The fetal response to acute perinatal hypoxia and the pathophysiological mechanisms behind hypoxic-ischemic encephalopathy

Stud.med. Maia Blomhoff Holm

Faculty of Medicine
UNIVERSITY OF OSLO

2012

Supervisor: Tore Henriksen
Professor dr.med.

Abstract

Perinatal hypoxic-ischemic encephalopathy (HIE) is an important cause of brain injury in the newborn. It may lead to a variety of different long-term neurological sequelaes; from mild behavioral deficits to severe compromise with seizures, mental retardation and cerebral palsy (CP).

The purpose of the present thesis is to provide an overview of the fetal physiological responses to acute perinatal hypoxia and the pathophysiological mechanisms behind the development of HIE. Different terms related to this condition will also be discussed, as well as normal fetal physiology and methods of fetal surveillance during labor. Finally will future challenges and possible strategies for preventing HIE be suggested. The information presented in this paper is a summary of current knowledge, and has primarily been obtained by searches in medical databases for relevant reviews and articles.

A thorough insight into the normal physiology of the fetus and the process of labor, as well as knowledge of the possible pathophysiological mechanisms involved, are crucial for understanding how HIE may arise. It also forms the basis for understanding and interpreting the fetal heart rate monitoring and other modes of surveillances used to detect endangered fetuses during labor. Through this we may improve the individual management of every labor and thereby reduce the risk of development of birth asphyxia and possible fetal injury.

Content

1 Introduction 3
1.1 Method 3
1.2 Hypoxic-ischemic encephalopathy 3
1.3 Chemical and physiological parameters related to hypoxia 6
1.3.1 Fetal hypoxia – definitions 6
1.3.2 Metabolic changes associated with hypoxia 6
1.3.3 pH and base excess 2
1.4 Normal fetal oxygenation 3
1.5 More about the fetal heart rate 4
1.6 Fetal surveillance during labor 4
2 The fetal physiological responses to acute perinatal hypoxia 6
2.1 Responses of the healthy term fetus to hypoxia during labor 6
2.1.1 Reduced oxygen supply during normal, uncomplicated labor (hypoxemia to moderate hypoxia) 6
2.1.2 Development of severe hypoxia 7
2.2 Responses to hypoxia during labor in fetuses afflicted by prenatal placental insufficiency 9
2.2.1 Fetal adaptation to chronic placental insufficiency 9
2.2.2 Placental restricted fetuses' response to acute hypoxia during labor 10
2.3 Current Norwegian guidelines for fetal monitoring during labor 13
3 Pathophysiological mechanisms behind HIE 14
3.1 Development of brain ischemia 14
3.2 Development of brain inflammation 15
3.3 Brain damage due to neuronal cell death 15
3.4 Localization of brain damage 16
3.5 The relationship between fetal asphyxia, HIE and CP 17
4 Future perspectives and challenges 19
4.1 Identify the fetuses that are particularly vulnerable to hypoxia during labor 19
4.2 Improve fetal monitoring during labor 19
4.3 Examine the connection between perinatal hypoxia and development of chronic disease later in life 20
4.4 Improve the knowledge of fetal physiology in order to more correctly interpret present and future modes of fetal surveillance during labor 20
References 21

1 Introduction

Hypoxia plays a significant physiological role in fetal development. It is important in major embryonic processes like angiogenesis, hematopoiesis, chondrogenesis, and placental development. Excessive acute or chronic hypoxia, however, may adversely affect the fetus in various ways in any state of the development process. The purpose of the present thesis is to provide an overview of the physiological responses of both a) the healthy term fetus, and b) the term fetus exposed to prenatal placental insufficiency, to sudden oxygen deprivation during labor. It will also describe how such hypoxia, in some cases, may cause the development of hypoxic-ischemic encephalopathy (HIE). A thorough insight into the normal physiology of the fetus and the process of labor, as well as knowledge of the possible pathophysiological mechanisms involved, are crucial for understanding how HIE may arise. It also forms the basis for understanding the fetal heart rate monitoring and other modes of surveillances used to detect endangered fetuses during labor, and to aid in the interpretation of these monitorations. Through this we may improve the individual management of every labor and reduce the risk of development of birth asphyxia and possible fetal injury.

Before reviewing the current knowledge of the fetal physiological response to acute perinatal hypoxia and the pathophysiological mechanisms behind the development of HIE, a short introduction to HIE and to the chemical and physical parameters related to hypoxia will be given. Normal fetal oxygenation in utero and the regulation of the fetal heart rate will be introduced, as well as a short presentation of CTG and STAN, methods of fetal surveillance during labor.

1.1 Method

The present thesis is a summary of the current knowledge of the physiology of fetal perinatal hypoxia and the development of HIE. It is not a systematic review. The knowledge presented in this paper has been obtained by searches in Pubmed/Medline and the Cochrane Library for relevant articles, using relevant terms, mainly focusing on recent reviews published in journals with high impact factor. This was done to ensure quality and the most up to date research. Some original articles have also been included. The search was performed from August 2011 to February 2012. Some key reviews were initially provided by the supervisor, Prof Dr. Med Tore Henriksen. In addition, medical textbooks and Norsk Veileder i Fødselshjelp have been consulted in order to obtain information of current practical guidelines.

1.2 Hypoxic-ischemic encephalopathy

Neonatal hypoxic-ischemic encephalopathy (HIE) is an acute, nonstatic encephalopathy caused by brain hypoxia and ischemia during or closely associated with labor. It may cause a variety of long-term neurological sequelaes in the affected children, varying from mild behavioral deficits to severe compromise like cerebral palsy and other dyskinesias, visual impairment, epilepsy, and poor cognitive development (1) (2) (3). The incidence of HIE for nonanomalous term infants in developed countries was in 2008 estimated to be 2.5 of 1000 live births (range 1.2-7.7/1000) (4). The incidence in resource-poor countries is probably

much higher, but little data are available (5). The diagnosis of HIE is based on a number of clinical criteria, together forming a clinical syndrome that describes the causality of the encephalopathy (see figure 1). These criteria include evidence of fetal distress immediately before or during delivery, severe fetal metabolic acidosis, and the need for newborn resuscitation. They also rely on clinical signs or indications of acute neurological abnormalities on imaging or electroencephalography (EEG). In addition, all other identifiable causes of neonatal encephalopathy must be excluded (1) (6) (7). Common symptoms of HIE are hypotonia, poor feeding, seizures and a reduced level of consciousness. According to Sarnat and Sarnat (8), the severity of HIE can be graded in three stages, from 1-3, based on the infant's neuromuscular control, autonomic function, level of consciousness, the presence of seizures, and findings on the electroencephalography (EEG) (table 1).

Clinical criteria for HIE

- Fetal distress before or during birth detected on fetal monitoring
- Severe fetal metabolic acidosis, i.e. fetal blood pH < 7 and/or BE <-12
- Apgar score of 0-3 for longer than 5 minutes with need for resuscitation
- Neonatal neurologic sequelae, e g seizures, coma, hypotonia
- Neurological abnormalities on EEG and/or images
- Exclusion of other identifiable causes of neonatal encephalopathy

Figure 1 Clinical criteria for HIE

The prognosis of HIE is often difficult to assess. It is influenced by the severity and the duration of the baby's symptoms as well as other factors, like the degree of fetal acidosis during labor, the Apgar score and whether it was need for resuscitation after birth, the duration of time before the neonate's first breath, and how early any seizures started. The pattern of injury on MRI is also important (1) (2) (7). In cases of mild stage 1 encephalopathy, a good outcome with no long-term adverse effects may be expected, but when the infant is considered as moderately or seriously affected, i.e. Sarnat stage 2 or 3, respectively 15-27% and 50-100% will die in the neonatal period or develop severe and permanent neuropsychological sequelaes (2) (6) (7) (3).

	Stage 1	**Stage 2**	**Stage 3**
Level of consciousness	Hyperalert	Lethargic/obtunded	Stuporus
Neuromuscular control			
Muscle tone	*Normal*	*Mild hypotonia*	*Flaccid*
Posture	*Mild distal flexion*	*Strong distal flexion*	*Intermittent decerebration*
Strength reflexes	*Overactive*	*Overactive*	*Decreased/absent*
Segmental myoclonus	*Present*	*Present*	*Absent*
Complex reflexes			
Suck	*Weak*	*Weak/absent*	*Absent*
Moro	*Strong; low threshold*	*Weak; incomplete; high threshold*	*Absent*
Oculovestibular	*Normal*	*Overactive*	*Weak/absent*

Tonic neck	*Slight*	*Strong*	*Absent*
Autonomic function	Generalized sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	*Mydriasis*	*Miosis*	*Variable, often unequal. Poor light reflex*
Heart rate	*Tachycardia*	*Bradycardia*	*Variable*
Bronchial and salivary secretions	*Sparse*	*Profuse*	*Variable*
GI motility	*Normal/ decreased*	*Increased; diarrhea*	*Variable*
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
EEG findings	Normal (awake)	– Early: low voltage continuous delta and theta – Later: periodic pattern (awake) – Seizures: focal 1-to 11/2-Hz spike and wave	– Early: periodic pattern with isopotential phases – Later: totally isopotential
Duration	< 24 h	2-14 days	Hours-weeks

Table 1 Sarnat Clinical Stages of Perinatal Hypoxic Ischemic Brain Injury

The relationship between HIE and cerebral palsy (CP) has been extensively studied throughout the years. Cerebral palsy is a group of disorders in the development of movement and posture that are attributed to non-progressive disturbances in the developing brain, affecting approximately 2 infants per 1000 live births. Disease develops before the age of two, and it is often accompanied by impairment of cognition, perception, sensation, and communication. 25-50% of the affected children are in addition suffering from associated seizures (13).

