Circulatory and metabolic studies of normally grown and growth restricted fetal sheep before and during spontaneous labor and at delivery

By

Weiping Tan

M.D., The No. 4 Medical University of The Army, Xian, China, 1976
M.Sc., The Capital Institute of Medical Science, Beijing, China, 1985
M.Sc., The Free University of Brussels, Brussels, Belgium, 1990

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We accept this thesis as conforming to the required standard

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Department of Obstetrics & Gynaecology

The University of British Columbia
Vancouver, Canada

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ABSTRACT

To examine the fetal responses to labor, 2 groups of chronically instrumented fetal sheep were studied. The groups were separated on the basis of femoral arterial pH being greater (Group II) or less (Group I) than 7.15 at delivery. Fetal and placental weights in Group I (n = 6) were significantly lower than in Group II (n = 6), associated with a lower placental/fetal weight ratio, higher brain/liver weight ratio, higher adrenal weight and slightly shorter gestation length. In the antepartum period, Group I was hypoxemic, acidemic and hypoglycemic compared to Group II. Umbilical blood flow and fetal O₂ delivery were also lower in Group I, but O₂ consumption was also reduced, so that there was maintenance of a balance between O₂ consumption and delivery. This was perhaps due to a programmed reduction in growth, thereby resulting in only modest hypoxemia and acidemia prior to labor onset. However, during labor Group I developed severe hypoxemia and lactic acidemia, whereas blood gas and acid-base status were maintained in Group II until delivery. In Group I, there was increased blood flow to most organs and tissues during labor; however, there was a fall in flow at delivery. In contrast, in Group II there was maintenance of perfusion to most organs which persisted through labor and delivery. In both groups, fetal O₂ delivery fell. In Group II, this was accompanied by a marked increase in O₂ extraction, so that O₂ consumption was maintained. In contrast, there was only a minimal change in O₂ extraction in Group I, with the result that O₂ consumption fell significantly at delivery. The increase in lactate concentration during labor in Group I was associated with a marked rise in fetal lactate uptake from the placenta, thus contributing to fetal lactic acidemia, perhaps as a consequence of inadequate oxygenation. The terminal
fall in metabolic rate, coupled with the decreased perfusion to most fetal organs and tissues at delivery suggests that the growth restricted fetuses were severely compromised by the end of labor. They were thus less able to tolerate labor than the normally grown fetuses. The reduced placental weight may be involved in both the antepartum and intrapartum fetal compromise.
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<tr>
<td>ACTH</td>
<td>adrenocorticotrophic hormone</td>
</tr>
<tr>
<td>AGA</td>
<td>appropriate for gestational age</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>AVP</td>
<td>arginine vasopressin</td>
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<td>BE</td>
<td>base excess</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<td>BSA</td>
<td>bovine serum albumin</td>
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<td>CA</td>
<td>carotid artery</td>
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<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>CaO₂</td>
<td>umbilical arterial oxygen content</td>
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<td>CRH</td>
<td>corticotropin-releasing hormone</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<td>CVO</td>
<td>combined ventricular output</td>
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<td>Cvo₂</td>
<td>umbilical venous oxygen content</td>
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<td>d</td>
<td>day</td>
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<td>DO₂</td>
<td>oxygen delivery</td>
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<td>ECoG</td>
<td>electrocorticogram</td>
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<td>EoG</td>
<td>electro-oculagram</td>
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<td>FA</td>
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<td>FAP</td>
<td>fetal arterial pressure</td>
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<td>Abbreviation</td>
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<td>FBM</td>
<td>fetal breathing movements</td>
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<td>fetal heart rate</td>
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<td>g</td>
<td>gram</td>
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<td>GI</td>
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<td>h</td>
<td>hour</td>
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<td>Hb</td>
<td>hemoglobin</td>
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<td>HCO$_3^-$</td>
<td>bicarbonate ion concentration</td>
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<td>i.d.</td>
<td>inside diameter</td>
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<tr>
<td>IGFs</td>
<td>insulin-like growth factors</td>
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<td>IGFBPs</td>
<td>insulin-like growth factor binding proteins</td>
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<tr>
<td>I.U.</td>
<td>international unit</td>
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<td>IUGR</td>
<td>intrauterine growth restriction</td>
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<td>kg</td>
<td>kilogram</td>
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<td>KOH</td>
<td>potassium hydroxide</td>
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<td>LBW</td>
<td>low birth weight</td>
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<td>LGA</td>
<td>large for gestational age</td>
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<td>MA</td>
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<td>min</td>
<td>minute</td>
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<td>MLCK</td>
<td>myosin light chain kinase</td>
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<td>NCSS</td>
<td>number cruncher statistical system</td>
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<td>o.d.</td>
<td>outside diameter</td>
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<td>Pco₂</td>
<td>partial pressure of carbon dioxide</td>
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<tr>
<td>pg</td>
<td>picogram</td>
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<td>PGE</td>
<td>prostaglandin E</td>
</tr>
<tr>
<td>PGF</td>
<td>prostaglandin F</td>
</tr>
<tr>
<td>Po₂</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>PVN</td>
<td>paraventricular nucleus</td>
</tr>
<tr>
<td>Qum</td>
<td>umbilical blood flow</td>
</tr>
<tr>
<td>r</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movements</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEE</td>
<td>standard error of the estimate</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SGA</td>
<td>small for gestational age</td>
</tr>
<tr>
<td>Tco₂</td>
<td>total Co₂ content</td>
</tr>
<tr>
<td>TV</td>
<td>tarsal vein</td>
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<tr>
<td>UV</td>
<td>umbilical vein</td>
</tr>
<tr>
<td>VLBW</td>
<td>very low birth weight</td>
</tr>
<tr>
<td>VO₂</td>
<td>oxygen consumption</td>
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I wish to dedicate this thesis to the memory of my Dad, whose glorious life will always be an inspiration to me.

“Do not follow where the path may lead. Go instead where there is no path and leave a trail.” M. Strode
1. **INTRODUCTION**

1.1 **Fetal Growth and Fetal Growth Restriction**

1.1.1 **Definition of Fetal Growth and Growth Restriction**

The term *fetal growth* refers to the normal increase in fetal body size *in utero*. The most commonly used criterion for fetal growth is weight gain. Normal intrauterine growth is the fetal expression of a genetically determined potential to grow, which is neither abnormally constrained nor promoted by internal or external factors. However, some have suggested that the fetus rarely expresses its full genetic potential for growth because of placental and maternal constraints (Gluckman and Liggins, 1984).

Experimentally, normal growth of an individual within an animal population can be defined using standard statistical methods such as a measured weight that falls within 2 standard deviations of the mean for that population (Owens et al. 1987a). However, in most sheep studies, fetuses are often categorized as growth-restricted when the mean weight of animals with experimental intervention is reduced by $> 20\%$ with statistical significance, as compared with the mean for control fetuses of the same population (Creasy et al. 1972; Harding et al. 1985; Block et al. 1989; Block et al. 1990b).

Clinically, there are many terms in common use that describe variations in fetal growth. The terms low birth weight (LBW) and very low birth weight (VLBW) describe human
infants with birth weight less than 2500 gm and 1500 gm, respectively. These terms do not incorporate a concept of gestational age. Low birth weight may occur because a baby is born preterm or is small for gestational age (SGA) (Battaglia, 1970; Yerushalmy, 1970). SGA refers to those neonates below the 10th percentile for weight adjusted for gestational age, whereas large for gestational age (LGA) refers to neonates above the 90th percentile, again adjusted for gestational age. Those between the 10th and 90th percentile are appropriate for gestational age (AGA) (Brenner et al. 1976).

