Renal and cerebral circularity responses to hypoxemia in the ovine fetus

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Chapter 1

General Introduction
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Undernutrition in utero is associated with cardiovascular and other diseases in adult life. It has been suggested that abnormal programming in a critical stage of fetal development is responsible for the adult disease, but the mechanism has not been elucidated. Obviously, the placenta has a key role in the transfer of nutrients and oxygen to the fetus and insufficient transfer by the placenta is the most common cause of fetal undernutrition e.g. growth retardation. The fetus, however, responds to undernutrition by a number of short and long term adaptations. These adaptations include the cardiovascular system, the central nervous system and the fluid regulation (kidneys). Many of the fetal responses have been attributed to the hypoxemia which results from the insufficient placental transfer. However, placental insufficiency not only results in a reduced oxygen supply, but causes a reduction in the supply and removal of a variety of substances from the fetus. Consequently, complex changes occur in the ‘milieu interieur’ of the fetus. This makes it practically impossible to identify which factors are specifically responsible for the adaptive changes in the various organ systems in the growth retarded fetus. In the present study we aimed to unravel these mechanisms by studying the effects of pure hypoxemia without all the accompanying disturbances resulting from insufficient placental transfer. We focused on the circulatory adaptation to sustained hypoxemia of the fetal kidney and the fetal brain, which are in a critical stage of development in the near term ovine fetus.

1.1 Intrauterine environment

Growth and development of the mammalian fetus is a complicated sequence of events. Fetal growth and development is mainly controlled by genetic and environmental factors, the latter by altering the expression of fetal genes.

The diversity of size and weight of babies born after normal pregnancies is remarkable. Studies of the birthweight of relatives, together with evidence from animal cross breeding experiments, have led to the conclusion that this diversity is essentially determined by the intrauterine environment, and that genetic factors play a relatively weak role (Desai and Hales 1997), as was shown in the well-known experiments of Walton and Hammond (1938). In
cross breeding between Shetland and Shire horses, foals were smaller at birth when a Shetland pony was the mother than when a Shire horse was the mother. Both crosses are genetically the same, which implied that the Shetland mother had constrained the fetal growth. Studies of animals show that the supply of nutrients and oxygen is one of the most relevant factors of the intrauterine environment in determining the limits of fetal growth. In humans, studies show that the degree of fetal growth constraint is actually set when the mothers themselves are in utero, since low maternal birthweight was the biological factor showing the highest risk associated with small babies (Ounsted et al. 1986).

Low birthweight for gestational age, disproportion between head and abdominal circumference, disproportion between length and weight, and the ratio between placental weight and birthweight, are markers for lack of nutrients at particular stages of gestation (Barker 1997; Desai and Hales 1997). They reflect adaptations that the fetus made to sustain its development; adaptations that, it seems, may permanently programme its physiology and metabolism (King and Loke 1994; Barker 1997).

The process whereby disturbances at a critical or sensitive period can modify early fetal development with possible long-term outcomes is called programming. There are four essential principles which underlie the concept of programming (Desai and Hales 1997):

1. Nutritional (or non-nutritional, such as hormonal signals during critical periods) manipulations cause different effects at different times in early life.
2. Rapidly growing fetuses and neonates are more vulnerable to these manipulations.
3. Manipulation in early life has permanent effects.
4. The permanent effects include reduced cell numbers, altered organ structure and resetting of hormonal axes.

Evidence for programming during critical periods of development in animals dates back over 100 years ago (Spalding 1873). One of the classical examples is the exposure of female rats at a critical period of fetal life to a single dose of exogenous testosterone, which reorientates sexual behaviour of these animals permanently (Ounsted et al. 1986).

Events that subtly retard intrauterine growth may determine common disorders, such as hypertension (Dennison et al. 1997) and non-insulin-dependent diabetes (Seckl 1997), raised serum cholesterol, abnormal blood clotting and autoimmune diseases in adult life (Kannisto et al. 1997).

Recently, it was found that asymmetrical growth retardation is associated with a substantial reduction in the number of nephrons, and that there is no postnatal compensation for this (Hinchcliffe et al. 1992). Furthermore, a prospective study on preterm infants has shown that a brief period of early dietary management (formula feed verses human milk) has a major impact about 8 years later on brain growth, neurodevelopment and bone mineralization, in that human milk promotes these processes (Desai and Hales 1997). It is not that surprising that especially neuronal and renal processes are candidates for programming during fetal life, since at birth virtually all brain neurons and renal glomeruli are already present (Hinchcliffe et al. 1991).

There is considerable evidence now that undernutrition during prenatal life may have lifelong consequences (Gluckman 1993), indicating that lifespan is at least partially determined before birth (Barker 1995). Therefore, fetal growth retardation should be considered a major focus for those interested in reducing morbidity in adult life.