It was for a long time believed that the overwhelming majority of CP cases were attributed to fetal compromise during labor (2) (4) (9) (10) (11). This comprehension has changed, however, and intrapartum asphyxia, prematurity, and other perinatal complications, are now believed to account for only a smaller percentage of the cases. Still, the actual number is highly controversial, varying in different reports from under 10% to around 35% (2) (4) (9) (10) (12) (13). This will be further discussed in section 3.5 of the present thesis. The most frequent causes of CP are prenatal conditions, such as disturbances in brain development, fetal malformations, metabolic diseases, intrauterine infections, cerebrovascular events (stroke etc.), and exposition to toxic substances. CP may also be a result of postnatal occurrences, for instance hypoglycemia, meningitis and septicemia, inadequate neonatal resuscitation, injuries, and, again, cerebrovascular events. In addition, a large portion of children with CP has no identifiable cause of brain impairment (11).

Nonetheless, HIE is a significant cause of CP, as well as other disabilities, and these impairments constitute a major socio-economic burden world-wide, especially in resource-poor countries. HIE substantially affect the global burden of disease, measured in disability-adjusted life-years, because the condition contribute to both premature mortality and long-term disability (5). Thus, it is a major goal for doctors and other birth attendants to detect and avoid situations that may lead to development of HIE.

1.3 Chemical and physiological parameters related to hypoxia

1.3.1 Fetal hypoxia – definitions

With respect to perinatal oxygen deprivation, it is important to differentiate between several different terms. The first phase of poor fetal oxygenation leads to hypoxemia (figure 2). The oxygen saturation in the arterial blood falls, but the function of cells and organs is usually not affected. If the oxygen supply decreases further, hypoxia develops (figure 3). Hypoxia is a state of reduced oxygen tension and subsequent anaerobic metabolism, mainly in peripheral tissue. In asphyxia (figure 4) the hypoxia and anaerobic metabolism extends to central organs like the heart, brain and adrenal glands, potentially leading to metabolic acidosis (14) (15). Ischemia is also an important term in this context. This refers to oxygen deficiency due to reduced blood flow and hypoperfusion of an organ or a tissue, and may operate in combination with hypoxia.

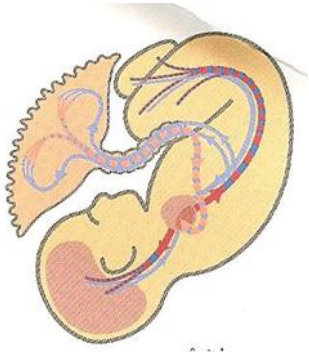

Figure 2 Hypoxemia, from Fosterovervåking og ST-analyse © Neoventa Medical (14)

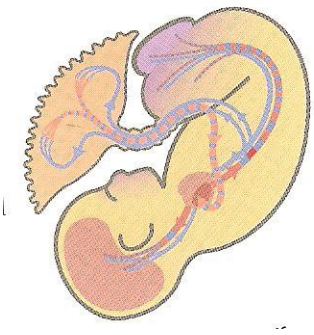

Figure 3 Hypoxia, from Fosterovervåking og ST-analyse © Neoventa Medical (14)

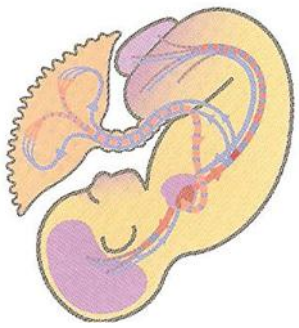

Figure 4 Asphyxia, from Fosterovervåking og ST-analyse © Neoventa Medical (14)

1.3.2 Metabolic changes associated with hypoxia

When a tissue is exposed to reduced oxygen supply, it loses its ability for efficient oxidative phosphorylation. As a consequence, the cells switch from aerobic to anaerobic metabolism. This leads to an energy-inefficient state with depletion of adenosine triphosphate (ATP)

reserves, accumulation of lactic acid and hydrogen ions (i.e. acidosis), and reduced cellular functions (3) (9) (16) (17). The ATP-dependent sodium-potassium pump fail, and the exchange of ions across the cell membrane is disrupted, initiating cascade reactions leading to cell injury and death.

In the case of neurons, which are important in the pathophysiology of HIE, the above mentioned events lead to intracellular accumulation of sodium, calcium and water, i.e. cytotoxic edema, and to depolarization of the membrane potential with a release of excitatory neurotransmitters from axon terminals, glutamate in particular. The increased level of calcium within the neuronal cell induces production of the free radical nitric oxide by activation of the neuronal nitric oxide synthase. It is also established that free fatty acids accumulate in the cytoplasm due to activation of phospholipases and increase turnover of membrane phospholipids. Some of these fatty acids are further peroxidized by oxygen free radicals mainly generated by reductive processes in the mitochondria. The combined effects of cellular energy failure, acidosis, glutamate release, intracellular calcium accumulation, lipid peroxidation and nitric oxide neurotoxicity will damage important components in the cell and lead to cell death (3) (16). Further details regarding neuronal cell death will be given in section 3.2.

1.3.3 pH and base excess

Acidosis is an important consequence of cellular hypoxia. The acidity or alkalinity of a solution is expressed by the pH logarithm, a measurement of the concentration of hydrogen ions in a solution, defined as pH = -lg [H3O+]. Solutions at 25°C with a pH less than 7 are considered acidic, while those with a pH greater than 7 are basic or alkaline. A pH level of 7.0 is defined as neutral because the concentration of hydronium ions then equals the concentration of hydroxide ions (18) (19).

The normal values of pH in arterial blood lie between 7.35 and 7.43 (19). Values lower than 6.8 and greater than 7.8 is not considered consistent with life. It is therefore extremely important that the body maintain the pH within the normal range. To accomplish this, the body has several buffer systems coping with changes in the hydrogen concentration, like bicarbonate ions ($H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow CO_2 + H_2O$), hemoglobin and other plasma proteins, as well as phosphate (18) (19).

The hydrogen ions in the body are under normal physiological conditions derived from carbon dioxide, from organic acids like fatty acids and ketonic acids, and from nonorganic acids like phosphoric acid. The lungs eliminate large quantities of the acid produced in the body through the very efficient ventilation of carbon dioxide. To a lesser and slower extent, the kidneys also contribute to the maintenance of the acid base balance by glomerular filtration of acidic anions, tubular secretion of hydrogen ions, and by reabsorption and production of bicarbonate ions. (18) (19)

Acid base disorders (table 2) arise if there is a shift in the acid base balance outside the normal range. Acidosis is the term used for any situation where the concentration of hydrogen ions in the blood is increased and the pH in arterial blood is below 7.35, whereas alkalosis is due to reduced hydrogen ion concentration with arterial pH greater than 7.43. Acidosis and alkalosis may further be classified according to the cause of the change in pH. Respiratory acid base disorders are a result of inexpedient ventilation of carbon dioxide by the

lungs, whereas acid base disorders are considered metabolic if generated in any other way. Hence, metabolic acid base disorders are often a result of changes in the capacity of the buffer systems, measured as the concentration of bicarbonate in the circulation (18) (19).

Base excess (BE) is an expression of the metabolic component of the acid base disorder. BE is defined as the amount of strong acid that must be added to each liter of fully oxygenated blood to return the pH to 7.40 at a temperature of 37°C and a carbon dioxide partial pressure of 5.3 kPa. A positive BE arises in situations with excessive amounts of bicarbonate, i.e. metabolic alkalosis, while a negative value is a sign of reduced bicarbonate levels, i.e. metabolic acidosis. The BE value in blood is normally between 3 and -3 (19).

Acid/base disorder	Conditions in arterial blood				Compensation mechanisms
	pH	CO2	HCO3-	BE	
Respiratory acidosis	↓	↑	↑	–	Kidney: increase H+ secretion and HCO3- reabsorption
Respiratory alkalosis	↑	↓	↓	–	Kidney: increase HCO3- secretion and H+ reabsorption
Metabolic acidosis	↓	↓	↓	↓	Lungs: hyperventilation
Metabolic alkalosis	↑	↑	↑	↑	Lungs: hypoventilation

Table 2 Acid/base disorders

Both pH and BE is useful in measuring the degree of fetal metabolic acidosis that may arise during labor. Though pH is most commonly used, it is not the best parameter for estimating the cumulative exposure to perinatal hypoxia, as its logarithmic scale does not provide a linear measurement of acid accumulation. A shift in pH from 7.0 to 6.9 is associated with almost twice the change in concentration of hydrogen ions compared to the change associated with a pH shift from 7.3 to7.2. BE however, changes linearly with the degree of accumulation of metabolic acid, and is in addition adjusted for variations in the partial pressure of carbon dioxide. pH on the other hand, is influenced by both respiratory and metabolic alterations (9) (20).

