well as having a robust parameter for measurement and quantification of changes (Norén *et al* 2010). More commonly a clinical DS tool would start as a technological development with a subsequent focus on its clinical applicability. An example would be to use specific mathematical models to test their ability to separate deliveries with a normal outcome from those with signs of hypoxia and on the basis of such findings provide a model of data interpretation. Siira *et al* tested whether term fetuses born with marked cord artery acidaemia (pH <7.05) would reveal differences in FHR spectral analysis during the last hour of the recording compared with a non-acidotic control group. They reported that marked fetal acidosis was associated with frequency specific changes (mainly an increase in the 0.04 – 0.15 Hz band, which in turn modify the fetal sympathovagal balance) in FHRV. Thus, FHRV spectral analysis provides further information about the compensation ability of fetal ANS responding to the stress of labour (Siira *et al* 2005).

Power spectrum estimation methods are used to classify the frequency components of FHR associated with vagal and sympathetic activity applying fast Fourier transform (FFT) modelling algorithm. The FFT algorithm imposes certain limitations in analysing this kind of signal, because an evenly sampled, infinite and stationary time series is required (Altimiras

1999). The FHR signal recorded during labour opposes to these requirements. As an alternative, wavelet transform (WT) has proved to be potentially useful techniques for the analysis of signals like FHR, even under non-stationary conditions. It does not, however, provide precise estimates of the frequency and time localization of low-frequency structures and further developments with the use of matching pursuit techniques have instead been applied with some success (Salamalekis *et al* 2006).

As an analytical concept is developed further, more and more detailed testing is done applying a multi-disciplinary approach with physiological, mathematical, statistical, bioengineering as well as clinical knowledge and experience. However, very little is achieved unless there is a database consisting of index cases as well as reference cases. In our situation, an index case is defined as a case providing a pattern of reactivity in relation to fetal state and outcome. The problem in developing DS for situations such as loss of reactivity is those cases that provide adequate data are extremely rare – usually the patterns in question are acted upon and the case would have a good outcome. The comment may then be that due its rarity little would be gained by identifying such a pattern. On the other hand patterns that should warrant prompt action may require specific flags to secure identification. A DS tool would reduce uncertainty in high risk situations during actual monitoring but also be of use in training.

The EU program on "Safe dissemination of the ST analysis through the Centre of Excellence model" generated a unique database containing clinical as well as FECG data obtained from ten obstetric units across Europe. An essential part of the work behind this thesis was the quality control of the data which is always crucial to setting up such a large database of > 7 GB. Obviously, such an asset should be used with some care simply because the large numbers may by themselves create a problem in that statistical significance may be obtained on relationships that may not be clinically relevant.

Residuals and outcome

Although Paper I does not provide detailed information on how the mathematical model was developed, it provides an insight into an important aspect of the validation process – the optimization of parameter settings for the main RR trend, i.e. the design of the polynomial function. The outcome of this analysis was the use of two sets of polynomials to test for two different models of low variability. The first was to test for persistently low reactivity (LR1) of 70 minutes duration and the second was to test for loss of reactivity for a minimum of 30 minutes (LR2). As discussed previously, there was a need to reduce the impact of changes in baseline HR by normalising the Residual measurement at a standard HR of 150 bpm. The aim of the Residuals development was to calculate the main RR trend with as little influence as possible from rapid

changes in baseline FHR. Thus, based on the initial tests, we chose to apply the more robust polynomial of 12th degree in the subsequent analysis. By adding a new parameter, the variance of two minute 95th Pct Residuals, we only needed to work with one polynomial function. As our work continued the 95th Pct of 20 minutes running data was renamed and called ResPct as this parameter became the predominantly used parameter in papers II and III. The LR1 and LR2 definitions and settings from the first paper were also redefined in paper II when REvents were introduced.