1.2 Fetal growth retardation

Fetal growth depends on a stable supply of oxygen and other nutrients from the mother. Impairment of the uteroplacental and/or the fetoplacental circulation has been reported to cause chronic hypoxia in the fetus, which results in fetal growth retardation (or intrauterine growth retardation (IUGR)), fetal distress (Block et al. 1984; Mori et al. 1993), and possibly preterm labor (Valenzuela et al. 1992).

IUGR occurs in ~3% of all human pregnancies and is associated with a significant increase in both perinatal morbidity and mortality (Hooper et al. 1991; Kamitomo 1993). Although multiple etiologies of IUGR exist, many are associated with limited nutrient and oxygen delivery to the fetus (Lueder et al. 1995). For example, umbilical cord occlusion, maternal cigarette smoking, and reduced uteroplacental circulation all result into fetal hypoxia (Hooper et al. 1990).

Oligohydramnios, a shortage of amniotic fluid, is often associated with chronic fetal hypoxia as found in IUGR, suggesting that hypoxia affects the fetal fluid balance (Wlodek et al. 1993).

Such a decrease in amniotic fluid is associated with an increase in fetal morbidity and mortality (Veille et al. 1993). Animal research revealed that oligohydramnios causes an increase in spinal flexion, leading to compression.
of abdominal content, upward displacement of the diaphragm, and loss of lung liquid due to lung compression (Harding et al. 1990). This impairs fetal lung development, and can result in permanent lung hypoplasia, in particular when oligohydramnios is present in early pregnancy.

### 1.3 Fetal adaptations to hypoxemia and the consequences

The plasticity of the fetus enables it to adapt itself to life threatening events. In case of acute hypoxemia, the fetal circulation is rearranged in such a way that oxygenated blood is preferentially distributed to the cerebral circulation, to the heart and to the adrenal glands (Block et al. 1984; Jensen et al. 1987; Rudolph 1984; Jensen and Berger 1991), a phenomenon called ‘centralization’ or ‘brain sparing’. The influence of the mother and/or placenta in this centralization is thought to be of minor importance, but difficult to proof. Recently, Mulder et al. reported that fertilized eggs respond to hypoxia in the same way as the mammalian fetus, in that the cardiac output is redistributed to the most vital organs in the chick embryo. This all happens without any influence of the mother (Mulder et al. 1998).

In the adult human the cardiovascular responses to hypoxia appear to be caused largely through the activity of the sympathetic part of the autonomic nervous system. In some species adrenal secretion of catecholamines influences the response to hypoxia, and may be as important as enhanced sympathetic nerve activity. Because sympathetic innervation in the fetus is incomplete, tissues such as the heart have a supersensitivity to exogenous catecholamines (Jones et al. 1988). It is known that acute fetal hypoxemia causes substantial changes in the fetal endocrine status: the plasma concentrations of epinephrine, norepinephrine (Jones and Robinson 1975), arginine vasopressin (AVP) (Wlodek et al. 1995), adrenocorticotropic hormone (ACTH), and cortisol (Bocking et al. 1986) increase. All of these responses presumably aid the fetus in surviving hypoxia (Hooper et al. 1990).

The growth-retarded fetus is thought to maintain a degree of redistribution of oxygenated blood similar to that of a normal fetus exposed to acute hypoxemia (Block et al. 1984). A consequence of this chronic redistribution of blood flow is that other organs and tissues, that are less vital for intrauterine survival, such as the lungs, the skin and the carcass get a reduced blood supply. Hence, growth and development of these organs is impaired. The development of oligohydramnios in IUGR is explained by the presumed decrease in renal blood flow leading to a decreased glomerular filtration rate (GFR) and hence decreased fetal urine production rate (Chevalier 1996). However, the precise mechanism leading to the decrease in amniotic fluid during IUGR has not been clarified. Indeed, in the growth retarded human fetus urine production rate is reduced, although overall data on fetal urine production rate are not univocal (Nicolaides et al. 1990; Wladimiroff and Campbell 1974; Oosterhof 1993). Doppler ultrasound studies on renal blood flow in the human fetus, estimated by flow velocity waveforms of the renal artery, show conflicting results (Arduini and Rizzo 1991; Vyas et al. 1989; Oosterhof 1993).

It is becoming more and more apparent that survival of premature infants weighing less than 1000g is related in part to a good understanding of the limitations imposed by the immature kidney (Robillard et al. 1989). Since the survival of these infants has increased dramatically over the past two decades, knowledge of developmental renal physiology has assumed heightened clinical relevance (Avner 1990).