1.4 Normal fetal oxygenation

In uterine life, the oxygen tension and drive for effective fetal gas exchange is quite different from the adult population. Sir Joseph Barcroft summarized this in 1946 with the phrase "Mount Everest in utero", comparing the partial pressure of oxygen in the fetus with what we find in humans at the top of Mount Everest (17) (21). However, this does not mean that the fetus spends nine months in lack of adequate oxygenation. Normal fetal physiology involves several adaptive mechanisms, many similar to those known from acclimatization to high altitude. These adaptive mechanisms permit the fetus to achieve a level of oxygen consumption similar to extra uterine life, in fact exceeding its needs under normal conditions.

An important mechanism for sustaining adequate fetal oxygenation is the maintenance of high blood flow rates in fetal tissue. This is a result of the high fetal cardiac output, mainly due to the rapid fetal heart rate (see below). An optimized gas exchange across a large respiratory surface, i.e. the placenta, is also significant, as well as improved oxygen transport by

hematologic adaptations. Such hematologic adaptations include high fetal hemoglobin concentrations, increasing oxygen binding capacity, and a shift of the hemoglobin dissociation curve to the left (as opposed to the right-ward shift seen in adults exposed to high altitudes), resulting in enhanced oxygen affinity in the blood. The combined increase in both capacity and affinity provides the fetus with high blood oxygen stores, something that may defer anaerobiosis in periods of reduced oxygen supply. To some degree this will also compensate for the slight impaired tissue oxygenation resulting from the shift of the hemoglobin dissociation curve.

In order to ensure sufficient oxygenation of vital fetal organs, there are also metabolic adjustments in the tissues leading to reduced oxygen demands. The metabolic rate of the immature fetal brain is particularly low compared to adult tissues. This is partly due to the lower cell membrane permeability resulting in delayed depolarization, but is also caused by reduced release of excitatory amino acids from nerve terminals (17) (22).

Another significant contribution to adequate fetal oxygenation in utero, as well as after birth, is the precise and complex regulation of the above mentioned FHR. Chemoreceptors in the aorta and the carotid artery monitor changes in the circulating concentration of oxygen, carbon dioxide, and hydrogen ions. Any changes in arterial pressure are registered by baroreceptors in several of the large systemic arteries in thorax and the neck, the aortic arch and the carotid artery in particular. Together with medullary cardiorespiratory centers, these receptors feed the autonomic nervous system with information about the body's circulatory and respiratory state, helping to adjust the FHR to the body's need.

1.5 More about the fetal heart rate

As mentioned above, the FHR is regulated by the autonomic nervous system. The sinoatrial and atrial ventricular nodes are the main sites for impulse generation and conduction in the heart, and with advancing gestation, the FHR decreases due to increasing parasympathetic vagal nerve input to these nodes. The sympathetic nervous system innervates the fetal heart through nerves distributed throughout the myocardium, thus increasing the FHR. Catecholamines, like adrenalin and noradrenalin, from the adrenal gland execute the same stimulating effect. A rapid (30-180 seconds) rise or fall of the FHR, however, is usually caused by change of vagal input to the heart rather than the effect of increased sympathetic nerve stimuli, as the latter is a slower mode of altering the FHR.

1.6 Fetal surveillance during labor

During labor, the fetus experiences episodes of reduced oxygen supply and even lower oxygen tension than the conditions in utero. Although usually completely normal, this may in some cases create potentially harmful situations for the baby. In order to reveal these and other possible complications, it has long been routine practice in developed countries to monitor the FHR during labor (9) (14). Despite several limitations as a method for fetal surveillance, the FHR provides valuable information about the fetal state and how it manages the process of labor.

The first report of hearing the fetal heartbeat in utero occurred in the middle of the 17^{th} or 18^{th} century. More than 100 years later, DeKergeradee was the first to suggest that listening to the

fetal heart beat might be of clinical utility. During the 20th century, intermittent auscultation of the fetal heart became conventional during hospital deliveries, and from the 1960s and 70s, cardiotocography (CTG), the recording of the FHR together with the uterine contractions, has been in widespread use (9).

The interpretation of CTG is based on guidelines from the International Federation of Gynecology and Obstetrics (FIGO), and consists of the evaluation of baseline FHR, the variability in the baseline, and the presence of decelerations and accelerations. The CTG recording is according to these parameters classified as normal, deviating, pathological or preterminal (15).

Later efforts to further improve fetal surveillance during labor have led to the introduction of automatic ST-analysis of the fetal electrocardiography (STAN) (figure 5). STAN provides continuous information on the ability of the fetal heart muscle to respond to the stress of labor. An elevation of the ST segment and T wave, quantified by the ratio between the T wave and QRS amplitudes (T/QRS), identifies fetal heart muscle responding to hypoxia by switching to anaerobic metabolism. This leads to utilization of glycogen stored in the heart and a subsequent release of potassium ions that in turn alters the ST segment and the T wave in fetal ECG. An ST segment depression may indicate a situation where the heart is not fully able to respond to the hypoxic insult (14) (23).

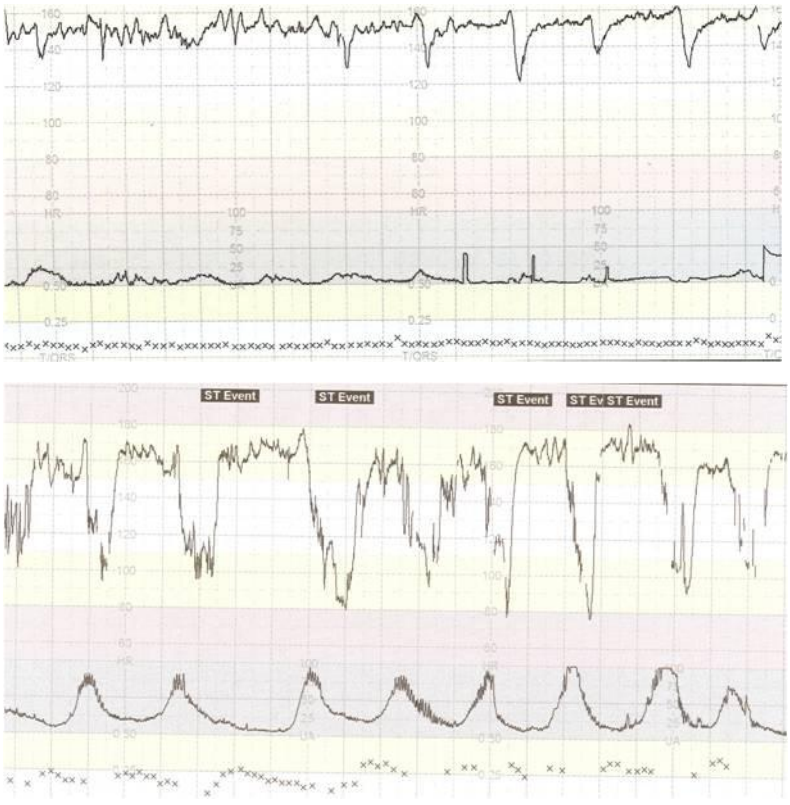

Figure 5 Above: Normal CTG recording with stable T/QRS © Neoventa Medical (14)

Below: Pathological CTG recording with ST-events (significant changes of the ST segment from the T/QRS baseline) © Neoventa Medical (14)

2 The fetal physiological responses to acute perinatal hypoxia

2.1 Responses of the healthy term fetus to hypoxia during labor

2.1.1 Reduced oxygen supply during normal, uncomplicated labor (hypoxemia to moderate hypoxia)

Even during the normal, uncomplicated labor, the fetus undergoes a number of repeated periods with reduced oxygen supply. This is caused by the uterine contractions and the consequent reduction in uterine and placental blood flow. However, these brief hypoxic episodes are usually well tolerated, as the fetus has several defense mechanisms helping it to cope with the impaired oxygenation (20) (24).

The initial fetal response to an episode of low oxygen supply is a slowing of the FHR. This bradycardia is a vagally mediated chemoreceptor response, seen as decelerations on the CTG. It is a direct effect of the uterine contractions during labor and completely normal, particularly during the second stage. The bradycardia is considered as a protective reaction as it helps to reduce myocardial work and thereby diminishes the heart's oxygen requirements.

Though the FHR decreases during hypoxic episodes, there is a rise in fetal blood pressure. This is due to peripheral vasoconstriction, another important fetal defense mechanism, as it shunts blood from peripheral tissues to central organs like the brain, heart and adrenals. Through this the fetus maintains adequate oxygen tension in the most vulnerable organs enabling them to continue to work aerobically, while the more resistant peripheral tissues are driven into anaerobic metabolism. The shunting is a result of the release of catecholamines like adrenalin and noradrenalin, induced by the physiological stress of labor, and further triggered by the hypoxia. After a while however, the increasing release of catecholamines gradually makes FHR return back to baseline, or even drives it into a slight tachycardia. This allows the cardiac output to contribute to the maintenance of the blood pressure, and the peripheral vasoconstriction is consequently reduced, permitting a greater perfusion also of peripheral organs (14) (17) (21) (22) (24) (25).