For the REvents, a very low ResPct (<2.0 ms) with no variance (<0.06 ms^2) for 28 minutes became the marker of a situation where no FHRV was evident and a somewhat higher ResPct (<2.3 ms) with low variance (<0.08 ms^2) over a longer period (60 minutes) became the marker of persistently low variability. However, in neither of these defined LR1 and LR2 situations have we included the occurrence of accelerations, which by manual analysis were found to occur in 49% of the cases. Those accelerations may be used to identify a fetus with low FHRV but still with a capacity to react. The importance of accelerations as a marker of normality is illustrated by the findings of Williams and Galerneau (Williams and Galerneau 2003) showing that with normal bandwidth, accelerations had no impact on the level of cord artery acidosis whereas with a bandwidth of <5 bpm the absence of accelerations was associated with a significant decrease in pH.

A "hibernating" pattern with much reduced reactivity/variability may also be regarded as a functional response to stress in the perinatal period (Singer 1999) and it is of interest to note the significant increase in the rate of admissions to neonatal care among those displaying low or decreasing Residuals. The connection between neonatal admissions and low Residuals may be a consequence of decreased reactivity and reduced ability to adapt at time of birth. The actual cause for the increased need for neonatal admission in cases with reduced reactivity has not been investigated. Perinatal infections has also been associated with low reactivity and Griffin *et al* have reported on abnormal heart rate patterns consisting of short lasting decelerations, reduced heart rate variability emerging prior to clinically verified sepsis or sepsis-like conditions (Griffin *et al* 2003). Furthermore, the experimental study in the present thesis showed low Residuals during basal condition in the preterm lamb fetus likely to reflect an immature ANS.

To elucidate the relationship between changes in fetal reactivity and the impact of infections, an experimental study was undertaken investigating the relationship between LPS, gestational age and fetal myocardial function. Preterm fetuses exposed to >90 ng/kg LPS died within 8 hours of LPS administration, a response not seen in near-term fetuses. In both surviving

and non-surviving preterm fetuses cardiovascular responses were characterised by decreased arterial pressure, negative T waves and tachycardia accompanied by an increase in FHRV, in contrast to the near-term fetus showing no changes after LPS. The increase in Residuals may require additional analysis simply because the development of ST depression with negative T waves would sooner indicate reduced myocardial reactivity.

ECG ST waveform response to LPS

It has previously been shown by our group, that both the preterm and near-term fetal sheep demonstrate an elevated T wave with increase in T/QRS ratio as the initial response to intrauterine asphyxia (Rosén *et al* 1984, Welin *et al* 2005), while ST depression/negative T waves have been associated with growth retardation and acute hypoxia with a blunted catecholamine response (Widmark *et al* 1990) as well as terminal situations with cardiac injury following severe hypo perfusion (Wibbens *et al* 2005). In the present study preterm fetuses developed negative ST after LPS administration as part of a progressively developing pattern of tachycardia, lowered blood pressure and decreased T wave amplitude, while elevation of the T wave was not observed. ST elevation requires the fetus to elicit an active myocardial response and it is possible that the preterm fetuses did not maintain this capacity after LPS exposure, as LPS may have direct effects on myocardial function as discussed below. No changes in the ST waveform were seen in the near-term fetuses. Although the negative ST patterns were observed in both surviving and non-surviving preterm animals, the magnitude of ST change was more marked in the non-surviving fetuses. These ST changes were associated with a marked tachycardia indicating that the myocardium was performing under maximal stress. Interestingly, the ST pattern was found to normalise with occasionally normalised FHR, presumably allowing for increased diastole and improved venous return and end diastolic pressure. Activation of cardiac volume receptors with vagally mediated bradycardia (Bezold- Jarisch reflex) may be the direct mechanism behind these rapidly occurring decelerations (Thorén 1973).