### 1.4 The fetal circulation

In the fetus there are several vascular shunts to meet with the different circulation of oxygenated blood compared with the adult situation: during intrauterine life, the placenta serves as the site of gas exchange rather than the lungs (Fig.1). The most important features of the fetal circulation are the relatively large proportion of the combined output of both cardiac ventricles distributed to the umbilical cord and the placenta and the small proportion distributed to the lungs (Jensen and Berger 1991). This requires special vascular channels: the ductus venosus, the foramen ovale and the ductus arteriosus. The ductus venosus is a relatively large vessel through which a large part of the umbilical venous return bypasses the liver tissue, and is delivered straight to the fetal heart. In the heart itself, the foramen ovale is shunting well-oxygenated inferior caval blood from the right to the left atrium, without passing through the lungs. Blood from the right ventricle, flowing towards the relatively high-resistance pulmonary circulation is bypassed into the systemic arch via a short but large-diameter blood vessel, the ductus arteriosus (Mott 1982). The fetal circulation is not only anatomically different,
it also works under conditions very different from those found in adult mammals. Arterial pressure is low and similar in all circuits, and systemic arterial blood gas tensions are asphyxial compared to the adult standards.

Quantitative information about the fetal circulation has been acquired almost entirely from the fetal lamb in the last one-third of gestation. About 60% of the combined cardiac output goes to the oxygen-consuming circuits of the fetal body, and 40% to the umbilical circulation (Mott 1982). Under control conditions, the umbilical vessels, which are widely patent in utero, transport blood at ~200 ml·kg⁻¹·min⁻¹ at an arterial blood pressure of 40-50 mmHg. The average cerebral blood flow is 100-200 ml·100 g⁻¹·min⁻¹ at PO2 and CO2 values in the normal fetal range, which is 2 to 4 times higher than in adult humans (53 ml·100 g⁻¹·min⁻¹). The pulmonary circulation receives 5-6% of the combined cardiac output (Mott 1982). In the adult, the relatively small kidneys (about 1% of total body weight in humans) receive a remarkable large amount of blood, carrying about 20-25% of the total cardiac output (Eckert and Randall 1983), or about 1.2-1.3 l/min (Guyton and Hall 1996). Kidney blood flow in the near term fetus is much less, estimated as 2-4% of the combined ventricular blood output in last trimester of gestation. In the fetal sheep, renal blood flow is in the order of 1.5 to 2.0 ml·min⁻¹·g⁻¹ kidney weight (Aperia et al. 1977; Robillard et al. 1981a) in this period of gestation.

1.5 Fetal versus adult renal characteristics

Nephrogenesis begins at 8 weeks of gestational life and continues until 34 weeks in humans (Peters et al. 1992; McGory 1972). During nephrogenesis, morphological and functional differentiation of nephrons happens centrifugally. Nephrons at the inner cortex are perfused to a greater proportion, and are more differentiated morphologically compared to the nephrons located in the outermost region of the cortex (Kahane et al. 1995; Lumbers 1995). Renal tubular maturation begins in the late first trimester, and continues after birth (Peters et al. 1992).

As described above, the blood flow to the adult kidney is much higher than during fetal life. In the adult situation, the blood flow in the renal cortex (4-5 ml·g⁻¹·min⁻¹ kidney tissue) is much greater than in the medulla (0.3-2 ml·min⁻¹·g⁻¹). However, even the medullary flow is relatively large on a tissue weight basis. For comparison, the normal blood flow to the adult brain is 0.5 ml·min⁻¹·g⁻¹ (Guyton and Hall 1996). In the fetal situation, this distribution of blood flow per tissue weight between the brain and kidney is practically the same (1-2 ml/min/g). Within 48 hours of delivery, renal blood flow increases by an amount disproportionate to the rest of the body, primarily due to decreased vascular resistance. This increase in RBF is critical to the emergence of the kidney as the organ responsible for the maintenance of body fluid homeostasis (Corey and Spitzer 1992).

The mature kidney is capable of maintaining a relatively constant renal blood flow despite alterations in perfusion pressure over a range of 80-150 mmHg. This autoregulation is thought to be operable in the fetus also, despite the much lower perfusion pressure to which the fetal kidney is exposed (40-60 mmHg) (Smith 1982).

Since the time of Hippocrates, it has been recognized that the fetus produces urine (Needham 1931), which contributes substantially to the
accumulation of amniotic fluid (Robillard et al. 1989; Ervin et al. 1986). Since the maintenance of amniotic fluid volume within the normal range is important for promoting fetal well-being (Brace and Wolf 1989), regulation of the extracellular fluid volume by the immature kidney is of major importance (Avner 1990). Without sufficient amniotic fluid, the development of the fetal lung, limb and gut may be impaired, sometimes to the point where life after birth is not possible (Lumbers 1995). So, in contrast to the adult kidney, the fetal kidney plays an essential role in providing the environment that surrounds the fetus (Lumbers 1983).

Research on the possible factors influencing the production of urine by the fetal kidney were investigated in anesthetized fetal sheep more than 40 years ago (Alexander et al. 1958). In fetal sheep, urine passes into the amniotic cavity through the urethra and into the allantoic cavity through the urachus. In the human, the allantois normally degenerates early in gestation so that fetal urine flows only into the amniotic fluid cavity (Campbell et al. 1973).