SGA is often used synonymously with the term intrauterine growth restriction (IUGR). Thus, the generally accepted definition for IUGR is birth weight less than the tenth percentile for gestational age. An alternative definition is birth weight more than two standard deviations below the mean for gestational age, corresponding to approximately the third percentile (Gruenwald, 1966). Although these are useful schemes and the most frequently used clinical methods for defining fetal growth restriction, there are some drawbacks with these methods. One of major sources of potential error is that the prevalence of fetal growth restriction will vary markedly, depending on the fetal growth curve used (Goldenberg et al. 1989; Alexander et al. 1996). Another major problem is that these definitions are only statistical considerations. They do not denote a pathophysiologic process resulting in restriction of fetal growth. Strictly speaking, SGA describes a population of fetuses by weight distribution alone without specific reference to the presence of underlying pathology, while IUGR refers to a specific pathologic term describing a group of neonates with a weight at or below the 10th percentile for gestational age and sex occurring as a result of a pathologic process that inhibits the
realization of the normal intrinsic growth potential (Manning, 1995). The same definition should also apply to animal studies to define that a fetus is intrauterine growth restricted. IUGR may be the result of maternal, fetal, or placental disorders occurring alone or in combination. Therefore, the term IUGR should not be used as a substitute for SGA.

1.1.2 Symmetric versus Asymmetric Intrauterine Growth Restriction

There are two distinct patterns of slowed growth: asymmetric and symmetric intrauterine growth restriction (Gruenwald, 1965). In asymmetric IUGR, growth of the fetal trunk is restricted compared with that of the head (relative brain sparing) and the baby is of normal length for gestational age but body weight is below normal. On the other hand, in the symmetric pattern, growth of the entire fetus is restricted and both the length and weight of a baby are below normal. Therefore, the reliance on only gestational age and birth weight appears to neglect the pattern of fetal growth restriction. The commonly used method to evaluate this issue is the ponderal index (Miller and Hassanein, 1971), calculated by the formula: \[ \frac{\text{birth weight (gm)}}{\text{crown-heel length (cm)}}^3 \times 100. \] The ponderal index is normal in symmetric IUGR babies and low in asymmetric IUGR babies.

1.1.3 Factors Influencing Weight Heterogeneity in the Sheep

Fetal growth in sheep is influenced to varying degrees by numerous factors including fecundity, sex, parity, breed or breed cross, heat or cold stress, and maternal nutrition. It has been shown that for a constant placental weight, male lambs tend to be heavier than
female lambs, later born lambs heavier than first born lambs (i.e. birth weight of fetus increases with increased parity), and single lambs heavier than twin lambs (Alexander, 1964a). Also, birth weights are decreased by heat stress or poor maternal nutrition and increased by cold stress (see section 1.1.5). Furthermore, using embryo transfer techniques, there is strong evidence for maternal constraint of fetal growth in sheep. Lambs of the same genotype differed in birthweight according to whether their uterine environment was large or small (Hunter, 1956), thus confirming the elegant and classic study by Walton and Hammond (1938) about the maternal influence on fetal birth weight in horses.

1.1.4 Normal Growth of the Fetus and Placenta

1.1.4.1 Fetal Growth in the Sheep

The general patterns of fetal growth in sheep under different environmental and nutritional conditions have been studied in animals killed at different stages of pregnancy or observed at birth (Wallace, 1948; Alexander and Williams, 1971; Koong et al. 1975; Mellor, 1983). Also, serial measurement of fetal growth using an in utero crown-rump length measuring device (Mellor and Matheson, 1979; Mellor and Murray, 1982) or implantable ultrasound transducers (Taylor et al. 1983; Mesiano et al. 1988) has been reported. In addition, fetal growth can be assessed indirectly from measurements of blood or plasma volume (Caton et al. 1975; Kwan et al. 1995a). Recently, external ultrasound has been used successfully
to describe both fetal and placental growth in the sheep (Kelly et al. 1987; Barbera et al. 1995a; Barbera et al. 1995b).

In the many mammalian species that have been studied including sheep, weight of the fetus increases exponentially throughout gestation. The exponential model \( W = W_0 e^{(b_1 - b_2 t)t} \) has been used to describe fetal growth (Koong et al. 1975); where \( W \) = weight in grams, \( W_0 \) = initial weight, \( b_1 \) = initial growth rate per day, \( b_2 \) = change in growth rate per day, and \( t \) = day of gestation. The classic fetal sheep growth curve was established by Koong et al. in 1975, and describes a typical pattern of fetal weight versus gestational age, giving the equation \( W = 0.000103e^{0.614N - (0.1281 - 0.00038)t} \), where \( N \) = number of fetuses (Koong et al. 1975). According to this curve, fetal weight increases slowly during early gestation and quite rapidly during the later stages, but the equation also indicates that the relative rate of fetal growth (the proportional increase per day), which is represented by the exponent of e, decreases as gestation advances. Thus, the model for fetal growth in sheep indicates an initial growth rate (b1) of 12.81% per day, which decreases by 0.038% per day (b2) as gestation advances. This model fits the actual relative rate of fetal growth well and accurately predicts the sigmoidal pattern of fetal growth.

Despite the continual decrease in the "relative" rate of fetal growth (percentage per day), the "absolute" rate of fetal growth (g/d) increases exponentially throughout gestation, because of the large increase in fetal weight as gestation advances. In addition, fetal weight increases most dramatically during the last half of gestation. About 90% of birth weight is achieved during the last 40% of gestation (Koong et al. 1975). The rapid
changes in fetal weight suggest the need for substantial changes in maternal circulation and metabolism as well as major changes in uteroplacental function to meet fetal needs for growth and development in late gestation.

Although the regulation of fetal growth is not yet well understood, three major components are involved: the fetal genome, the placenta, and maternal factors (Robinson et al. 1994b). Obviously, these components are closely interrelated. However, the placenta plays a central role in the regulation of fetal growth through its regulation of substrate supply and its production and transport of hormones and other substances to the fetal and maternal systems.

1.1.4.2 Placental Growth in the Sheep

The sheep placenta is classified as a cotyledonary, epitheliochorial placenta (Battaglia and Meschia, 1986a) in which highly vascularized, oval structures of endometrial thickenings known as caruncles are distributed over the mucosal surface of the uterus. The non-pregnant uterus contains about 60 to 150 caruncles. These are potential sites for chorionic attachment, which takes place at about 30 days after conception and usually occupies 70-80% of available caruncles. During pregnancy, caruncles form connections with localized villous projections of the chorion, termed cotyledons. The caruncle and cotyledon combined are referred to as placentomes. Thus, the fetal and maternal tissues together constitute the placenta (Alexander, 1964a; Stegeman, 1974). The numbers of cotyledons per fetus are rather variable, ranging from ~ 20 to 100 in sheep, which depends upon
several factors such as breed and strain of sheep, litter size, parity of ewe, sex of lamb and
the number of caruncles in the uterus (Wallace, 1948; Alexander, 1964a; Stegeman,
1974). The overall growth of the sheep placenta varies widely in healthy individuals, the
ranges in size at term being 100-650 g (Mellor, 1983). Most of this variation remains
unexplained.