Endotoxemia is associated with insufficient myocardial function, including a depressed Frank-Starling and diastolic pressure-volume relationship (Figure 2) and contractile insufficiency (Aghajani *et al* 2004, Sugi *et al* 1991). However, in neonatal lambs, LPS has been shown to depress myocardial function, not primarily as a result of a decrease in preload as the decrease in contractility preceded the decrease in end-diastolic and stroke volume (Sosa *et al* 1994). A direct effect of LPS on the heart, via the LPS receptor toll-like receptor-4, has been associated with heart failure, suggesting a functional role in the heart (Frantz *et al* 1999). Furthermore, combined observations from both previous studies on the effect of cord occlusion and the present on LPS exposure, suggest that negative ST changes associated with myocardial dysfunction in the fetus

may be caused by alterations of central haemodynamics rather than being a direct effect of LPS *per se.* Regardless of the mechanisms, the LPS impact is gestational age dependent and seems to correlate to the depressed Frank-Starling curve of the premature myocardium and reduced margins of safety.

Some preterm fetuses demonstrated an interesting sequence consisting of a drop in FHR in parallel to the disappearance of negative T waves. Assuming this is an active response to compensate for a depressed Frank-Starling curve through increase in diastolic filling and end diastolic pressure, we may assume that this Bezold-Jarisch reflex is constantly activated causing rapid variations in FHR identical to what also was noted as part of an arousal/alarm reaction in response to a vaginal delivery. The experimental studies thus illustrate possible mechanisms that may be of importance in the interpretation of fetal reactivity in the clinical situation.

Increased reactivity

Apart from a predominant increase in Residuals towards a normal vaginal delivery, episodic increases also occurred during first stage. Those situations of increased reactivity are characterised by an increase in circulating catecholamines (Reid *et al* 1990) and fetal activity indicating a change in sleep state. Such reactions are part of normal behaviour and constitute the reactivity pattern recorded during the antenatal period (Wheeler *et al* 1980, Visser *et al* 1982, Dawes *et al* 1992).

The normal fetal response to a vaginal delivery is characterized by an arousal reaction. Remarkably high levels of catecholamines are found in the umbilical blood of newborn infants (Lagercrantz *et al* 1977, Nylund *et al* 1979) and extremely high levels are seen after instrumental delivery and/or asphyxia (Hägnevik *et al* 1984). It is, however, unclear to what extent an increase in FHRV and the Residual parameter ResPct may serve to distinguish a normal arousal from the alarm reaction of developing fetal hypoxia. Judging from the patterns recorded in this study it appears that fetuses that developed metabolic acidosis had a more marked rise of ResPct including magnitude, rate and peak value. This suggests the possibility of capturing an arousal reaction developing into an alarm reaction by more pronounced increases in the FHRV. As noted, no differences occurred between cases with metabolic acidosis and controls with regard to the onset of ResPct rise in relation to the onset of active pushing, or in baseline ResPct. Thus, the duration of the alarm reaction seemed less relevant as a marker of metabolic acidosis than the extent of a ResPct change and the level of reactivity reached.

The ability of a fetus to respond and manage stress of labour is essential to intact survival. Thus, it was of interest to note the clinical outcome data associated with fetuses unable to maintain the arousal/alarm reaction as characterized by increased FHRV. The loss of FHRV in

this group was accompanied by lower Apgar score as well as a significantly more pronounced metabolic acidosis suggesting that the more pronounced neonatal depression in this group was indeed secondary to fetal hypoxia. These findings may help to identify a pathological pattern of reaction with a FHRV rise followed by a decrease that is associated with aggravated hypoxia and eventually resulting in lack of reactivity and preterminal FHR.

In agreement with our findings, studies from the 1970s found an increase in FHRV followed by a decrease during experimental hypoxia (Dalton *et al* 1977). This has been verified by Yu *et al* (Yu *et al* 1998) who, using power spectrum analysis, showed an increase in spectral energy in the LF band during mild and moderate hypoxemia in fetal lamb. Similar to our findings, Siira *et al* (Siira *et al* 2005) demonstrated that during the last hour of labour the LF band initially increased, but near delivery decreased in acidotic fetuses with an arterial pH<7.05 compared to non-acidotic fetuses. Thus, it appears as if the changes in FHRV during developing hypoxia are characterized by an initial rise followed by a gradual decrease. Figure 18 provides the Residual data from an adverse outcome case where the fetus had signs of cardiomyopathy