During fetal life glomerular filtration rate (GFR) is low (1.4 ml·min⁻¹·kg⁻¹) compared to the mature kidney (2.4 ml·min⁻¹·kg⁻¹) (Robillard et al. 1989; Lumbers 1995), and urine is rather hypotonic to plasma (Ervin et al. 1986). Furthermore, the regulation of the fluid balance in the fetus is substantially different from that in the adult; the late-gestation fetus urinates and drinks a volume equal to 20-30% of body weight per day, whereas comparable values in the adult are 2-3% of body weight per day (Brace 1989). Despite the lower renal perfusion pressure and the higher renal vascular resistance, the filtration fraction (FF) is not significantly different from the adult (Lumbers 1995), although the total filtering capacity, as seen in the mature kidney, is by far not yet reached. It is important to recognize that the principal activity of the fetal kidney is growth and development, and that the placenta is the chief homeostatic organ during intrauterine life (Aperia et al. 1977).

Since renal tubular maturation is a continuing process which goes on after birth, it is not surprising that the fetal kidney is not yet capable of concentrating urine, representing only 20-30% of the adult potential (Robillard and Nakamura 1995). Furthermore, it is suggested that the structural immaturity of the medulla, characterized by a short loop of Henle and a the relatively high blood flow to the inner nephrons and medulla, may limit the build-up of an osmotic gradient (Robillard and Nakamura 1995).

During development, certain functional characteristics become apparent within a relatively short period of time, which are seen as “critical periods” (Solomon 1982). Developmental changes of the kidney are associated with a change in regulation rather than in renal function per se. For example, in the sheep fetus, it has been shown that the capacity of the immature kidney to secrete H⁺ is not a limiting factor in urinary acidification, but instead some regulatory component is absent (Solomon 1982). Also, the antidiuretic hormone, vasopressin (AVP) is present in fetal animals and as early as the 11th week of gestation in the human, and is capable of concentrating the fetal urine. However, vasopressin is shown to have less effect on the fetal nephron compared to the adult kidney (Robillard et al. 1982).

1.6 Animal research on fetal hypoxemia

Through the years, much research has been done on the fetal responses to asphyxia/hypoxemia. The early studies focused on short, acute and severe forms of hypoxemia, in animals under general anaesthesia. In the late 1960s, the first studies on fetuses in utero after recovery from surgery and anaesthesia were performed, using chronic implantation of catheters and probes (Jensen and Berger 1991). There are few species apart from the sheep in which it is possible to perform the chronic instrumentation necessary to study the development of fetal responses. Small laboratory animals such as the rat and guinea pig are just too small, and other farm animals, such as the pig, have large numbers of fetuses (Cheung and Brace 1988).

The results of the reported studies on fetal responses to hypoxemia are difficult to compare, because of the great variety in methods used to apply hypoxia: the severity, duration, mode of induction, and whether or not concomitant changes in arterial pH and PCO₂ occur, show considerable variations (Robillard et al. 1981). Furthermore, the techniques used to measure blood flow during fetal hypoxemia also differs.

Fetal hypoxia can be induced by reducing arterial oxygen content, by restricting uterine blood flow, or by reducing umbilical blood flow by cord compression. While maternal hypoxemia only reduces oxygen supply to the fetus, reducing uterine or umbilical blood flow also interferes with fetal PCO₂ elimination as well as with energy substrate supply to the fetus (Rudolph 1984).
Table I. Brief overview of hypoxemia studies on fetal lambs, regarding renal blood flow and function and cerebral blood flow.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fetal age (days)</th>
<th>Method</th>
<th>Duration</th>
<th>PHa</th>
<th>PaCO2</th>
<th>CBF</th>
<th>RBF</th>
<th>UFR</th>
<th>GFR</th>
<th>RV R</th>
<th>Technique</th>
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<tr>
<td>1972 Dunne et al.</td>
<td>138</td>
<td>cord occl.</td>
<td>15-45 sec</td>
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<td>e.m. flowmeter</td>
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<td>1974 Cohn et al.</td>
<td>122-142</td>
<td>Mat. O2</td>
<td>4-44 min</td>
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<td>1979 Millard et al.</td>
<td>125-140</td>
<td>Mat. O2</td>
<td>10 min</td>
<td>↑</td>
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<td>e.m. flowmeter / Doppler Ultrasound</td>
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<td>1979 Peeters et al.</td>
<td>130-145</td>
<td>Mat. O2</td>
<td>1 h</td>
<td>↓</td>
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<td>microspheres</td>
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<td>1985 Iwamoto et al.</td>
<td>124-133</td>
<td>Mat. O2</td>
<td>40 min</td>
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<tr>
<td>1985 Jensen et al.</td>
<td>130</td>
<td>arrest UBF</td>
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<td>1985 Nakamura et al.</td>
<td>132-143</td>
<td>Mat. O2</td>
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<td>1988 Bocking et al.</td>
<td>127 d</td>
<td>RUBF</td>
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<td>microspheres</td>
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<td>1989 Wlodek et al.</td>
<td>127-135</td>
<td>Mat. O2</td>
<td>3 h</td>
<td>↓</td>
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<td>1990 Rurak et al.</td>
<td>132</td>
<td>Mat. O2</td>
<td>8 h</td>
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<td>microspheres</td>
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<td>1990 Block et al.</td>
<td>124-132</td>
<td>Mat. O2</td>
<td>20 min</td>
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<td>microspheres</td>
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<td>1993 Wlodek et al.</td>
<td>125</td>
<td>RUBF</td>
<td>24 h</td>
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<td>1994 Asano et al.</td>
<td>110-113</td>
<td>Mat. O2</td>
<td>8 h</td>
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<td>1994 Cock et al.</td>
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<td>1996 Cock et al.</td>
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CBF, cerebral blood flow; RBF, renal blood flow; UFR, urine flow rate; GFR, glomerular filtration rate; RVR, renal vascular resistance; Technique, method used to measure blood flow.
1.6.1 Renal and cerebral responses to hypoxemia