Placental weight tends to increase with age of the ewe (Alexander, 1964a). Stegeman
(1974) showed that placental weight is correlated with uterine weight. She speculated
that incomplete uterine development before mating may explain lower placental weights in
young ewes. The combined weight of twin placentae is usually greater than that of single
fetuses, due to a greater number of available caruncles being occupied by cotyledons (80%
in twins vs. 70% in singles) and a greater average weight of individual cotyledons.
However, the weight for individual twins is almost always less than that for singles,
indicating an important but incomplete compensation for the smaller number of cotyledons
associated with a twin (Alexander, 1964a). These general relationships extend to triplets
and quadruplets (Robinson et al, 1977a), in which the increase in total placental weight
with increasing litter size is apparent as early as 50-60 days post conception. The stimulus
for such early manifestation of placental compensatory growth is unknown. Furthermore,
Alexander (1964a) reported that male fetuses tend to have heavier placentae than females,
but Stegeman (1974) found no significant effect of fetal sex.

The ovine placental weight increases rapidly in early gestation and exceeds fetal growth
until mid-gestation when it abruptly ceases growing, while fetal weight continues to
increase and exceeds that of the placenta. Earlier studies indicated that placental weight reaches a maximum by 90 days of pregnancy and then tends to decline slowly until term at 145-150 days (Wallace, 1948; Alexander, 1964a; Rosenfeld et al. 1974; Stegeman, 1974). However, more recent data demonstrate an even earlier peak of placental size at day 75-80 of gestation when it attained its maximum in terms of placentome diameter (Kelly et al. 1987) and both wet and dry placental weights (Ehrhardt and Bell, 1995). Also, the latter changes are paralleled by changes in placental protein and DNA content (Ehrhardt and Bell, 1995).

Thus, at different stages of gestation the relative growth rates of the placenta and its fetus vary greatly. Hence, the potential demands on placental function and metabolism and their influences on fetal growth, may depend on a combination of the stage of gestation, and both intrinsic and extrinsic factors (Robinson et al. 1995).

1.1.5 Experimental Manipulation of Fetal Growth in the Sheep

1.1.5.1 Maternal Undernutrition

Maternal undernutrition (reduction in the dietary digestible crude protein or metabolizable energy) has been used extensively to reduce growth in fetal sheep. In most studies, placental growth is also reduced. For instance, moderate to severe undernutrition during the period of placental growth between 4 and 13 weeks of gestation significantly reduced placental weight at or near term (Wallace, 1948; Mellor, 1983). This effect was apparent
at 90 days of gestation after severe underfeeding during early and mid pregnancy (Everitt, 1964). There is also some indication that underfeeding during late pregnancy reduces placental weight in twin-bearing ewes but not in ewes with single fetuses (Mellor and Murray, 1981). However, there are a few reports of enhanced placental growth in early or mid pregnancy with moderate undernutrition. In these studies, placental weight was increased by maternal undernutrition during mid-pregnancy (Faichney and White, 1987; McCrabb et al. 1991; Haggarty et al. 1997). Furthermore, the effect of maternal undernutrition on placental development appears to be strongly influenced by maternal body weight at conception, with ewes of low body weight exhibiting a greater increase in placental weight than heavier ewes (Robinson et al. 1994a). Thus, when pregnant ewes have been subjected to a period of undernutrition, growth of the placenta would be either reduced or enhanced. These differences in the response of the placenta may be due to the timing, severity and duration of undernutrition, as well as level of body reserves in the ewe. Nevertheless, maternal undernutrition for most of pregnancy does reduce fetal growth by approximately 30% (Mellor, 1983). Mellor (1983) found that in six groups of pregnant ewes subjected to widely different levels and patterns of nutrition, the regression of fetal weight on loge placental weight at 142 days of gestation accounted for 69 to 91% of the variance in fetal weight, and 63% of variance when all data were pooled, thus suggesting that placental size limits fetal growth. In general, nutritional deprivation restricts fetal growth more severely in late pregnancy than similar undernutrition limited to early or mid-pregnancy (Mellor and Matheson, 1979; Mellor, 1987). Also, poor maternal nutrition prior to conception may seriously impair fetal growth and produce symmetrically
small, low birth weight lambs, whereas undernutrition in early gestation may cause asymmetrical intrauterine growth restriction (Mellor, 1983; Godfrey and Barker, 1995).

1.1.5.2 Maternal Hypoxaemia

Decreased oxygen transfer to the fetus may contribute to fetal growth restriction. Both long-term (from 30 days to 135 days gestation) and short-term (from 120 to 141 days gestation) hypobaric hypoxia produces a reduction in fetal body weight, but with slightly less than an effect compared to maternal undernutrition (Jacobs et al. 1988a). Placental weight is not affected by short-term hypoxia, though long-term exposure reduces the average size of cotyledons. The adrenal glands are significantly heavier in the hypoxic fetuses than in controls (Jacobs et al. 1988a). The mechanisms by which reduced oxygen availability limits fetal growth are not yet known.

1.1.5.3 Maternal Heat Stress

Prolonged environmental heating of pregnant ewes (such as 38-40°C for 9h or 30-32°C for 15h daily), sufficient to elevate deep body temperature, can cause dramatic reductions in placental size and fetal growth with a characteristic morphometric pattern of asymmetrical intrauterine growth restriction (Alexander and Williams, 1971; Bell et al. 1987). In heat stressed-ewes, placental weights at 130-136 days of gestation ranged from 93 to 253 g, compared with 250 to 400 g in unheated ewes from the same flock. There is a direct curvilinear relationship between the reduction in placental and fetal weights with
an increase in the fetal/placental weight ratio (Bell et al. 1987; Bell et al. 1989). Total placental DNA content is also found to be significantly reduced in the heated ewes, suggesting a reduction in cell number (Vatnick et al. 1991). Fetal growth restriction was most marked when heat exposure was continued through mid and late pregnancy, but significant reductions also occurred when heating was confined to mid (50-100 days) or late gestation (100-150 days) only (Alexander and Williams, 1971; Bell et al. 1987). Heat stress episodes as short as the final 25 days of gestation also lead to reduced fetal growth (Brown et al. 1977). Twelve hours of elevated temperature per day during the second half of gestation is sufficient to reduce lamb birth weights. The latter, however, are larger than lambs from ewes subjected to continuous heat (Shelton and Huston, 1968). Early termination of pregnancy does not appear to be an essential component of heat stress induced IUGR in sheep. One study found that a 7 days shortening of gestation in heat stressed ewes accounted for two-thirds of the 50% reduction in lamb birth weights (Shelton and Huston, 1968). Other studies, however, indicate that heat stress IUGR lambs occur with full-term deliveries (Brown et al. 1977) as well as when delivered at a constant gestational age by Caesarian section (Alexander and Williams, 1971). It also appears that heat-induced IUGR is independent of maternal feed intake in sheep. Lambs from heat-stressed ewes are significantly smaller than lambs from thermoneutral ewes even when the latter are restricted to the feed intake of the heat-stressed ewes (Cartwright and Thwaites, 1976; Brown et al. 1977).
1.1.5.4 Maternal Cold Exposure

As compared with heat stress, cold stress has an opposite effect on fetal growth. For instance, chronic maternal cold exposure induced by winter shearing or at -10°C with wool-clipped during the last 4-8 weeks of gestation can cause a significant increase in lamb birth weight, independent of any effect on feed intake (Thompson et al. 1982; Symonds et al. 1986; Symonds et al. 1992). This was attributed to elevated fetal plasma insulin levels associated with moderate maternal and fetal hyperglycemia (Thompson et al. 1982).