The term fetus also has an increased anaerobic capacity. This is partly because of the elevated storages of glycogen in the liver and myocardium, but is in addition facilitated by the improved clearance of lactate from the fetal circulation, mainly by the placenta. The latter is the result of a continuous wash-out of acid metabolites by the well maintained cardiac function. However, the fetal tolerance to hypoxia is not only a result of increased anaerobic capacity, but also a consequence of mechanisms maintaining aerobic metabolism as long as possible, despite limited oxygen supplies. This is achieved by a reduction in the metabolic rate, not only due to the reduced myocardial work as mentioned above, but to an overall decrease in fetal activity, including less body and eye movements, as well as a fall in body temperature. The reduced metabolic rate also contributes to an improved fetal gas exchange and diffusion of oxygen into the tissues (14) (17) (22).

The periods of reduced placental blood flow related to the contractions during labor are associated with a fall in pH, BE and oxygen tension, and a rise in the concentration of carbon dioxide in the fetal circulation. The high levels of carbon dioxide produced by the aerobic cellular respiration accumulate in the blood due to the reduced placental clearance, creating a respiratory acidosis. However, this acidosis dissolves quickly after birth with the baby's first breaths, and is not associated with neonatal complications (9) (12) (14) (20).

2.1.2 Development of severe hypoxia and asphyxia

Although the healthy term fetus has an outstanding ability to adapt to sudden episodes of reduced oxygen supply during labor, situations that exceed the fetal capacity sometimes arise. There are several potential causes of severe hypoxic episodes during labor. Maternal hypoxemia or hypotension due to acute blood loss, regional anesthesia or systemic illness is rather rare. More common is reduced uterine blood flow seen in cases of excessive uterine contractions often following oxytocin hyper stimulation or prolonged second stage, or umbilical cord occlusion (9) (20).

i. When does hypoxia become dangerous for the fetus?

Fetal asphyxia arises when anaerobic metabolism, due to lack of adequate tissue oxygenation, extends to central organs, leading to accumulation of lactate and hydrogen ions. It is the subsequent metabolic acidosis, not the respiratory acidosis mentioned above, that is associated with increased risk of adverse effects and neonatal complications (6). However, it is noteworthy that fetal asphyxia not necessarily is synonymous with *severe* metabolic acidosis and the possible development of HIE (14). Some degree of metabolic acidosis develops in almost every labor, but it is not considered clinically important until umbilical artery pH falls below 7.0 and BE falls below-12. It is only at these levels the incidence of adverse clinical events starts to rise significantly (4) (9) (12) (16) (20) (24). Several studies have demonstrated an incidence of umbilical artery BE <-12 to 2% of births in a normal obstetric population (6) (9). In a review in American Journal of Obstetrics and Gynecology from 2008 it was reported an incidence of umbilical artery pH <7.0 at birth of 3.7/1000 (range 2.9-8.3/1000) (4). Of the infants with this degree of acidosis, 23.1% died or suffered from associated neonatal neurologic morbidities, like low Apgar scores or early seizures. The great majority of the newborns were, however, perfectly healthy and suffered no short or long term injuries. This is important to notice, as most neonates with umbilical cord blood samples showing low pH or even severe metabolic acidosis during labor, never develop any signs of neurological illness or other adverse effects (2) (4) (6).

ii. Hypoxia leading to metabolic acidosis and hypotension

As described in section 2.1.1, the initial fetal response to sudden hypoxic episodes is characterized by a rapid vagally mediated bradycardia with decelerations on the CTG, eventually followed by a return of FHR to baseline. The reduction in FHR is mainly related to the severity of the hypoxia (21) (24).

A series of bradycardic events with variable decelerations on CTG, though usually well tolerated by healthy term babies, may ultimately lead to severe metabolic acidosis and hypotension if the insults trigging these events are repeated sufficiently frequent and are of a

long enough duration. This has been demonstrated in experiments on near term fetal sheep, the main source of most of our knowledge of fetal physiology during labor. In a study by Gunn and coworkers (21) (24) the effect of 1 minute umbilical cord occlusions repeated every 5 minutes, consistent with the contractions in the latent phase of first stage labor, was examined on fetal sheep FHR, blood pressure and blood pH. This was compared to the effect of 1 minute occlusion every 2.5 minutes, similar with the fetal strain seen in the active part of the first stage and in the second stage of labor. The fetal sheep adapted fully to the low frequency of repeated hypoxic episodes, but the more rapid occlusions resulted in development of progressive metabolic acidosis and hypotension. The frequent occlusions were in this study associated with focal neuronal damage, while no injury was seen after less frequent occlusions.

The same results are seen when exposing fetal sheep to one prolonged episode of severe hypoxia, instead of repeated insults of shorter duration. In one such study, Gunn and coworkers examined the effect of complete umbilical cord occlusion for 15 minutes on near term fetal sheep (26). The first minutes of the occlusion, the fetuses responded normally, with an initial bradycardia and hypertensive response, followed by an increase in FHR. At the same time there was a considerable decrease in fetal femoral artery blood flow, reflecting a centralization of fetal blood flow. No significant acidosis was detected at this point. After 4 minutes, however, the femoral artery blood flow started to increase, followed after a few minutes by a progressive drop in FHR and fetal blood pressure, persisting throughout the hypoxic period. At the end of the 15 minute occlusion, severe metabolic acidosis was present.

Development of metabolic acidosis

On average will term infants have a mean umbilical artery pH of 7.27 +/- 0.07 and BE of -2.7 +/- 2.8 mmol/L (4). A mild acidosis develops in nearly all labors. Experiments have estimated the total reduction of BE in the uncomplicated labor to be approximately 3 mmol/L (9) (20). BE does not change significantly during the latent part of the first phase of labor, but the normal physiological stress of the active phase may decrease fetal BE by 1 mmol/L per 3 hours. In the second stage, BE normally drops 1 mmol/L per hour.

Ross and coworkers have studied the effect of increasing frequencies of complete umbilical cord occlusions, i.e. a severe hypoxic event, on fetal bovine acid base status, and the rate of BE decrease in response to the altered frequencies of the occlusions, as well as the recovery of BE afterwards (9). They found that 1 minute of severe umbilical cord occlusion decreased fetal BE by approximately 0.5 mmol/L, regardless of the frequency of the occlusion. It was the total time of occlusion versus time of recovery that determined the degree of metabolic acidosis. This is thought to be due to insufficient restoration of fetal cellular metabolism and replenishment of glycogen stores between the periods of reduced oxygen supply (24). The study also showed that BE normalizes with approximately 0.1 mmol/L per minute after an umbilical cord occlusion or another hypoxic event causing bradycardia. This is consistent with the findings from Gunn and coworkers (21) (24), as a frequency of 1 minute complete umbilical cord occlusion every 5 minutes allows for adequate time for recovery, while more frequent occlusions, as well as prolonged hypoxic episodes, may result in rapid development of metabolic acidosis.

Development of hypotension

As demonstrated in the studies by Gunn and coworkers presented above, severely asphyxiated fetuses develop sustained bradycardia and profound hypotension when exposed to repeated or

prolonged episodes of severely reduced oxygen supply (21) (22) (24) (26). This phenomenon is known as fetal cardiovascular decompensation, and is thought to be a direct effect of severe acidosis and hypoxia on the myocardial cells. This is opposed to the slight interhypoxic tachycardia seen in fetuses able to cope with episodes of poor oxygen tension. The depletion of glycogen, creatine phosphate and ATP stores in the myocardium reduces the duration of the action potentials and thereby disrupts myocardial cell function reversibly (6) (21). The subsequent reduction in FHR and cardiac output may be reinforced by a depletion of the catecholamines in blood as the acidosis progresses. This leads to a failure in maintaining the fetal blood pressure at adequate levels, and hypotension follows (9).

2.2 Responses to hypoxia during labor in fetuses afflicted by prenatal placental insufficiency

Until now, the focus in the present thesis has been on the physiological reactions of the *healthy* term fetus exposed to sudden hypoxic episodes during labor. Do the previously described mechanisms also apply to fetuses that in the prenatal period have been exposed to, and potentially distressed by placental insufficiency (PI)? PI is a diverse condition and may be defined in many ways. In experimental settings and research it is often referred to as reduced capacity to transfer or exchange nutrients and/or oxygen between the maternal and fetal circulations, whereas in a clinical setting the definition of PI is based on size and growth of the fetus, often combined with fetal cardiovascular parameters assessed by Doppler techniques. The causes of PI are multiple. These include "poor placentation", i.e. insufficient transformation of the uterine spiral arteries that supply maternal blood to the intervillous space (maternal side) of the placental membrane. Vascular malformations on fetal side, thrombosis, placental bleedings, infections, and possibly immunological processes may also cause PI. Clinical risk factors for this condition include maternal hypertension, diabetes, infections, autoimmune diseases like systemic lupus erythematosus (SLE), smoking and prothrombotic states. From clinical experience it is recognized that a placental restricted fetus is more likely to be compromised by labor related strain well tolerated by a healthy fetus. Prenatal hypoxia is associated with an increased incidence of fetal asphyxia with severe metabolic acidosis during labor and possible neonatal neurologic morbidity and mortality (6) (21) (24) (25) (26).