Table I shows an (incomplete) overview of fetal studies on renal and cerebral blood flow and urine flow rate, in response to hypoxemia, which have been published over the last 25 years. In the first experiments on fetal sheep, the hypoxic condition lasted no longer than several minutes. Durations of 30 min were considered as a chronic situation. However, it became more and more clear that in human pregnancy the most harmful effects of hypoxemia are the result of chronic fetal hypoxemia instead of acute hypoxemia. Therefore, chronic hypoxemia studies with a duration of several hours, became more and more in favour (Hooper et al. 1990).

Acute hypoxemia, no matter how it is induced, is associated with bradycardia and increased blood pressure, the latter being more pronounced in case of severe hypoxemia (Cohn et al. 1974). Also, fetal body movements and breathing movements are decreased during the hypoxic insult. Acute hypoxemia induces large increases in stress hormones such as catecholamines which are known to rise 50-100 fold in the hypoxic ovine fetus, and 50 fold increases in vasopressin (Jones et al. 1988). Increases in cortisol and adrenocorticotropic hormone (ACTH) are also seen (Hooper et al. 1990). During chronic hypoxemia (lasting several hours to days), fetal body movements and heart rate gradually return to control levels, but the initial redistribution of cardiac output is, in part, sustained. In addition, plasma concentrations of ACTH decline to near control levels (Challis 1986; Hooper et al. 1990), cortisol has either the tendency to decrease (Challis 1986) during the course of hypoxemia, or, together with prostaglandin E₂ concentrations, remains elevated (Hooper et al. 1990). Norepinephrine remains elevated throughout the hypoxic period, while epinephrine and AVP concentrations return to control levels during course of hypoxic period.

Studies in which a large decrease in fetal renal blood flow was found during hypoxemia, concern situations with severe hypoxemia, lasting for minutes to several hours, and were usually accompanied by progressive acidemia. Other studies show that during hypoxic periods of 120 min to 24 h in duration, no changes are seen in renal blood flow compared to control values (Włodek et al. 1989; McLellan et al. 1992). If the hypoxemia is severe, a decrease in renal blood flow is reported (Daniel 1975), while in case of mild hypoxemia with a duration of 48 h an increase in renal blood flow was seen (Bocking et al. 1988).

It has been shown for various organ systems that the responses of the fetus to acute and chronic hypoxemia are different (Jensen and Berger 1991; Iwamoto and Rudolph 1985). Furthermore, it is important to distinguish hypoxemia with and without acidemia: hypoxemia accompanied by acidemia causes a decrease in fetal renal blood flow (Rudolph 1984; Cohn et al. 1974), whereas fetal renal blood flow remains constant or increases when acidemia is absent (Bocking 1988; Towell et al. 1987; McLellan et al. 1992). Another factor influencing the fetal responses to hypoxemia is an elevated arterial PCO₂ level. Hypercapnia itself is known to reduce fetal renal blood flow by increasing resistance to flow (Beguin et al. 1974). The reduction in fetal renal blood flow during acidosis and hypercapnia is essentially reflex mediated (Beguin et al. 1974). This indicates that many factors concomitant with the induced hypoxemia have their reflect on the renal response to decreased arterial oxygen levels.