1.1.5.5 Maternal Hypovolemia

Since maternal body mass and plasma volume are correlated, it has been shown that reduced plasma volume or prevention of plasma volume expansion in late gestation in the pregnant sheep by intermittent withdrawal of maternal blood (25 ml/day) and/or chronic administration of diuretics can lead to mild fetal growth restriction in fetal sheep. The experimental animals have lower mean birth weight than controls, but the difference is not statistically significant. In contrast to birth weight, the ratio of birth weight to maternal weight is significantly lower in the experimental group (Daniel et al. 1989; Daniel et al. 1996; Daniel et al. 1997). No placental weight data are available in this model.
1.1.5.6 Maternal Exercise

In a small group of ewes which exercised daily for 30 min throughout late pregnancy and fed at the same level as non-exercised controls; there was an increase in lamb birth weights of exercised compared with sedentary ewes. This was attributed to stimulation of maternal feed intake or fetal hyperinsulinaemia during recovery from maternal exercise (Bell et al. 1983).

1.1.5.7 Restriction of Implantation Area

In sheep, a sufficiently large reduction in the number of placental attachment sites or endometrial caruncles prior to implantation, by direct excision, hemi-hysterotomy or ligation of a uterine horn, reduces placental size in the subsequent pregnancy (Alexander, 1964; Cefalo et al. 1977; Robinson et al. 1979; Caton et al. 1984). However, the majority of visible caruncles have to be removed to significantly restrict total placental weight. The placental compensation for the reduced number of implantation sites includes extension of the implanting conceptus to the extremes of the uterus, and an increase in size of the individual placentomes (particularly in the body of the uterus), a process that is apparent as early as day 77 of pregnancy. The stimulus to this compensatory overgrowth of placentomes is unknown. When placental size is sufficiently reduced in this way, fetal weight in late gestation or at term is also reduced and highly correlated with the reduced placental weight (Alexander, 1964b; Robinson et al. 1979; Harding et al. 1985; Owens et al. 1986). The associated fetal morphometric profile with carunculectomy is clearly that
of asymmetrical IUGR and the fetal weight/placental weight ratio is increased as the placental weights decline (Robinson et al. 1979; Harding et al. 1985; Owens et al. 1986).

### 1.1.5.8 Interference with Uterine and Umbilical Circulation

Data obtained in the pregnant ewe suggests that both the uterine and umbilical circulations are hyperperfused relative to the metabolic needs of the tissues at normal rates of flow (Battaglia and Meschia, 1986e). Thus when uterine blood flow is acutely reduced by 30-50% for a short time such as one hour, tissue oxygen uptake is maintained due to a simple reciprocal relationship between the rate of blood flow and the amount of oxygen extracted from it (Clapp, 1979; Gu et al. 1985). This fairly large, but short-term, decremental change in uterine blood flow has little impact on fetal growth. On the other hand, a chronic reduction of utero-placental blood flow in the sheep by repetitive embolization of the utero-placental circulation with microspheres produces fetal growth restriction with a classical morphometric profile of asymmetrical IUGR (Creasy et al. 1972; Clapp et al. 1982b; Charlton and Johengen, 1987). Grossly abnormal changes were observed in at least one-third of the cotyledons which were reduced in size. Placental weight was also reduced and fetal/placental weight ratio increased (Creasy et al. 1972). Serial measurements of utero-placental blood flow demonstrate that there is a direct relationship between the rate of utero-placental blood flow and the weight of the uterus and its tissue contents as well as growth of the fetus (Clapp et al. 1982b). Mechanical occlusion of a maternal iliac artery in the pregnant sheep in late gestation also reduces utero-placental blood flow (Clark et al. 1982), but only in the longer term restricts fetal growth, also
producing the classical morphometric profile of asymmetrical fetal growth restriction (Lang et al. 1997).

Embolization of the umbilical circulation in sheep by repetitive administration of microspheres in late gestation increases vascular resistance and reduces flow within the umbilical circulation (Trudinger et al. 1987; Gagnon et al. 1994). Longer term embolization (about 3 weeks) of the umbilical circulation can also induce asymmetrical fetal growth restriction as indicated by a reduction in the ponderal index and an increase in the brain/liver weight ratio (Gagnon et al. 1997). In this model, fetal and placental growth appears to be affected equally. The reduction in placental weight is in proportion with the reduction in fetal body weight and there is no consistent directional change in the fetal/placental weight ratio (Dr. Gagnon, personal communication). In contrast, ligation of one umbilical artery in the fetal sheep, in mid-gestation, reduces growth substantially, and in late gestation, can result in fetal death (Emmanouilides et al. 1968).

1.1.5.9 Fetal Factors Controlling Growth

The adjustment of fetal metabolism and growth to the available substrates requires a finely tuned regulatory system. The fetal endocrine status seems to play an important role in the coupling between supply of substrates and fetal growth (Owens, 1991). Among the hormones involved, insulin, thyroxine, triiodothyronine, and the insulin-like growth factors (IGFs) including IGF-I and IGF-II as well as IGF binding proteins (IGFBPs) seem to have a prominent place (Owens, 1991; Gluckman and Harding, 1994; Harding et al. 1994;
Gallaher et al. 1995). For example, ablation of some fetal organs such as thyroid and pancreas in fetal sheep alters and restricts growth (Fowden, 1992; Fowden and Silver, 1995). Thus, fetal growth is clearly dependent on thyroid hormones and insulin, which may act partly via circulating IGFs (Owens, 1991). Recently, severe fetal hemorrhage (40-45%) has also been found to result in impaired fetal growth. The effect may be due to inhibition of insulin by increased circulating catecholamine concentrations in these fetuses (Kwan et al. 1995a).

1.1.6 Hemodynamic, Metabolic and Endocrine Changes in Experimental Restriction of Fetal Sheep Growth

1.1.6.1 Hemodynamic Changes

Studies on the effect of undernutrition during late pregnancy on uterine and umbilical blood flow and uteroplacental metabolism have shown that uterine blood flow is reduced by 25-30% and that fetal placental blood flow is decreased by 20% (Morris et al. 1980). During fasting there is also a redistribution of maternal cardiac output away from the utero-placental circulation (Morris et al. 1980). Chronic heat stress imposed from 60-70 days of pregnancy in sheep is associated with reduced uterine (Alexander et al. 1987; Bell et al. 1987) and umbilical blood flows (Bell et al. 1987), compared to age-matched controls. There is a direct linear correlation between the reduction in both uterine and umbilical flow and the reduction in placental weight (Bell et al. 1987). Decreased uterine perfusion also occurs with long-term maternal hypovolemia (Daniel et al. 1989). In small
fetuses of the carunculectomy model, both umbilical and uterine blood flows are significantly lower than in controls at 121 and 130 days gestation. Also, near-term microsphere measurements of both utero-placental and umbilical flow demonstrate a direct linear relationship between absolute flow rates in the two circulations and both placental and fetal weight (Owens et al. 1986).

In the uteroplacental circulation embolization model, the growth restricted sheep fetuses demonstrate a redistribution of the circulation to provide preferential perfusion to the vital organs (Creasy et al. 1972; Block et al. 1984). Blood flow to the adrenal glands, brain, and heart is significantly higher in these fetuses as compared with the controls, but umbilical perfusion is significantly lower. Studies on circulatory responses to spontaneous hypoxemia in growth restricted fetuses show that (1) combined ventricular output (CVO) tends to be increased; (2) CVO is redistributed so that oxygen delivery to the heart is increased by over 50%; (3) oxygen delivery to the lung and placenta is decreased, but maintained to other organs, including the brain (Goetzman et al. 1984). On the other hand, in response to acutely imposed hypoxemia, growth restricted fetuses have more pronounced blood flow responses to all vital organs than normally grown fetuses, with the exception that the proportion of combined ventricular output perfusing the placental does not change significantly (Block et al. 1984).
1.1.6.2 Metabolic Changes

Maternal undernutrition is associated with a significant decrease in uteroplacental oxygen uptake, but fetal oxygen uptake is not affected. Undernutrition also causes large decreases in uterine, umbilical and uteroplacental net uptakes of glucose, and similar declines in uterine and umbilical glucose/O\(_2\) quotients, and also a reduction in umbilical net uptake of lactate (Chandler et al. 1985; Leury et al. 1990a). These changes are accompanied by a decrease in placental glucose transfer capacity (Leury et al. 1990a). Furthermore, in underfed ewes, fetal glucose utilization rate greatly exceeded net umbilical uptake of glucose, suggesting induction of a substantial rate of fetal endogenous glucogenesis (Leury et al. 1990b).