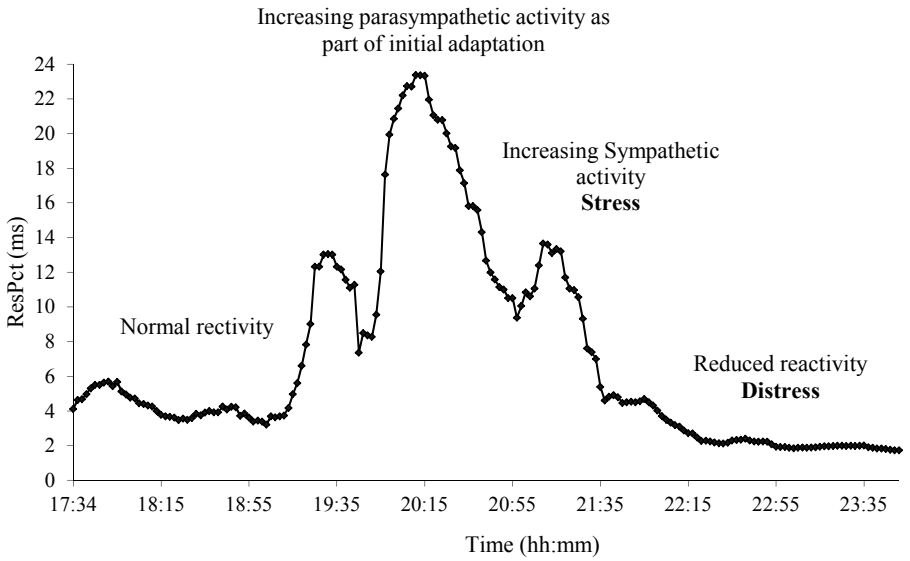

Figure 18. ResPct data obtained during labour in an adverse outcome case. At 22:00 treatment with intravenous antibiotics was started because of maternal pyrexia. At autopsy, alterations in the fetal myocardium showed signs of cardiomyopathy (for further information on this case, see Westerhuis *et al* 2007, case 2).

In our study, 19/23 cases with metabolic acidosis and FECG data available within 38 minutes of delivery, had a marked increase in reactivity affecting both ResPct and T/QRS, the latter emerging on average 26 minutes after the onset of the increase in FHRV. These cases serve to illustrate the timing of ANS reactivity in relation to myocardial metabolic reactivity response during the development of metabolic acidosis and how this reactivity is reflected by changes in FHR as well as the ST waveform. Assuming the rise in ResPct is an active response with rapidly occurring alterations in consecutive RR intervals, such a response would require detailed control of the heartbeat as a consequence of rapidly changing central haemodynamics. Such situations may occur in connection with uterine contractions and/or cord entanglement and beat-to-beat based alterations in venous return and central blood volume with intermittent cord vein compression. Further, the Bezold- Jarisch reflex may serve to illustrate a mechanism whereby a marked onset of bradycardia may be noted with a decrease in venous return and the filling pressure of the heart (Giussani *et al* 1997). This spinal reflex originates in cardiac sensory receptors with non-myelinated vagal afferent pathways (Thorén 1979). Stimulation of these inhibitory cardiac receptors by stretch increases parasympathetic activity and inhibits sympathetic activity and thus provides a mechanism whereby parasympathetic activity may affect the FHRV. The most likely mechanisms of increased sympathetic activity would be the mechanical strain of labour as well as fetal hypoxia (Walker *et al* 1973). From these mechanisms one may expect marked and rapid changes with an increase in both sympathetic and parasympathetic autonomic nervous system reactivity during labour, thus providing a physiological base for the observations made in connection with the Residual method.

The clinical relevance of obtaining more detailed information of FHRV during the arousal of normal delivery may be limited, since these patterns of reactivity are easily identified visually by clinical CTG. There is, however, a risk of these reactive patterns being regarded as a sign of normality when they in fact identify the beginning of an alarm reaction associated with intrapartum hypoxia and metabolic acidosis. With the Residual method there is also the potential of combining automated FHRV analysis with ST analysis providing user support during second stage and not restricting the aim with automated FHRV to identify loss of reactivity only. Further research is needed to fully understand the physiological mechanism behind changes in FHRV during labour and to what extent those changes are associated with fetal compromise of different origin.