2.2.1 Fetal adaptation to chronic placental insufficiency

PI has, beside endocrinological dysfunction, two main consequences for the fetus. Firstly, transfer of energy and specific nutrients is impaired. Secondly, a sustained decrease in fetal oxygen partial pressure and a mild increase in the partial pressure of carbon dioxide may occur. The fetal pH, however, does not change (27). The alterations leads to compromised fetal nutritional status, including reduced glycogen stores, and increased fetal levels of circulatory catecholamines and cortisol in the blood (see below). The combined results of PI are cardiovascular adaptations that spare cerebral, cardiac and adrenal functions on the cost of other organs, with a reduction in fetal body growth (27).

The features of PI are based on both experimental studies and clinical experience. Several animal studies have shown significantly higher plasma noradrenalin concentrations in near term fetal sheep exposed to PI compared to normoxic fetuses. The relationship between fetal

plasma noradrenalin and the partial pressure of oxygen is found to be inversely proportional. The source of this increase in noradrenalin concentration is unclear, but studies suggest that it is a consequence of increased fetal sympathoadrenal activity rather than reduced clearance from the blood. One possibility is that low oxygen concentrations acts via chemoreflex mechanisms to stimulate catecholamine secretion from sympathetic neurons. The contribution of the carotid chemoreflex to the cardiovascular and catecholamine responses to prolonged periods of hypoxia during late gestation is, however, not well understood in the chronically hypoxic fetus (27).

In experimentally placental restricted fetal sheep, the arterial pressure is maintained at the same levels as in fetuses with adequate placental function**.** The effect of PI on the FHR, however, varies according to the method used to induce the insufficiency. Pulgar and coworkers report a development of significant tachycardia when a chronic global (fetal *and* maternal) hypoxia is induced experimentally by maternal intratracheal administration of nitrogen gas for 5 days (25). On the other hand, placental restriction induced by a surgical reduction of placental volume, creating only fetal hypoxia, leads to a lower FHR in the hypoxic fetus than in the normoxic controls. This has been demonstrated by experiments from Danielson and coworkers (27).

Another study from Danielson and coworker (27), found that the activation of peripheral vascular alpha-adrenergic receptors plays a more important role in the maintenance of blood pressure in the chronically hypoxic sheep fetus than it does in normoxic controls. This was demonstrated by a greater and more sustained hypotensive response to alpha-adrenergic blockage in a group of fetuses with induced placental insufficiency compared to controls with a normally functioning placenta. It is thought that noradrenalin acts on the alpha-adrenergic receptors, thereby increasing the peripheral vascular tone and subsequently the blood pressure. This would explain the direct relationship between the hypotensive response to alpha-adrenergic blockade and the degree of fetal hypoxia that was seen in the same study. Danielson and coworkers have also reported a consecutive activation of the central nervous system and the renin angiotensin pathway in chronically hypoxic fetuses, maintaining the basal blood pressure in the last weeks of the pregnancy. Infusion of an angiotensin-converting enzyme (ACE)-inhibitor in near term fetal sheep resulted in a greater hypotensive response in the fetuses exposed to PI than in the healthy control group (27).

The resulting increased peripheral sympathetic vascular tone is not only important for maintaining blood pressure, but plays a crucial role in redistributing cardiac output in placental restricted fetuses. As mentioned above, this sparing effects help to maintain fetal brain growth and function, despite the reduction in overall fetal body growth.

2.2.2 Placental restricted fetuses' response to acute hypoxia during labor

Several studies have explored the clinical experience of a reduced tolerance to acute hypoxic episodes during labor in placental restricted fetuses. The results are, however, somewhat conflicting.

In a review by Gunn and coworkers, the response of chronically hypoxic fetuses to 1 minute umbilical cord occlusions repeated every 5 minutes was reported. This rate is, as discussed above, usually well tolerated by normoxic fetuses (24). However, the fetuses with preexisting PI experienced severe, progressive metabolic acidosis (pH 7.07 +/- 0.14, vs. 7.34 +/.0.07 in

the normoxic group) and hypotension after 4 hours of occlusion. These results are consistent with the clinical experience that preexisting hypoxic fetuses are vulnerable even to the relatively infrequent episodes of further reduced oxygen supply in early labor.

Shah and Perlman reported the neonatal characteristics and adverse outcome rates of infants with HIE following 1) prolonged partial asphyxia, 2) acute near-total perinatal asphyxia, and 3) the two combined (28). Their results showed that the prolonged partial group demonstrated the least severe incidence of neonatal illness, i.e. death or severe disability (61%), the group with acute near-total asphyxia had intermediate severity (67%) and the combined group had the highest incidence of severe illness (79%). Similar results were found in the estimation of adverse long-term outcomes.

Pulgar and coworkers studied the effect of mild prolonged hypoxemia on the fetal sheep cardiovascular and neural responses to 5 minutes umbilical cord occlusions repeated every 30 minutes (25). They also considered the degree of neuronal damage following the occlusions. Their results showed that although the usual initial vagally mediated bradycardic response was present to the same degree in both groups, the preexisting hypoxic fetuses had a greater fall in FHR compared to previously normoxic fetuses during the last minute of the second, third and fourth occlusions. The chronically hypoxic fetuses also demonstrated a more severe terminal hypotension in the final occlusion. A temporary hypertensive response to the umbilical cord occlusions was seen at the same time in both groups. However, it was significantly greater in the chronically hypoxic group.

Preexisting hypoxia was in the same study also found to enhance the depression and alter the pattern of the fetal electrocorticography (ECoG) during and after occlusions, indicating that chronic hypoxia alters the brain response to super-imposed acute hypoxic events and is associated with an increased neuronal loss. The study concluded that preexisting mild hypoxia has a detrimental effect on the cardiovascular and neural responses to repeated umbilical cord occlusion, and is associated with neuronal loss (25).

In one of the previously referred studies by Gunn and coworkers, they examined how chronically prenatal hypoxia would alter hemodynamic responses and changes in T/QRS ratio and ST waveform shape on STAN during acute severe asphyxia in near-term fetal sheep (26). They found that a severe asphyxic episode was induced by a complete occlusion of the umbilical cord for 15 minutes, creating fetal distress comparable to abruptio placenta or umbilical cord prolapse. In agreement with the results obtained by Pulgar and coworkers (25), they also reported a similar initial bradycardia in both the preexisting hypoxic group and the controls when exposed to an acute severe hypoxic event, followed by a brief increase in FHR and blood pressure. However, the reaction was, also consistent with the findings by Pulgar and coworkers, significantly greater and of longer duration in the preexisting hypoxic fetuses. Furthermore, the fetuses with preinduced hypoxia showed in addition a quicker initial fall in femoral blood flow and vascular conductance during the occlusion, i.e. a more rapid centralization of circulation (26). Both the increased rise in FHR and blood pressure, and the more effective centralization of circulation, is thought to be due to the enhanced fetal sympathetic responses together with the increased circulating blood levels of catecholamines. The enhanced sensitivity to noradrenalin in the peripheral vasculature in chronically hypoxic fetuses is also contributing, as well as an increased sensitivity of the alpha-adrenergic receptor-mediated peripheral chemoreflex. The increased peripheral vascular tone was, however, only sustained for approximately 4 minutes of severe hypoxia. The subsequent loss of peripheral vasoconstriction was associated with a further progressive fall in FHR, and later with hypotension. This is probably a result of the same pathophysiologic mechanisms

described for healthy term babies, i.e. vascular decompensation; depletion of catecholamines and impaired cardiac functions due to direct cardiomyocyte injury. The effect is further triggered in the chronic hypoxic fetuses due to the great degree of posthypoxic vasodilatation, which is a normal physiologic response seen in all tissues after a period of reduced oxygen supply.

The umbilical cord occlusions were associated with a significant rise in T/QRS ratio in both groups. The presence of elevation of the ST segment of the fetal ECG, typically measured relative to the QRS complex (the T/QRS ratio), is a result of anaerobe metabolism in myocytes and hence an indication of myocardial hypoxia. The preexisting hypoxic fetuses had a significant delay in this rise, and a slower rate of fall. This suggests that chronic hypoxia alters myocardial cellular responses during acute asphyxic events. ST waveform elevation is thought to reflect anaerobic cardiac metabolism, which means that the subsequent fall in ST waveform height reflects depletion of cardiac glycogen, the major substrate for anaerobic metabolism. The sustained elevation of the ST wave in the preexisting hypoxia group and the associated higher and more prolonged rise in FHR, suggests that preexisting hypoxia may improve the fetus' ability to maintain anaerobic cardiac metabolism. In this study by Gunn and coworkers, chronically prenatal hypoxia did not affect the timing of hypotension or metabolic acidosis during occlusion (26).

The results from the presented studies indicate that chronic fetal compromise may have both positive and negative impact on various fetal responses to acute hypoxic episodes during labor. Chronically hypoxic fetal sheep exposed to a sudden reduction in oxygen supply exhibited more pronounced centralization of circulation, associated with greater increases in plasma catecholamine concentrations. They also showed an enhanced sympathetic response and improved abilities to maintain anaerobic cardiac metabolism during prolonged severe hypoxia. In contrast, when exposed to repeated umbilical cord occlusions, the preexisting hypoxic fetuses developed severe hypotension and metabolic acidosis much more rapidly than the normoxic control group. The terminal hypotension was also greater and more severe. Preexisting hypoxia was also associated with an increased neural loss and poorer outcome, both in the neonatal period and long-term.