IUGR induced by restriction of placental growth in sheep using surgical excision of endometrial caruncles prior to pregnancy is characterized by chronic fetal hypoxaemia (Harding et al. 1985; Owens et al. 1987a; Owens et al. 1987b; Phillips et al. 1996), hypoglycemia (Harding et al. 1985; Owens et al. 1987b) and hyperlacticemia (Owens et al. 1987b), and is associated with reduced rates of both oxygen and glucose delivery to and consumption by the gravid uterus and fetus (Owens et al. 1987a; Owens et al. 1987b). While both respond by increasing oxygen extraction, only the fetus attempts to maintain glucose consumption by increasing extraction. Utero-placental oxygen consumption is significantly lower in small fetuses compared to that in controls. Despite these changes, oxygen consumption by the gravid uterus and fetus, per kg of tissue mass, is similar in both groups of carunclectomized and control sheep. Utero-placental oxygen consumption
per kg of utero-placental mass in small fetuses is not significantly different from controls, although it averages only 25% of that in controls (Owens et al. 1987a). This is most probably because of a large variability in the estimates of utero-placental oxygen consumption. On the other hand, utero-placental glucose consumption is significantly lower in small fetuses compared to that in control ewes, both as a total and per kg of placenta. Fetal glucose consumption per kg of fetus is similar in control and carunclectomized sheep, but fetal glucose extraction increases as fetal weight decreases. Moreover, utero-placental production of lactate per kg of placenta is significantly higher in carunclectomized ewes compared to controls and increased as fetal femoral arterial oxygen content decreased. Consequently, a greater proportion of this increased lactate is released into the fetal circulation. Fetal lactate consumption per kg of fetus increased as the concentration of lactate in blood from the common umbilical vein increased (Owens et al. 1987b). As a result of these qualitative changes in placental metabolism, the partition of oxygen and glucose within the gravid uterus was dramatically altered in favor of the fetus.

Similar changes also occur when placental size in sheep is reduced by heat exposure throughout mid-and into late pregnancy (Alexander and Williams, 1971; Bell et al. 1987). Reduced placental weight during chronic heat stress is associated with decreased maternal to fetal transfer of oxygen and glucose (Bell et al. 1987). Placental glucose transfer capacity is also significantly reduced (Thureen et al. 1992). These effects are accompanied by an enlargement of the PO₂ difference between uterine and umbilical venous blood, a decrease in the PO₂ and oxygen saturation of fetal arterial blood, and fetal hypoglycemia.
Uteroplacental rates of oxygen and glucose utilization are significantly correlated with placental weight. From this, it has been concluded that heat-induced fetal growth restriction is secondary to a primary reduction in placental size, and that a suppression of placental function is important in heat-induced fetal dwarfing (Bell et al. 1987; Bell et al. 1989).

When vascular damage is experimentally produced in the sheep placenta late in gestation by microsphere embolization, both the total and weight specific rates of oxygen, glucose and lactate consumption by the fetus are also reduced (Clapp et al. 1981). Chronic fetal placental embolization also produces progressive fetal hypoxemia (Gagnon et al. 1994; Gagnon et al. 1997). Similarly, a prolonged uterine blood flow reduction with external occlusion of the terminal aorta in sheep results in a significant decrease in fetal oxygen delivery and fetal arterial oxygen content, but there are no differences in the uptakes of oxygen, glucose, and lactate by the fetus (Boyle et al. 1996). Positive correlations were found between linear growth rate and fetal arterial oxygen content and between linear growth rate and fetal oxygen delivery. The authors conclude that the correlations between linear growth rate and fetal oxygenation provide strong evidence of the central role of oxygen in the regulation of fetal growth (Boyle et al. 1996). Chronic hypobaric hypoxia has no effect on the supply of glucose to the fetus, but it is associated with a chronic elevation in the concentration of plasma lactate (Jacobs et al. 1988b). Mild growth restricted fetuses, produced by prevention of expansion of maternal plasma volume, are also hypoglycemic during early and late gestation but hypoxemic only during late gestation (Daniel et al. 1996).
In hypothyroid fetuses with growth restriction caused by either fetal thyroidectomy or hypophysectomy, umbilical O\textsubscript{2} uptake expressed on a weight-specific basis is reduced. This is accompanied by fetal hypoxaemia and significant reductions in the rates of glucose oxidation, CO\textsubscript{2} production from glucose carbon and O\textsubscript{2} utilization for glucose oxidation (Fowden and Silver, 1995). In growth restricted sheep fetuses caused by pancreatectomy, there are reductions in the rates of umbilical uptake, fetal utilization and oxidation of glucose (Fowden, 1992).

Thus when placental and hence fetal growth is restricted, a qualitative reduction in the utilization of substrates by the placenta occurs, helping to conserve oxygen and in particular glucose for the fetus. At the same time, increased extraction of these substrates by the gravid uterus and certainly the fetus suggests that a reduction in the margin of safety between supply and demand exists (Owens et al. 1987a; Owens et al. 1987b). As a consequence, perturbations which lead to reduction in supply or increased demand, such as uterine contractures (Sunderji et al. 1984), fetal breathing movements (Rurak and Gruber, 1983a) and in particular the stress of labor, may be potentially more threatening to the viability of the growth restricted fetus than to a normally grown fetus.

1.1.6.3 Endocrine Changes

Chronic restriction of substrate and chronic hypoxaemia are the probable causes of the wide variety of endocrine changes that have been documented for the growth restricted fetus. Hormones whose blood concentrations are reduced in growth restricted fetuses
include insulin (Robinson et al. 1980; Harding et al. 1985), thyroxine, triiodothyronine (Mellor and Pearson, 1977; Robinson et al. 1980; Harding et al. 1985; Jones, 1985), and the IGF-I and II (Owens et al. 1994; Kind et al. 1995). The concentrations of insulin and IGF-I correlate closely with plasma glucose (Owens, 1991). IGF-II concentrations are reduced in the most severely growth-restricted fetuses (Owens et al. 1994). In late gestation the plasma concentration of cortisol (Robinson et al. 1980; Clapp et al. 1982a; Harding et al. 1985; Phillips et al. 1996; Gagnon et al. 1997), catecholamines (Jones and Robinson, 1983), and glucagon (Jones, 1985) are high in the growth restricted fetus.