Data on fetal urine flow during hypoxemia are also inconsistent throughout the literature, again probably due to differences in methods of inducing hypoxemia and the duration and severity of hypoxia. Acute fetal hypoxemia, induced by lowering the inspired oxygen concentration of the ewe, caused fetal urinary output to decrease (Nakamura 1985; Robillard 1981). During the fall in urine flow found during acute or short periods (30 min) of hypoxemia, fetal glomerular filtration rate (GFR), filtration fraction (FF) and free water concentration of ACTH decline to near control levels (Challis 1986; Hooper et al. 1990), cortisol has either the tendency to decrease (Challis 1986) during the course of hypoxemia, or, together with prostaglandin E₂ concentrations, remains elevated (Hooper et al. 1990). Norepinephrine remains elevated throughout the hypoxic period, while epinephrine and AVP concentrations return to control levels during course of hypoxic period.

Studies in which a large decrease in fetal renal blood flow was found during hypoxemia, concern situations with severe hypoxemia, lasting for minutes to several hours, and were usually accompanied by progressive acidemia. Other studies show that during hypoxic periods of 120 min to 24 h in duration, no changes are seen in renal blood flow compared to control values (Włodek et al. 1989; McLellan et al. 1992). If the hypoxemia is severe, a decrease in renal blood flow is reported (Daniel 1975), while in case of mild hypoxemia with a duration of 48 h an increase in renal blood flow was seen (Bocking et al. 1988).
1.7 Blood flow measurement techniques

From Table I it is obvious that there is great variety in fetal blood flow responses to hypoxemia. This can be partly explained by the way hypoxemia was induced, the severity, and the duration of the hypoxic period. However, yet another point of difference between the studies needs attention, that is the way of measuring blood flows to the fetal organs of interest.

The most commonly used technique to study blood flow in fetal organs is radiolabelled microspheres. This technique allows blood flow studies of many fetal organs, but only intermittently at few time points during a study.

Electromagnetic and ultrasonic flowprobes have the advantage that recordings can be made continuously, and for the entire duration of the study. The disadvantage is that only the blood flow to a specific organ can be measured, and that the probes have to be implanted.

Doppler-ultrasound is non-invasive, but has the disadvantage that not the blood flow itself but the blood velocity in a particular vessel is measured. A brief overview of the different techniques is described below.

1.7.1 Microspheres

Injections of indicators of different types into the intravascular compartment has been applied for the study of the circulation for many years. Solid foreign particles have been used as indicators to measure distribution of organ blood flow, distribution of cardiac output, and the presence of shunting through various organs. The ability to label these particles with radio nuclides makes them therefore easily detectable or quantified.

The most commonly used nuclide-labelled microspheres are made of an inert plastic. The nuclide is incorporated into the plastic, and therefore the number of counts per minute determined will be related to sphere volume. The microspheres are trapped in the arteriolar or capillary vascular system on the first circulation after injection. In order to measure organ blood flow, the microspheres must be well mixed at the site of the injection and their concentration in all arteries downstream from the site of injection should be similar. Distribution of the microspheres will then be similar to the distribution of blood flow to the organs. It is essential that when measuring flow, the number of microspheres injected be carefully calculated to satisfy the criteria for the organ or portion of organ with the lowest flow that is to be measured.

A further prerequisite for the use of microspheres to measure distribution of blood flow is that all microspheres are entrapped in the peripheral microcirculation and that no significant number bypass the organs. To quantitate the organ blood flow, the most common method used is that of a surrogate organ flow known as the reference sample technique. Before, during, and after injection of the microspheres, blood is collected from an artery at a constant rate. The volume of the blood withdrawn is not critical as long as more than 400 microspheres are present. Flow to any organ can now be calculated using the relationship:

$$\text{Unknown organ flow (ml/min)} = \frac{\text{known organ flow (ml/min) } \times \text{ No. of microspheres in organ with unknown flow}}{\text{No. of microspheres in organ with known flow}}$$

where the “known” organ is the reference sample (Heymann et al. 1977).

A disadvantage of the use of microspheres to measure blood flow is that only a limited number of measurements can be made during a study period in one and the same animal. The use of color “coded” microspheres instead of radio-nuclide labeling is an attractive alternative which allows to take measurements at more different time points during a study period.

1.7.2 Electromagnetic flowprobes

Electromagnetic flowprobes are one of the possibilities to measure blood flow continuously.

When a magnetic field is applied at right angles to the direction of motion of a conduction fluid, a potential difference is set up at right angles to both the flow direction and the magnetic field. Fabre (1932) suggested that blood flow could be measured using electromagnetic flowmeters and Kolín (1936) showed results from in vitro measurement on an isolated carotid artery. Wetterer (1937) developed a flowmeter independently of Kolín and published results from in vivo measurements on the rabbit aorta (Woodcock 1975).

There are several different types of electromagnetic flowmeter, differing from each other in the way the magnetic field is produced. However, there are basically two main types, namely, the direct current and the alternating current flowmeters. Early electromagnetic flowmeters were all direct current instruments. In general, the advantages of alternating current excitation of the
magnet are, first, that electrode polarization is avoided, and second, it is easier to amplify alternating current signals than direct current and consequently they can be used to measure very low flows. Clark and Wyatt (1969) discuss the design of electromagnetic flow probes and distinguish three main categories: 1) perivascular, 2) cannular, and 3) intravascular probes (Woodcock 1975).