These apparently ambiguous results demonstrate that fetuses exposed to PI in the prenatal period have a very complex physiological response to acutely reduced oxygen supply. This may explain why the subject still is poorly understood, in spite of considerable research. In addition to complex pathophysiology, differences in methods used in the experimental studies (i.e. repeated acute hypoxic events vs. prolonged periods of induced hypoxia) may account for some of the divergent findings. Gunn and coworkers states in the review referred to in the beginning of this section that "experimental studies seem to suggest improved or greater cardiovascular adaptation to moderate induced hypoxemia, i.e. greater centralization and noradrenalin concentrations, but these studies used moderate hypoxia rather than labor like hypoxia-ischemia" (24). As shown throughout the present thesis, fetuses may cope very well with both chronic and acute reduced oxygen supply due to their extensive defense mechanisms, but at some point the stress becomes too severe, the mechanisms fail, decompensation begins, and the fetus is in danger of being adversely affected.

2.3 Current Norwegian guidelines for fetal monitoring during labor

Although the fetal physiological responses to perinatal hypoxia are extensive, it is often difficult to clinically assess when the fetus becomes compromised during labor. The Norwegian "veileder i fødselshjelp" from 2008 provides guidelines for fetal monitoring during labor in order to help detect endangered fetuses, and thereby "decrease the perinatal mortality and the neurological long-term morbidity related to intrapartal fetal asphyxia" (15). The guide recommends the following steps based on systematic reviews from The Cochrane Library, a systematic search in acknowledged medical databases, and the British guidelines on the use of electronic fetal monitoring from 2001:

1. Healthy women with uncomplicated pregnancies should be offered *intermittent auscultation* with a Pinard stethoscope, Doppler or external CTG during labor.
2. If the intermittent auscultation reveals a deviant FHR or the labor originally is considered as risky, *continuous electronic fetal monitoring with externally (Doppler) or internally (scalp electrode) recorded CTG* is indicated.
3. If the CTG is regarded as deviating or pathological, it may be a sign of possible development of fetal asphyxia. In these situations, additional monitoring can help identify the fetuses in actual need of intervention to prevent them from further progression and worsening of the asphyxia and metabolic acidosis, and thereby avoid potential fetal injury.
    a. *Analysis of fetal blood gas from the fetal scalp,* including pH, base excess and lactate, may provide useful additional information about the fetal condition.
    b. *STAN* combined with the analysis of internally recorded CTG is also an important tool, giving information about the oxygenation of the fetal heart.

    Comparisons between these two methods show good correspondence. This means that if the labor is monitored with CTG-STAN, there is a lesser need for additional scalp blood gas analysis.

4. *Umbilical cord blood gas analysis* is the only way of objectively assess whether the fetus has been exposed to significant hypoxia during labor or not. It may also provide information about the duration and the severity of the hypoxic insult (14).

3 Pathophysiological mechanisms behind HIE

The studies reviewed in the present thesis highlight two main pathophysiological responses following sudden episodes of reduced oxygen supply during labor as potentially detrimental to the fetus; *metabolic acidosis* and *hypotension*. In the following section, the relationship between these phenomenons and the development of HIE will be further elaborated. The general effects of low oxygen concentration in a tissue and how hypoxia may cause cell damage and death was presented early in the present thesis. With respect to the events responsible for development of HIE, the effect of hypoxia on cerebral blood flow and neuronal cell death is particularly interesting.

3.1 Development of brain ischemia

The depletion of ATP and primary cellular energy failure due to hypoxia is known to precede the initiation of cascade reactions leading to cell dysfunction and death. This is also the case in the pathogenesis of hypoxic ischemic brain damage. As described in the introduction, metabolic acidosis itself may cause neuronal injury and death, particularly due to the excessive release of glutamate, which creates an energy-requiring excitotoxic cascade leading to neuronal degeneration (see below) (3) (4). However, in addition to a direct harmful effect on neurons, metabolic acidosis may cause fetal hypotension resulting in ischemia, hence the term hypoxic-*ischemic* encephalopathy. The failure of the fetus to maintain basal blood pressure is due to hypoxic and acidotic disturbances of vascular vasomotor regulation and impairment of myocardial function resulting in reduced cerebral blood flow (28). This effect is further enhanced by the excessive lactate production that impairs auto regulation of the cerebral blood flow, thereby eliminating an important fetal defense mechanism that normally maintains stable cerebral blood flow regardless of variations of systemic arterial pressure (3) (29). The oxygen concentration in fetal blood is already poor under circumstances with hypoxia-induced metabolic acidosis, and the fall in cerebral blood flow will decrease the oxygen supply to the brain even further, reinforcing the hypoxic cell damage. The hypoperfusion of the brain tissue also leads to a reduced nutrient supply. The lack of glucose is particularly detrimental, as glucose is fundamental for brain energy metabolism (29). Experimental studies confirm that hypotension and consequent failure of brain perfusion is one of the major factors associated with neural injury in HIE (21) (24) (25).

However, it is not only the initial hypoxic ischemic phase with primary energy failure due to ATP depletion that may cause cerebral damage. A subsequent period of reperfusion often deteriorates the brain metabolism further by increasing the oxidative stress damage (3). This second phase of cerebral injury arises 2-6 hours after the primary insult, as the restoration of cerebral blood flow increases oxygen tension in the tissues and leads to generation of oxygen free radicals through activation of xanthine oxidase, and cyclooxygenase enzymes and “spill over” of superoxide from mitochondria. There is also an increase in nitric oxide production due to neuronal and endothelial nitric oxide syntethase activation. The increased levels of free radicals are responsible for oxidative cellular damage, leading to secondary failure of production of ATP due to mitochondrial dysfunction (16) (29).

3.2 Development of brain inflammation

Another significant component in the progression of fetal brain injury in HIE pathophysiology, is the cerebral inflammatory response following the hypoxic ischemic insults. This inflammation is both generated by activation of local inflammatory cells in the affected brain tissue, and by recruitment of circulating immune cells (16) (30).

3.3 Brain damage due to neuronal cell death

Before describing neuronal cell death during HIE, a short introduction to the various forms of cell death will be given: There are two main forms of cell death, necrosis and apoptosis. Necrosis is a passive, ATP-independent process characterized by cellular swelling, mitochondrial damage, and chromatin condensation into irregular clumps. Eventually necrosis leads to cytotoxic edema and cell lysis with cytokine release and activation of inflammatory reactions. Apoptosis, on the other hand, is an active, ATP-dependent process of programmed cell death. It is characterized by cell shrinkage, chromatin condensation and DNA fragmentation, and no inflammatory responses are triggered (16) (29). It should be noted, however, that ischemic necrotic cell death often is preceded by apoptosis. Thus, cell death may manifest along a continuous spectrum with apoptosis at one end and necrosis at the other. Activation of different signaling pathways will result in a great diversity of cell death phenotypes with various combinations of structural and biochemical features, and it is therefore not always easy to categorize the type of death going on in a cell or a tissue (31).

The mechanisms behind neuronal cell death following hypoxic ischemic insults depend mainly on the severity of the insult and the maturation of the brain. The energy failure caused by the hypoxia prevents effective completion of the ATP-dependent process of apoptosis with a consequent displacement of cell death towards necrosis (31). The initial hypoxic ischemic phase of cerebral damage is therefore dominated by necrosis in the ischemic areas. In reperfused areas, however, cell death mainly consists of apoptosis, extending beyond the parts of the brain directly affected by the ischemia (3) (29). In cases of moderate asphyxia, cell damage is primarily apoptotic, while necrosis dominates in severe asphyxic insults (16) (29). The form of cell death that occurs also depends on the subtype of excitatory glutamate receptors that are activated, as glutamate-induced energy requiring excitotoxic neurodegeneration is important in the pathophysiology of HIE (3) (31). Excitotoxic cell injury primarily induces necrosis, but it may also trigger cytological features more like apoptosis. Glutamate receptors play a particular important role in the fetal brain in the perinatal period, making the fetal neurons more vulnerable to excitotoxicity (3) (31). As previously described in the present thesis, extracellular glutamate concentrations increase following hypoxic ischemic insults, and specific glutamate receptor channel blockers have experimentally been shown to limit brain injury in HIE (3) (31).

The described diversity of cell death seen following perinatal hypoxic ischemic episodes is one of the fundamental differences of injury-associated neuronal death in the immature and mature central nervous system, manifesting more often in the immature fetal brain (31).

3.4 Localization of brain damage

Some areas of the brain are more prone to cellular damage following perinatal hypoxic ischemic episodes than others. This is also the case for the different cerebral cell types, as the neurons are the most vulnerable, followed by oligodendrocytes and astrocytes, while microglia cells are less susceptible to hypoxic ischemic injury (3). As a consequence, selective neuronal loss is one of the most common brain injuries associated with hypoxic ischemic insults in the term fetus.

The localization of cerebral damage depends on the gestational age of the fetus, involving primarily grey matter in term infants and white matter in the premature. This is related to maturational events connected to development of the cerebral vascular system. As the focus of the present thesis is the pathophysiology of term infants, there will be no further details given related to the premature neonate and injuries in the white matter (29).