In summary, fetal growth has been experimentally restricted in a variety of ways either prior to pregnancy or at later stages of gestation in the sheep. The responses of the placenta and fetus are surprisingly consistent in many aspects. The experimental perturbations discussed above, such as a reduction in uterine blood flow, placental destruction, limitation of placental growth or maternal heat stress and maternal nutritional deprivation, all produce evidence of asymmetric growth restriction in the fetal lamb. The animal data in which metabolic measurements have been obtained indicate that when growth restriction is fully developed, all metabolic parameters are consistent with a restriction of substrate availability. These changes include a decrease in oxygen content and glucose concentration with an increase in lactate concentrations, and in most of cases, reduced rates of both oxygen and glucose delivery to and consumption by the gravid uterus and fetus. One difference is that when low birth weight is due to maternal undernutrition, chronic prenatal hypoxaemia is absent, but hypoglycaemia remains (Mellor, 1983; Mellor and Cockburn, 1986). Also, in these models, although placental
transfer and substrate uptakes are markedly reduced at this point, they remain closely matched to fetal weight. Thus, the reduction of fetal growth rate, as the response to noxious maternal and placental stimuli that ultimately limit placental growth and transfer function, has survival value as it immediately decreases absolute utilization of oxygen and metabolic substrates. Likewise, the change in the distribution of fetal cardiac output also has distinct survival value. In addition, from these studies it is suggested that the placental responses to a variety of negative stimuli initiate a restriction of fetal growth which is mediated by as yet incompletely understood mechanisms. As suggested by recent epidemiological studies, the potential impact of IUGR might well extend to adulthood with increased propensity to develop hypertension and ischaemic heart disease (Barker and Fall, 1993; Barker, 1996a; Barker, 1996b). Thus, a better understanding of the mechanisms involved in fetal growth as well as the short and particularly the long-term consequences of fetal growth restriction is of great importance.

### 1.2 Initiation of Parturition in the Sheep

In the sheep, it is now clear that parturition occurs as a result of many complex interactions between mother and fetus involving the sequential maturation of an endocrine organ communication system (Challis and Olson, 1988). The experiments on the sheep emphasized the role of the fetus in triggering labor (Liggins et al. 1973; Thorburn and Challis, 1979). Through increased activity of the hypothalamic-pituitary-adrenal axis and increased cortisol output from the adrenal gland during late gestation, the fetus provides the signal for the onset of parturition (Liggins et al. 1973). Hypophysectomy of the fetal
lamb in utero obliterates the normal prepartum increases in fetal adrenal weight and in plasma cortisol concentrations and prolongs gestation. On the other hand, fetal infusion of adrenocorticotropic hormone (ACTH) results in a precocious increase in fetal adrenal weight, and in plasma cortisol concentrations, and premature delivery that, endocrinologically, may resemble the sequence of events seen at full term (Liggins et al. 1973; Thorburn and Challis, 1979). McDonald and Nathanielsz (1991) showed that bilateral lesions of the fetal ovine hypothalamic paraventricular nucleus (PVN) prolong gestation significantly. In addition, following lesions of the fetal PVN, fetal plasma concentrations of both ACTH and cortisol failed to undergo the normal periparturient increase, further supporting a hypothalamic signal for parturition in sheep. The hormone secreted by PVN from the fetal hypothalamus is corticotropin-releasing hormone (CRH), which plays a central role in initiating the birth process.

Clear evidence for an endocrine organ communication system leading to birth is now available in the sheep. In this species, pituitary responsiveness to CRH increases between days 110 and 125 (Norman and Challis, 1987), and precedes the increase in adrenal response to ACTH, which occurs between days 125 and 135 (Challis and Olson, 1988). The production of ACTH in the fetal lamb is controlled by CRH. The increase in secretion of ACTH stimulates the fetal adrenal cortex to grow and to secrete more cortisol. The maturation of the fetal adrenal cortex is the key factor in the initial movement toward parturition. Fetal cortisol induces changes in placental steroid output at about 7 days prepartum, via inducing expression of the steroidogenic enzyme, 17α-hydroxylase. This results in a decrease in the output of progesterone from the placenta.
into the maternal circulation, and a later increase in the concentration of unconjugated estrogen (Thorburn and Challis, 1979). Maternal plasma estrogens rise during the last 36 to 48 hours of pregnancy, closely associated with elevated uterine output of stimulatory prostaglandins, especially PGF$_{2\alpha}$. The prostaglandins have effects on the placenta (release of relaxin, inhibition of progesterone production), on the ovaries (release of relaxin), on the uterus (relaxation of the cervix, contraction of the myometrium), and on the neurohypophysis (release of oxytocin) (Thorburn and Challis, 1979; Challis and Lye, 1986). Prostaglandin production within the uterine cervix itself appears to play an important part in the process of cervical softening, dilatation and effacement, by increasing the proportion of collagen in the tissues of the body of the uterus and of the cervix (Ellwood et al. 1979; Ellwood, 1980). The high levels of estrogen also lead to an increase in myometrial oxytocin receptors and gap junctions (Thorburn and Challis, 1979; Challis and Lye, 1986). The biochemical mechanisms controlling contractions in the myometrium are similar to those in other smooth muscle. Once labor is initiated myometrial contraction and relaxation proceed via the enzymatic phosphorylation and dephosphorylation of myosin and subsequent promotion and inhibition of myosin-actin interactions. The key enzyme is myosin light chain kinase (MLCK). Activity of MLCK is regulated by calcium, calmodulin, and cyclic adenosine monophosphate (cAMP)-mediated phosphorylation, which is in turn influenced by hormones and pharmacological agents (Huszar and Naftolin, 1984; Myers and Nathanielsz, 1993).

In summary, maturation of the fetal hypothalamic-pituitary-adrenal axis is the dominant event leading to preparation of the fetal and maternal systems for impending parturition in
the sheep. Fetal cortisol is involved in activating the fetal-placenta enzyme systems for the appropriate synthesis within the estrogen-progesterone complex. This initiates the whole cascade of hormonal events involving changes in the secretion of placental steroid hormones, PGF2α, oxytocin activation and softening and dilatation of the uterine cervix. In the sheep during pregnancy the ratio of progesterone:estrogen is high, and uterine contractions are limited and weak; they are called contractures (see section 1.3.1.1). Thus, systemic levels of progesterone act to maintain myometrial quiescence during pregnancy, while contractures appear to be due to the intrinsic contractility of the smooth muscle cells (Myers and Nathanielsz, 1993). Decreases in progesterone and increases in estrogen secretion close to term appear to exert a major influence on the myometrium, thus establishing conditions that are favorable to well-coordinated contractions of myometrial smooth muscle to initiate and maintain labor (Lye, 1996). Although the timing of parturition in the sheep is clearly dependent on a cascade of endocrine signals from an intact fetal hypothalamo-pituitary-adrenal axis, the nature of the stimulus and the source of central neural activation are still unknown. Recently, an interesting hypothesis has been proposed that fetal hypoglycaemia in late gestation may act as a chronic stressor at the fetal hypothalamus level to stimulate ACTH secretion, thus initiating birth events (McMillen et al. 1995).

1.3 Mechanics of Parturition in the Sheep

Successful parturition depends on two mechanical processes: the establishment of regular and effective uterine contractions and the ripening and dilatation of the cervix.
1.3.1 Myometrial Contractions

1.3.1.1 Patterns of Myometrial Activity

Myometrial activity occurs throughout pregnancy. In all mammalian species studied including the sheep, two distinct types of myometrial activity epochs during pregnancy have been distinguished (Hindson and Ward, 1973; Nathanielsz et al. 1980a; Harding et al. 1982; Figueroa et al. 1985; Figueroa et al. 1987). When recorded as intrauterine pressure changes or as myometrial electromyographic activity, labor is associated with contractile epochs lasting approximately 1 minute. These short epochs are referred as to contractions. The commonest form of myometrial activity, during pregnancy in sheep, is epochs of activity lasting about 5-10 minutes with a frequency of approximately one epoch every 20-60 minutes, associated with smaller amplitude intrauterine pressure changes (around 2-5 mmHg) than those that occur with labor contractions. The myometrial electromyographic bursts accompanying these epochs are also more fragmented than those occurring at labor. These longer lasting epochs are referred as contractures, which are analogous to Braxton-Hicks contractions in human pregnancy (Hindson and Ward, 1973; Nathanielsz et al. 1980a; Harding et al. 1982; Figueroa et al. 1985; Figueroa et al. 1987).