In summary, it appears as if changes in FHRV during developing hypoxia are characterized by an initial rise followed by a gradual decrease. The Residual method provides continuous information on FHRV and is able to capture the biphasic FHRV pattern associated with more marked metabolic acidosis. Further the Residual method allows for the non-stationary nature of the FHR during delivery and may be a useful additional tool to evaluate the CTG during labour.

Conclusions to given aims

- The Residual method can be used for quantifying FHRV regardless of alteration in baseline heart rate. By adding statistical parameters, such as the 20 minutes 95^{th} percentile and the variance of the 2 minute 95^{th} percentile, the method can separate low or lost reactivity from normal reactivity. Furthermore, continuous monitoring using the Residuals technique allows for both the identification of a gradual loss of reactivity occurring over several hours as well as rapidly occurring rise occurring over minutes.
- By using the Residual method, REvents settings have been identified from index cases and validated in a large clinical database. Cases with low FHRV were found to have an increased rate of operative deliveries and admission to neonatal care compared with cases without REvents.
- Increased FHRV, measured with the Residual method, was the most common response during second stage of labour regardless of neonatal outcome. Infants born with metabolic acidosis responded with a higher FHRV and faster increase in FHRV compared to infants with normal outcome and a decrease in reactivity following the initial increase was associated with neonatal depression and a more pronounced acidosis.
- Preterm lambs were more sensitive to LPS in terms of myocardial and cardiovascular response than the more mature fetuses were. High FHRV and negative ST waveform were seen as LPS-induced stress response in preterm fetuses.

Future perspective

The possibility to quantify FHRV with a polynomial function will allow further development of automatic CTG classification. The next step would be to adapt the method so that signs of reactivity (i.e. accelerations) can be automatically assessed and used to further distinguish between healthy and compromised fetuses. The method could also be used to quantify the reactivity at onset of recording and separate the pattern into different categories such as reactive or nonreactive FHR. With different classification the settings regarding reactivity events could be different, with longer timeframe for reactivity patterns. All these tests could be performed using the current database. To be able to fully validate the clinical outcome of an automatically assessed CTG a full scale randomised clinical trial is warranted. In the meantime it is suggested that the Residuals function is used to support the interpretation of FHR based reactivity features as part of a decision support features identifying specific situations where there is a need to check reactivity.