The site of cerebral injury is also related to the blood flow to the various brain regions and to their metabolic activity. For instance, the watershed areas in the periphery of the cerebral vascular supply are the most susceptible to reduced cerebral perfusion pressure and ischemia (29) (figure 6). Furthermore are the fetal cerebral regions with a high degree of glutamate receptor expression, like the hippocampus, cerebral cortex and deep nuclear structures, particularly vulnerability to perinatal hypoxic ischemic injury (31).

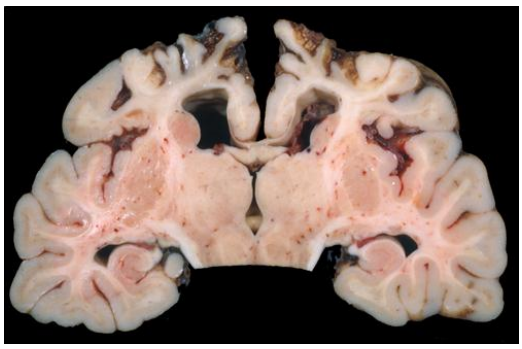

Figure 6 Cerebral atrophy in the watershed areas between the anterior and middle cerebral arteries, from http://neuropathology-web.org/chapter3

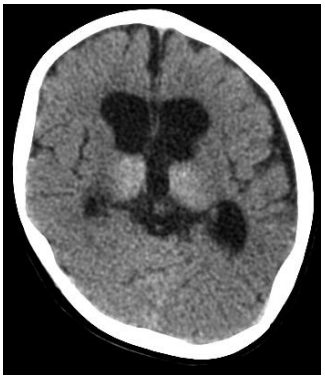

Figure 7 Thalamic affection following perinatal HIE, from http://neuropathology-web.org/chapter3

As expected will the intensity, timing and duration of the hypoxic-ischemic episode be important for determining the extension and severity of the brain damage. The more intense and long-lasting insult, the greater the number of neuronal and glial cells will die (29). Brain damage following acute, near total asphyxic insults is usually associated with a central pattern of focal neuronal injury, affecting mainly the thalamus, basal ganglia and the brain stem nuclei (figure 7). A cortical sparing is often seen in these situations. In contrast will prolonged, partial asphyxic insults characteristic for prenatal placental insufficiency, predominantly lead to cerebral cortical injury in the watershed areas and in parasagittal regions, with relative sparing of the central grey matter. Prolongation of either type of asphyxic insult, however, will result in more global damage (1) (24) (28).

3.5 The relationship between fetal asphyxia, HIE and CP

There are several different terms in clinical use related to fetal asphyxia and its potential sequelaes. Because of the great overlapping in occurrence between these conditions, they are often used synonymously. This is, however, incorrect, as each term stands for its own physiological or clinical condition with its own consequences, and may or may not follow some of the other conditions related to it. The relationship between the different terms can be illustrated as in figure 8.

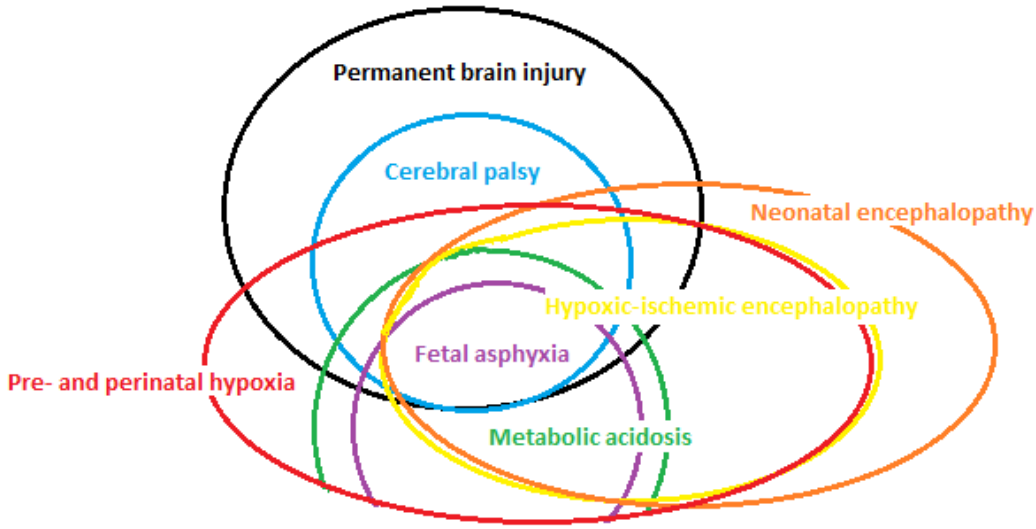

Figure 8 The relationship between different terms related to fetal asphyxia and its potential sequelaes (the proportions are only illustrative)

Although often referred to as "three sides to the same story" it is often difficult to prove the causal connection between intrapartal events like fetal asphyxia, neonatal affection like HIE, and long neurological adverse effects like CP. For instance will only a few of the babies with detected fetal asphyxia, develop neonatal HIE. Furthermore, not all of the HIE-babies will have indications of fetal compromise during labor. In some children who develop CP, fetal surveillance during labor did suggests a severe hypoxic event, but they never showed any sign of fetal injury in the neonatal period. And so on. Only in a small group of patients may an entire causal chain be established; fetal asphyxia with metabolic acidosis during labor, diagnosed HIE in the neonatal period and development of CP in the following years.

The complex interconnection between these conditions, as shown in figure 8, might be one of the reasons why it is still debated to what extent CP is a direct consequence of fetal asphyxia and/or HIE. When CTG was introduced in the late 60s and early 70s, there were great expectations of a significant reduction in the number of children developing CP, as perinatal causes was considered to account for the overwhelming majority of the cases (9). The CP rate has, however, remained almost unchanged the last 40-50 years, despite the marked changes in obstetrical practice aimed at reducing the risk of asphyxia, including a 5 fold increase in cesarean deliveries (4) (16) (32). This observation, together with the above mentioned fact that evidence of severe perinatal asphyxia is often absent in infants with neonatal encephalopathy, and conversely, that many infants who do have signs of fetal distress and

asphyxia do not develop neurological sequelae, have later led to the understanding that the main causes of such conditions occur before birth. The higher incidence of maternal illness, prenatal complications, and adverse social factors observed in infants with neonatal encephalopathy supports this view. It is thought that many neurological sequelae once attributed to perinatal asphyxia in reality are a manifestation of a fetal compromise during the prenatal period, and that the difficulties often documented in the course of labor are secondary to this prenatal deprivation (7) (10).

Resent research, however, has challenged this comprehension, suggesting that hypoxic-ischemic and other perinatal events do indeed play a significant role in the causation of cerebral palsy and other long term neurological sequelae. Cowan and coworkers have published several original studies and reviews supporting this view. A cohort of 351 term infants with neonatal encephalopathy, early seizures, or both, was analyzed with aim to distinguish between lesions acquired prenatally and those that were developed in the perinatal and early post-partum period (10). Their results showed that more than 90% of term infants with neonatal encephalopathy, seizures, or both, but without specific syndromes or major congenital defects, had evidence of perinatally acquired insults. There was a very low rate of established brain injury acquired before birth (10). A review from the same group from 2009, asserts that perinatal difficulties are responsible for 35% of term-born infants with both neonatal encephalopathy, and of those who develop CP (7). Cowan and coworkers claim that their results strongly suggest that events in the immediate perinatal period are most important in neonatal brain injury, and that identifying prenatal factors that may increase the risk of intolerance of stress in labor does not prove that the brain damage responsible for the adverse long term outcome did not occur in labor (10). This is supported by the widespread observation that babies born by pre labor cesarean section for non-fetal reasons rarely develop an encephalopathy unless they have a specific metabolic or developmental problem (7).

4 Future perspectives and challenges

In the future, it is important to continue to work towards clarifying the relative importance of fetal asphyxia and HIE in the causation of long term neurological sequelae. It is also important to increase our understanding of why some fetuses are more susceptible to deprivation due to acute perinatal hypoxia than others. This knowledge will help us to prioritize resources, and thereby reduce the incidence of devastating conditions like cerebral palsy and other long-term adverse effects. I would like to suggest four possible strategies or challenges that may help reach this goal.

4.1 Identify the fetuses that are particularly vulnerable to hypoxia during labor

Physiological hypoxia is a normal part of fetal life and may play an essential role in normal fetal development. In most cases, healthy fetuses also tolerate acute hypoxia during labor. However, in some cases, fetuses that seemingly are completely healthy suddenly demonstrate reduced tolerance to the stress of labor.

As discussed in section 2.2, fetuses that in the prenatal period are exposed to placental insufficiency are more likely to be impaired by acute severe hypoxic events during labor. These fetuses must, of course, be detected, and precautions taken during labor.

There are surprisingly little evidence of environmental and lifestyle risk factors for HIE. In future studies, it would be important to identify risk factors for reduced tolerance to hypoxia during labor. The risk factors may be related to genetic predisposition to hypoxic-ischemic injury, fetal imprinting due to risk factors of the mother (e.g. smoking, alcohol, obesity, hypertension etc.), or other environmental or lifestyle factors. If fetal tolerance to perinatal hypoxia is related to risk factors of the mother, tests for identification of vulnerable cases might be possible by analysis of maternal plasma or blood cells. Epigenetic changes of stress response genes caused by lifestyle factors are likely candidates.