Although continuous measurement of blood flow is possible, there are some factors that can disturb the blood flow measurement. First, for optimal performance the diameter of the cuff-type probe should be 10% smaller than the outside diameter of the blood vessel, which reduces the vessel diameter and moreover may result in vessel contractility. Second, the influence of haemoglobin concentration on blood flow measurement can be relatively large. Third, calibration of electromagnetic probes is preferably performed in vivo, and fourth, the wall of a blood vessel can influence the measurement in case the wall is pathologically thickened and in whether there is insulation around the vessel.

1.7.3 Ultrasonic flow probes

Another method to measure blood flow continuously and instantaneously is by means of ultrasound flow probes. This technique for measuring fluid velocity depends on the difference in phase between the emitted wave and a wave traveling opposite to or in the same direction as the fluid. This phase difference is proportional to flow.

Kalmus developed a more refined flow velocity meter which consisted of two transducers switched alternately to emitter and receiver at a rate of 10 Hz (Woodcock 1975).

These flow probes use an ultrasonic transit-time principle to sense liquid volume flow in vessels or tubing largely independent of flow velocity profile, turbulence and hematocrit. The method and theory of operation of nowadays used ultrasonic flow probes is as follows:

The flow probe consists of a probe body which houses two ultrasonic transducers and a fixed acoustic reflector bracket. The ultrasonic transducers are situated on one side of the vessel/tube under study and the reflector bracket is situated equidistant between the two transducers on the opposite side of the vessel or tube. Both transducers, transmit a plane wave of ultrasound of a minimal level which intersects the flowing liquid in upstream and downstream direction, bounces off the “acoustic reflector”, again intersects the vessel in either the upstream or downstream direction and is received by the opposing transducer where it is converted into electrical signals. The flowmeter then analyzes and records the signals as an accurate measure of the “transit-time” it took for both waves of ultrasound to travel from one transducer to the other. The downstream transit time is then subtracted from the upstream transit-time, and the difference is a measure of volume flow.

One ray of the ultrasonic beam records phase shift in transit time proportional to the average velocity of the liquid and the path length over which this velocity is encountered. With wide-beam ultrasonic illumination, the receiving transducer integrates these velocity-vessel chord products over the vessel’s full width and yields an average velocity time vessel inside area. The rays of the ultrasonic beam which cross the acoustic window without intersecting the vessel do not contribute to the volume flow integral. Volume flow is therefore sensed even when the vessel is much smaller than the acoustic window and is independent of the flow velocity profile. Misalignment of the vessel within the probe, has little consequence for the flow measurement because of the ultrasonic wave’s reflective pathway, whereby the sum of vector components of the flow does not change. (Transonic flowmeters 1991).
1.7.4 Doppler ultrasound

Blood-velocity waveforms can be transcutaneously recorded using the ultrasonic doppler-shift (Woodcock 1975). Doppler ultrasound therefore offers a noninvasive technology for investigating the circulatory system. Doppler velocimetry has been extensively used to investigate fetal, fetoplacental, and uteroplacental circulations via the human. Doppler velocimetry requires a comprehensive analysis of the Doppler signal involving applications of advanced technology with varying degrees of complexity and sophistication (Maulik, 1995).

The method is based on the following principle: When a beam of energy waves encounters a reflector smaller than its wavelength, the incident energy waves are reflected in all directions, known as scattering. A portion of the scattered energy will be reflected back to the source, known as backscattering. This phenomenon is also observed when an ultrasound beam intersects blood flow in a vessel (Shung 1976). In blood, the primary sources of ultrasonic scattering are the circulating red blood cells. The moving red cells also cause Doppler shift of the scattered ultrasound. The shift is proportional to the velocity of red cell movement.

For flow quantification, instantaneous flow can be measured by integrating the mean velocity across the vascular lumen with the vascular cross-sectional area. However, the clinical usefulness of Doppler velocimetry for quantification of flow is limited as it suffers from several sources of error (Gill, 1985). One of the critical concerns is the accuracy of measuring the vascular cross-sectional area, especially of the smaller fetal vessels. As estimation of the cross-sectional area involves squaring the radius, any error in measuring the vessel diameter is significantly amplified in the calculation of volume flow. Another important source of inaccuracy is the error in determining the angle of sonification.

Because of the problems related to volume flow measurement, there has been a need to seek alternative ways for investigating vascular flow dynamics using the Doppler method.