Sammanfattning

Friska fullgångna foster har en mycket stor kapacitet att hantera en kortvarig syrebrist som uppstår vid förlossningsvärkar. Forskning har visat att denna kapacitet minskar vid infektioner, hos omogna foster och vid återupprepade och långvariga situationer med syrebrist. Det finns då en risk att fostret blir påverkat och kan få permanenta skador. Elektronisk fosterövervakning under förlossningen är idag standard i de flesta länder. Målet är att identifiera foster som utsätts för en sådan belastning att de minskar sin förmåga att hantera ytterligare syrebrist under förlossningen. Ett vanligt sätt att övervaka fostret är att koppla en elektrod på huvudet efter vattenavgång, och därmed registrera fostrets elektriska hjärtsignal (EKG). EKG används sedan länge för att beräkna fostrets hjärtfrekvens, så kallat CTG. CTG kurvan innehåller också värkaktivitet och mönstret tolkas visuellt av förlossningspersonalen. Forskning har också visat att vågformen hos ett EKG (den så kallade ST-sträckan) ger information om hjärtmuskelns förmåga att svara på förlossningsstress. Denna information analyseras av en så kallad STAN apparat och utgör ett komplement till sedvanligt CTG. Även om STAN automatiskt analyserar förändringar i ST-intervallet så måste hjärtfrekvensen analyseras manuellt för att personalen skall få en komplett bild över fostrets tillstånd. Studier har visat att det finns en stor variation hur hjärtfrekvens tolkas vilket i vissa fall kan få allvarliga konsekvenser. För att undvika detta har det efterfrågats att även hjärtfrekvens mönstret skall ha stöd av automatisk tolkning. En viktig del av hjärtfrekvensen är den så kallade variabiliteten eller slag-till-slag variationen. Eftersom den regleras genom det autonoma nervsystemet så är det också ett mått på intakt cerebral funktion och att fostrets reaktivitetsförmåga är bevarad. Automatisk mätning av variabiliteten måste kunna ta hänsyn till de stora förändringarna som sker i hjärtfrekvensen under värkar och andra yttre omständigheter. Idag mäts variabiliteten visuellt genom en uppskattning av spridningen av hjärtfrekvensen under några minuter, s.k. bandbredd. Avhandlings syfte har varit att utvärdera en matematisk metod (så kallad Residual metoden) för att kvantifiera hjärtfrekvensvariabiliteten under förlossningen och studera hur man kan använda metoden för att hitta skillnader i fosters reaktionssätt vid infektion och syrebrist. I arbetet har en stor klinisk databas med över 7800 förlossningar används för att utvärdera den nya matematiska metoden som delvis liknar den gamla bandbreddmetodiken men tillåter en mer exakt och kontinuerlig registrering av hur hjärtslagen varierar sinsemellan. Som utgångspunkt valdes fyra fall där barnen varit mycket påverkade eller dödfödda vid födelsen och där fostren under förlossningen hade ett hjärtfrekvensmönster med mycket reducerad eller helt saknade slag-till-slag variation och därmed tecken till förmåga att reagera på förväntat sätt. Detta mönster kunde förekomma, antingen från början av registreringen eller så minskade variabiliteten då förlossningen fortskred. Med hjälp av

dessa fallen så bestämdes värden och gränser som sedan testades mot den stora databasen med flera tusen fall. Syftet var att hitta parametrar och gränsvärden så att referensfallen samt relativt få ”normala” fall ur databasen identifierades. Resultatet blev att när parametrar sattes så att samtliga referensfall kunde urskiljas, så identifierades även 2,6 % fall i den stora databasen. Dessa fall visade sig oftare ha hamnat på neonatalavdelningen för eftervård samt hade fler operativa förlossningar än i den grupp av fall som inte markerats. Analyserna visade också att metoden var tillämpbar även då hjärtfrekvensen kraftigt ändras t.ex. i samband med värk. Ett av fynden vid databasanalysen var att foster som enbart uppvisade påverkan i sina syra-bas värden vid förlossningen mycket sällan saknade hjärtfrekvensvariabilitet. I stället visade det sig att när vi analyserade foster som föddes med normal vaginal förlossning med eller utan metabol acidos (mått på genomgången syrebrist) så uppvisade dessa i stället en markant ökning av variabiliteten. Denna stegring var mer uttalad och skedde snabbare hos foster som uppvisade metabol acidos. Som tidigare visats så uppkom även ST förändringar i en ökad andel hos dess foster. I en experimentell studie användes Residual metoden och ST analysen för att undersöka hur fårfoster i olika gestations åldrar reagerade på inflammation. De foster som inte ännu var helt färdigutvecklade reagerade med en hög relativ variabilitetsstegring samt förändringar i ST intervallet, denna respons hittades inte hos de nästan fullgånga fostren.

Slutsatsen av arbete i denna avhandling är att med Residual metoden så är det möjligt att mäta hjärtfrekvensvariabilitet även i samband med förändringar i den basala hjärtfrekvensen och därmed få ett kontinuerligt mått under förlossningen. Det är också möjligt att identifiera avvikande mönster som oftast hittas hos foster som inte klarar av att hantera den kraftiga påverkan som förlossningen kan innebära. Residual-metoden ger hopp om att i framtiden hjälpa personalen att identifiera foster som utsätts för en belastning som de har svårt att klara av och som därmed skulle gynnas av en operativ förlossning.

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