4.2 Improve fetal monitoring during labor

The difficulties with proving the causal connections between fetal asphyxia, HIE and CP is partly due to the lack of good and accurate fetal surveillance. CTG is the most common obstetrical procedure in developed countries, but there is poor evidence of beneficial effects, both to mother or infant (9). CTG has a sensitivity of 85% with corresponding high negative predicative value in predicting the absence of fetal acidosis. The specificity and the positive predicative value of changes in the FHR pattern, however, are very low (24). Analysis of ST-segment of fetal ECG has improved the specificity.

It is interesting to notice that none of the modes of surveillance used to detect fetuses in danger of developing HIE, is actually monitoring the oxygen conditions in the brain. CTG and STAN only provide information of the heart. The analysis of blood from the fetal scalp during labor indicates the peripheral situation. Both these factors provide information on the overall condition of the fetus, but may indeed be misguiding with respect to the degree of brain

affection. It is a difficult task to unravel the causal pathway between fetal asphyxia, HIE and CP when we have no methods for directly measuring the effect of hypoxia and metabolic acidosis during labor on the fetal brain.

It is hoped that this will be possible in the future, and that there will be fetal brain imaging technologies available, e.g. EEG, functional MRI and other image modalities, to define cerebral metabolic activities, the patterns of brain lesions, and timing of lesion onset.

4.3 Examine the connection between perinatal hypoxia and development of chronic disease later in life

Epidemiological and animal studies show that various stress factors or environmental changes during fetal development can induce phenotypic changes affecting an individual's response to their later environment, and may alter the risk of chronic diseases in adult life. This theory is called the Developmental origin of health and disease (DoHAD) (33). It is intriguing to hypothesize that fetal hypoxic-ischemic insults, which predominately affects fetal brain and heart, also may affect the risk of developing common diseases in these organs later in life, e.g. dementia and Alzheimer's disease, or coronary heart diseases, respectively. Long term regulation of disease related genes through epigenetic modifications such as DNA methylation may possibly be involved in such effects. Further research is required to examine these interactions and unravel possible strategies for targeted interventions in order to prevent the longer-term adverse effects of exposure to a poor developmental environment and reduce the risk of chronic disease.

4.4 Improve the knowledge of fetal physiology in order to more correctly interpret present and future modes of fetal surveillance during labor

An important part of ongoing efforts to improve fetal surveillance in labor must be to improve our understanding of how different aspects of changes in FHR and other fetal physiological parameters relate to fetal condition. There is some evidence that significant improvements in the quality of intrapartum fetal assessment can be made by the more effective use of existing knowledge (24).These data support the potential for a simplified physiologic approach to improve perinatal fetal monitoring.

It is only through combining the information we receive from fetal monitoring with the whole clinical picture and our knowledge of basal physiology, that we may achieve the best comprehension of what is actually going on in each particular labor. Through this we may decide when intervention is required to avoid development of birth asphyxia and possible fetal injury, but it may also help us to avoid taking unnecessary actions.

References

1. Robertson CMT, Perlman M. Follow-up of the term infant after hypoxic-ischemic encephalopathy. Paediatr Child Health. 2006; 11: 278-282.

2. Hansen, TWR. Nevrologisk undersøkelse av nyfødte. In: Skjeldal OH, Helseth E, Gjerstad L, editors. Nevrologi og nevrokirurgi: fra barn til voksen. 4th ed. Nesbru: Forlaget Vett & Viten; 2007. p. 31-48.

3. Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. J Biomed Biotechnol. 2011; 2011: 609813.

4. Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. A systematic review of the role of intrapartum hypoxia-ischemia in the causation of neonatal encephalopathy. Am J Obstet Gynecol. 2008; 199: 587-595.

5. Mwanki MK, Atieno MA, Lawn JE, Newton CRJC. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012; 379: 445-452.

6. Low JA. Intrapartum fetal asphyxia: Definitions, diagnosis, and classification. Am J Obstet Gynecol. 1997; 176: 957-959.

7. de Vries LS, Cowan FM. Evolving understanding of hypoxic-ischemic encephalopathy in the term infant. Semin Pediatr Neurol. 2009; 16: 216-225.

8. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol. 1976; 33: 696-705.

9. Ross MG. Labor and fetal heart rate decelerations: relation to fetal metabolic acidosis. Clin Obstet Gynecol. 2011; 54: 74-82.

10. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. Lancet. 2003; 361: 736-742.

11. Phelan JP, Korst LM, Martin GI. Application of criteria developed by the task force on neonatal encephalopathy and cerebral palsy to acute asphyxiated neonates. Obstet Gynaecol. 2011; 118: 824-830.

12. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long term outcomes: systematic review and meta-analysis. BMJ. 2010; 340: c1471.

13.Reddihough D, Collins K. Cerebral palsy and neurodegeneral disease. In: Roberton DM and South M, editors. Practical paediatrics. 6th ed. New Zealand/Australia: Churchill Livingstone Elsevier; 2007. p. 593-602.

14. Neoventa. Fosterovervåking og ST-analyse. Mölndal: Neoventa Medical AB; 2007.

15.Eikeland T, Haugebert B, Henriksen T, Hjelle S, Yli BM, Blix E, et al. Overvåking under fødsel. In: Bjørge L, Henriksen T, Sand, Øian P, Hordnes K, editors. Veileder i fødselshjelp. Norsk Gynekologisk Forening; 2008.

16. Perlman JM. Summary proceedings from the neurology group on hypoxic-ischeamic encephalopathy. Pediatrics. 2006; 117: S28-S33.

17. Singer D. Neonatal tolerance to hypoxia: a comparative-physiological approach. Comp Biochem Physiol A Mol Integr Physiol. 1999; 123: 221-234.

18. Guyton AC, Hall JE. Regulation of acid-base balance. In: Guyton AC, Hall JE, editors. Textbook of Medical Physiology. 11th ed. Philadelphia: Elsevier Saunders; 2006. p. 383-401.

19. Stokke O. Forstyrrelser i syre/base-likevekten. In: Stokke O, Hagve TA, editors. Klinisk biokjemi og fysiologi. 3rd ed. Oslo: Gyldendal Norsk Forlag; 2006. p. 130-141.

20. Armstrong L, Stenson BJ. Use of umbilical cord blood gas analysis in the assessment of the newborn. Arch Dis Child Fetal Neonatal Ed. 2007; 92: F430-F434.

21. Bennet L, Gunn AJ. The fetal heart rate response to hypoxia: insights from animal models. Clin Perinatol. 2009; 36: 655-672.

22. Martin CB Jr. Normal fetal physiology and behaviour, and adaptive responses with hypoxemia. Semin Perinatol. 2008; 32: 239-242.

23. Rosén KG, Amer-Wåhlin I, Luzietti R, Norén H. Fetal ECG waveform analysis. Best Pract Res Clin Obstet Gynaecol. 2004; 18: 485-514.

24. Westgate JA, Wibbens B, Bennet L, Wassink G, Parer JT, Gunn AJ. The intrapartum deceleration in center stage: a physiologic approach to the interpretation of fetal heart rate changes in labor. Am J Obstet Gynecol. 2007; 197: 236.e1-236.e11.

25. Pulgar VM, Zhang J, Massmann GA, Figueroa JP. Mild chronic hypoxia modifies the fetal sheep neural and cardiovascular responses to repeated umbilical cord occlusion. Brain res. 2007; 1176: 18-26.

26. Wibbens B, Bennet L, Westgate JA, De Haan HH, Wassink G, Gunn AJ. Preexisting hypoxia is associated with a felayed but more sustained rise in T/QRS ratio during prolonged umbilical cord occlusion in near-term fetal sheep. Am J Physiol Integr Comp Physiol. 2007; 293: R1287-R1293.

27. Danielson L, McMillen C, Dyer JL, Morrison JL. Restriction of placental growth results in greater hypotensive response to alpha-adrenergic blockade in fetal sheep during late gestation. J Physiol. 2005; 563: 611-620.

28. Shah PS, Perlman M. Time courses of intrapartum asphyxia: neonatal characteristics and outcomes. Am J Perinatal. 2009; 26: 39-44.

29. Distefano G, Pratico AD. Actualities on molecular pathogenesis and repairing process of cerebral damage in perinatal hypoxic-ischemic encephalopathy. Ital J Pediatr. 2010; 36: 63.

30. Fathali N, Khatibi NH, Ostrowski RP, Zhang JH. The evolving landscape of neuroinflammation after neonatal hypoxia-ischemia. Acta Neurochir Suppl. 2011; 111: 93-100.

31. Northington FJ, Chavez-Valdez R, Martin LJ. Neuronal cell death in neonatal hypoxia-ischemia. Ann Neurol. 2011; 69: 743-758.

32. Ray-Santano C, Mielgo VE, Gastiasoro E, Murgia X, Lafuente H, Ruix-del-Yerro E, et al. Early cerebral hemodynamic, metabolic, and histological changes in hypoxic-ischemic fetal lambs during postnatal life. Front Neurosci. 2011; 5: 111.

33. Hanson MA, Gluckman PD. Developmental origins of health and disease: new insights. Basic Clin Pharmacol Toxicol. 2008; 102: 90-3.