Techniques have therefore been developed for analysing the Doppler frequency shift waveform in an angle-independent manner. Most of these analytic techniques involve deriving Doppler ratios from the various combinations of the peak systolic, end-diastolic and temporal mean values of the maximum frequency shift envelope. Because these parameters are taken from the same cardiac cycle, these ratios are virtually independent of the angle of sonification (Maulik, 1995).

The maximum Doppler frequency shift waveform represents the temporal changes in the peak velocity of the red cell movement during the cardiac cycle, and is therefore under the influence of both upstream and downstream circulatory factors (McDonald, 1974). The pulsatility of the flow velocity was originally investigated using Doppler ultrasound in the peripheral vascular system. Gosling and King were the first to develop the pulsatility index (PI) as a measure of the systolic-diastolic differential of the velocity pulse (Gosling 1975). A simplification of the PI, namely the peak-to-peak PI was introduced based on the peak systolic frequency shift, the end-diastolic frequency shift, and the temporal mean frequency shift over one cardiac cycle. The PI is presumed an index of vascular impedance downstream.

Vascular resistance is based on the Poiseuille equation:
\[ \text{vascular resistance} = \frac{\text{mean arterial pressure}}{\text{mean venous pressure}} \frac{1}{\text{vascular cross-sectional area}} \]

by blood flow. However, the formula is applied for steady-flow resistance calculations and may be unreliable in a pulsatile flow system (Milnor, 1972). Pulsatile flow and pressure are generated by cardiac contraction, and the pulsatility at a given point in the vascular bed is determined by the elasticity of the vessel walls, the distribution of the length and diameter of the vessels throughout the vascular bed and the degree of wave reflection. Wave reflections are created by the non-uniform elasticity of the vessels and by the change of vessel diameter and the total cross-section of the bed at each point of branching (McDonald, 1974).

Noninvasive Doppler is still the only option to the physician to monitor the human fetal circulation, and although much information about fetal blood flow characteristics can be obtained, the Doppler technique does not give quantitative information on blood flow. Furthermore, increased vascular resistance indicates increased dissipation of the energy of blood flow within the vascular bed, but it does not identify the nature, cause or exact site of this increase (Maulik, 1995).
1.8 Aim and outline of the thesis

Since there is a close relationship between the degree of growth retardation and perinatal morbidity and mortality, fetal growth retardation (FGR) is of major clinical concern. One of the causes of FGR is placental insufficiency, leading to a situation of chronic fetal hypoxemia, which is often accompanied by a decreasing amount of amniotic fluid, resulting in oligohydramnios. This shortage in amniotic fluid is ascribed to a reduction in fetal urine flow as a consequence of reduced renal blood flow. The presumed reduction in renal blood flow is thought to be the result of the redistribution of the cardiac output due to hypoxemia. However, experimental and clinical data on renal blood flow in the hypoxemic fetus do not show conclusive evidence. Therefore, the aim of the thesis was to study the responses of the renal physiology to chronic fetal hypoxemia without acidemia and hypercapnia.

Since urine production rate in the human fetus was found to be dependent on behavioral states, we first studied whether in the ovine fetus this was also the case (Chapter 1). During hypoxemia, fetal behavioral states change towards a higher percentage of high-voltage electrocortical activity (HV-ECoG). Changes in fetal urine production rate during hypoxemia could therefore be the consequence of behavioral state changes, rather than changes in fetal renal blood flow and physiology. Secondly, we studied the daily rhythm of fetal urine production rate and fetal renal blood flow (Chapter 2) to exclude the possibility to measure normal variances, in stead of changes due to chronic fetal hypoxemia. With this information in mind we studied fetal renal blood flow and renal function during 48 hours of isocapnic moderate hypoxemia (Chapter 3). These were strictly time dated studies in order to diminish the effects of daily rhythms. Finding a very quick response of the fetal renal blood flow to hypoxemia, we studied the fetal renal and cerebral blood flow responses during contractures, the latter representing spontaneous short periods of mild to moderate fetal hypoxemia (Chapter 4). The finding that during these short periods of hypoxemia the cerebral blood flow showed a decrease instead of a maintenance or increase in blood flow for compensation of decreased oxygen saturation and increased carbon dioxide, prompted us to study the response time of renal blood flow and cerebral blood flow during the onset of hypoxemia (Chapter 4). The possible influence of chronic moderate hypoxemia on the developing fetal brain was studied in Chapter 5. Most studies on hypoxic influences on the fetal sheep brain concern conditions of severe and acute hypoxemia, and consequently brain lesions. We looked for more subtle changes during chronic moderate hypoxemia without acidemia in the developing ovine brain using immunochemical staining technique for protein kinase C (PKC) γ, known for its multifunctional role in neurons. We studied one of the most vulnerable brain areas to hypoxemia, namely the hippocampus, for changes in PKC γ staining.

The gestational age of the fetuses during these studies (123-128 days) is known as a period in which both cerebral and renal development and function undergo major maturational changes.
1.9 References


Chapter 1 General Introduction


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