*Uterine contractures* provide a powerful source of repeated stimulation for the fetus throughout much of gestation, and have a number of effects on the fetus. In the chronically catheterized fetal sheep contractures are associated with a temporary fall in fetal Po$_2$, which in part is due to a reduction in uterine blood flow (Jansen et al. 1979a).
Also fetal pH falls during contractures in sheep while Pco₂ increases (Smits et al. 1986; Woudstra et al. 1995). Nathanielsz et al. (1980a) demonstrated changes in the fetal electrocorticogram (ECoG), electro-oculogram (EoG) and/or intratracheal pressure with 61 - 74 % of contractures, i.e., the ECoG changed from low-voltage, fast activity to high-voltage slow activity, the EoG pattern changed from rapid eye movements (i.e., REM sleep) to quiet sleep (i.e., non-REM sleep), and rapid irregular breathing to absent or sporadic breathing. Parer et al. (1984) used the microsphere method to assess fetal blood flow distribution before and during contractures. There was a mean increase of 19% in fetal carcass blood flow, a trend toward increased cardiac output, and myocardial and adrenal blood flow. In addition, there is a rise in fetal arterial and venous pressures during contractures, which is likely the result of translocation of blood from the placenta to the fetus, but fetal arterial pressure and heart rate decrease transiently just prior to a contracture (Brace and Brittingham, 1986). Contractures have also been shown to alter fetal hormone secretion (Woudstra et al. 1991), fetal dimensions (Nathanielsz et al. 1980a) and intracranial pressure (Walker and Harding, 1986). Recently, the effects of uterine contractures on the temporal organization of spontaneous motor activity in the fetal sheep were investigated during the last fifth of gestation. The results demonstrate that the stimulation associated with contractures may be an important influence on early behavioral organization, in that (1) uterine contractures might induce transient cerebral hypoxemia in the fetus, and (2) conditions established in the first few minutes of sustained uterine activity constitute the effective perturbation of fetal motor activity (Robertson et al. 1996).
Apart from the above changes in the fetus, the uterine contractures are also associated with maternal physiologic changes. During contractures there is a transient decrease in uterine blood flow (Nathanielsz et al. 1984). Approximately 70% of contractures are associated with a decrease in uterine blood flow of about 10% (Cabalam and Nathanielsz, 1981; Harding and Poore, 1984). In pregnant sheep, contractures are also associated with transient maternal bradycardia and slight hypertension (Rurak et al. 1993), similar to those reported in the fetus. The importance of these latter changes remains unknown.

1.3.1.2 The Switch from Contractures to Contractions

As discussed previously, labor is regulated by a multifactorial system of interconnected positive and negative feedback loops that involve endocrine and/or paracrine components. Myometrial contractility occurs throughout pregnancy and characteristic patterns of myometrial activity exist according to the endocrine status and the relationship to parturition. Throughout pregnancy, myometrial activity is of the contractures type, long-lasting, low-amplitude epochs of activity, but switching to predominantly contraction-type activity at term. This switch from contractures to contractions tends to occur at night (Nathanielsz et al. 1995). Eventually labor and delivery occur if the contractions last long enough and cervical dilation occurs. In terms of myometrial activity, labor is characterized by high-frequency, high-amplitude contractions with a relatively short duration of ~1 minute (Harding et al. 1982; Lye et al. 1983).
The shift of myometrial activity from predominantly contracture to predominantly contraction type activity is related to alteration in maternal plasma estrogen concentrations and maternal oxytocin function. It has been known that estrogen stimulates the release of oxytocin from the pituitary, as well as increasing the number of oxytocin receptors in the myometrium. Also, estrogens stimulate the production of prostaglandins (Challis and Lye, 1986; Jones and Challis, 1989; Myers and Nathanielsz, 1993). In sheep, during late gestation, there is also a good correlation between changes in gap junction formation in the myometrium and the estrogen:progesterone ratio (Garfield et al. 1979). Also, late in gestation the stress of food withdrawal increases both myometrial activity and plasma estrogens in the maternal circulation and may lead to preterm labor (Fowden and Silver, 1985; Milvae et al. 1987). Moreover, myometrial studies in the pregnant sheep demonstrated that the beginning of the rapid increase in concentration of maternal peripheral estrogens preceded the onset of parturient uterine contractility (Rawlings and Ward, 1976). All of these observations are in keeping with the hypothesis that estrogen plays a central role in preparing the myometrium for a periodic signal that causes the switch from contractures to contractions in the sheep (Nathanielsz et al. 1995).

1.3.2 Changes in the Cervix

A major function of the cervix during pregnancy is to retain the growing conceptus within the uterus. The cervix is firmer and more rigid than the uterine wall, which is due to the higher content of connective tissue (collagen). At labor, dilation of the cervix is due more to changes in the physical characteristics of cervical collagen (ripening) than to increased
intrauterine pressure. This is evident in species such as sheep, which have a rigid cervix (Fitzpatrick and Dobson, 1979). During spontaneous parturition there is a progressive loosening of the collagenous fiber bundles in the stroma of the ovine cervix, which change from an organ with predominantly thick collagen fiber bundles to one with a loose network of collagen fibrils (Owiny et al. 1987). Most of the softening of the ovine cervix appears to take place immediately prior to the onset of labor and delivery of the fetus (Owiny et al. 1991). Ripening of the cervix is hormone dependent and influenced by factors such as the elevated levels of estrogens and prostaglandins at the onset of parturition (Ellwood et al. 1979; Ellwood et al. 1980; Ledger et al. 1983). During labor, the sheep cervix changes from being long, hard and tightly closed, to being fully dilated (Fitzpatrick and Dobson, 1979). The cervix dilation occurs slowly and 1 or 2 hours before delivery little progress had been observed, suggesting that the final stages of dilatation must be rapid. This is most probably due to the mechanical advantage achieved once the amniotic sac is bulging through the cervix (Hindson et al. 1965). When the cervix is fully dilated the expulsion of the lamb appears to be accomplished for the most part by intense abdominal muscular contractions and straining movements. Accompanied by ripening and dilatation of the cervix during labor, cervical electromechanical activity decreases as the wall tension is reduced (Toutain et al. 1983; Ruckebusch, 1991).
1.4 Characteristics of Labor in the Sheep

1.4.1 Maternal Signs of Impending Parturition

1.4.1.1 Maternal Physical Signs and Behavioral Changes

The gestation length in sheep is about 145-147 days in domestic breeds. Pregnancy is usually shortened by one or two days if the ewe is carrying more than one lamb. Physical signs of approaching parturition include enlargement and swelling of the vulva and/or a discharge of mucus from the vulva (Houpt, 1991; Ruckebusch, 1991). These signs are useful as a guide, but they are too variable for an accurate prediction of the date of parturition. Also, just before lambing, certain behavioral signs are characteristic, such as restlessness, frequent lying down and getting up, pawing at bedding and frequent urination (Houpt, 1991; Ruckebusch, 1991). There was considerable variation in the length of preparturient behaviors observed in domestic sheep, but the same behaviors were seen regardless of breed or age of the ewe (Arnold and Morgan, 1975). Similar to the changes in maternal physical signs, these various behavioral signs present around the time of parturition can be used for a signal of impending parturition but not for accurately predicting the exact time of parturition.
1.4.1.2 Maternal Temperature

Rectal temperature changes have been studied as indicators of impending parturition in sheep under the assumption that certain hormones influence body temperature. For example, progesterone raises the basal body temperature because it causes an increase in the basal metabolic rate. In 80% of the ewes, body temperature drops about 0.5° C (below 39.4° C) during the last 48 hours prior to lambing (Ruckebusch, 1991). However, since this change is not dramatic, the temperature index has not been found to be a reliable indicator in the ewes.

1.4.1.3 Maternal Progesterone and Estrogen Profiles

One of the initial and important preparturient events observed in pregnant ewes is a hormone changeover from progesterone to estrogen production. There is a very significant decrease in the maternal plasma progesterone concentration during the last week of gestation (Bassett et al. 1969; Bassett and Thorburn, 1971) and a marked increase in maternal concentration of estrogens just prior to parturition (Challis, 1971). Thorburn et al. (1972) reported further observations on changes in estradiol-17β levels in uterine venous blood of sheep before parturition. The time course of the increase in estrogen follows the fall in progesterone concentration in the maternal blood, which begins at less than 5 days prior to parturition. This event is triggered by a rise in the level of cortisol in fetal blood, which begins 20-25 days before delivery (Magyar et al. 1980). A higher output of hypothalamic corticotropin-releasing hormone and adenohypophysial
ACTH is the cause of this higher level of cortisol in the fetal circulation (Challis and Olson, 1988).

As discussed earlier, the increase in estrogen level appears to convert the uterus from a state of quiescence to having potential for contractility. Hindson and Ward (1973) first pointed out the relationship of change in the type of uterine activity and an increase in the activity of the uterus with elevation in plasma estrogen concentrations. Rawlings and Ward (1976) investigated the association between hormonal changes and parturient uterine activity. The concentration of estrogens and progesterone in the peripheral plasma and the amplitude and frequency of uterine contractions were measured before and during parturition in six ewes. The concentration of progesterone in maternal peripheral plasma fell gradually over the last 7 days of gestation, with a more rapid decline in the last 2-3 days in some ewes. The maternal peripheral total estrogen concentration began to rise at about 50 h before fetal delivery, with an accelerating increase in the last 20 h. Thus, a dramatic increase in the plasma concentration of total unconjugated estrogens occurs shortly before parturition in sheep (Challis, 1971). The beginning of the rapid increase in concentration of maternal peripheral estrogens preceded the onset of parturient uterine contractility. Moreover, induction of preterm labor by infusion of ACTH to the sheep fetus resulted in a reduction of plasma progesterone concentration and increases in values of estradiol-17β in maternal plasma. The onset of myometrial contractile activity indicative of labor followed these endocrine changes (Olson et al. 1984; Lye and Freitag, 1990). Thus, a decline in maternal plasma progesterone concentrations and elevated plasma estrogen concentrations could be indicative of impending parturition in the sheep.
1.4.2 The Process of Labor

It is customary to divide the act of parturition in sheep into three stages (Ruckebusch, 1991). The first stage consists of uterine contractions that gradually force the allantoic and amniotic fluid sac against the uterine side of the cervix, causing it to dilate. In advanced first-stage labor, (at about 2 to 8 hours before lambing), uterine contractions and relaxation occur without pause, and the intrauterine pressure rises to an amplitude of 20 mmHg. As the fetus progresses through the cervix, the allantochorion ruptures, releasing allantoic fluid that marks the end of the first stage of labor. At this stage, parturient discomfort is evidenced by the animal's lying down, getting up, paddling with the hind feet, and restlessness. The distention of the cervix and vagina by the conceptus initiates a neurohumoral reflex, which produces the expulsive force of abdominal muscular contractions (straining) and the release of oxytocin, which in turn accentuates myometrial contractions. The combined forces of intra-abdominal and intrauterine pressure mark the beginning of the second stage of labor. When the allantoic and then the amniotic fluid escapes, some vigorous maternal abdominal straining, which is superimposed on uterine contractions, will lead to the eventual delivery of fetus. In the second stage of parturition there is close coordination between uterine and abdominal contractions and a further increase in uterine activity (Hindson and Ward, 1973). Most ewes are recumbent until the fetus is partially or completely expelled. The third stage is highlighted by the rupture of the umbilical cord and the expulsion of the fetal membranes from the uterus.
1.4.3 Duration of Parturition

According to Ruckebusch (1991), the electromechanical events recorded by an integrated electromyogram of the pregnant horn and cervix showed that about 48 h before parturition, uterocervical contractile activity decreases for the next 12 to 24 h. In the last 24 to 36 h before delivery, uterocervical contractile activity resumes and increases progressively to become almost continuous shortly before parturition. On the basis of the myometrial studies in the pregnant sheep, Hindson and Ward (1973) reported that a change in the type of uterine activity and an increase in the activity of the uterus occurs between 30 and 12 h before fetal delivery. They indicated that a qualitative change in contractions marked the beginning of first stage labor, from 10 to 18 h before the lamb was delivered. Cervical dilation of 4 cm occurs at around 3 h before delivery (Hindson et al. 1968; Hindson and Ward, 1973). The time when maternal abdominal straining begins represents the onset of second stage labor, with clearly coordinated and propagated contractions of one minute duration occurring at a regular frequency of 30 per hour (Fitzpatrick and Dobson, 1979), which is about 1-2 h prior delivery. Using a sensitive and precise method for recording uterine muscle activity (EMG), Harding et al. (1982) documented that in the sheep in which premature labor was induced, changes in the uterine EMG pattern could be observed 34 h before delivery. In the ewe undergoing spontaneous labor, the number of EMG bursts began to increase 67 h before delivery. Data from studies of Rurak et al. (1987a) showed that duration of labor ranges from 10 to 17 h. Thus, the duration of parturition in sheep varies widely, perhaps depending on the size of the fetus and presentation of the fetus, litter sizes as well as the type of study. In
addition, the duration of birth is longer in primigravid than in multigravid ewes. A likely reason is the smaller size of the birth canal related to the size of the fetus. Twin births are usually more rapid than singleton births, but the interval between delivery of twins varies from a few minutes to an hour or more (Ruckebusch, 1991).

1.5 The Effects of Uterine Contractions on the Uteroplacental Circulation during Labor

It has long been recognized that uterine contractions diminish uteroplacental blood flow. For instance, experiments conducted in sheep demonstrated that the flow of maternal blood to the gravid uterus is decreased during uterine contractions (Assali et al. 1958). Studies done in labor in pregnant sheep using the electromagnetic flowmeter technique have documented a fall in uterine blood flow that is roughly proportional to the magnitude of the contraction, whether spontaneous or induced by oxytocin (Assali et al. 1958; Assali et al. 1961; Greiss, 1965). Also, uterine blood flow is decreased on average by 50% or more within the first hours of parturition, although the patterns of change in blood flow varied considerably among the animals (Caton et al. 1980). Since in these studies perfusion pressures did not change during uterine contractions, it was concluded that the fall in uterine blood flow was secondary to increased vascular resistance within the uterus. On the other hand, measurements of uterine blood flow in pregnant ewes during ACTH-induced labor using radioactive microspheres showed no significant change in uterine blood flow. However, the partition of blood flow was altered; thus myometrial flow increased while uteroplacental blood flow decreased (Towell and Liggins, 1976).
The fall in blood flow during myometrial contractions during either spontaneous or oxytocin-induced labor is inversely related to the increase in intrauterine pressure (Assali et al. 1958; Assali et al. 1961; Greiss, 1965). The other important variables include frequency and duration of contractions, and the contour of the contraction waveform as measured by an intrauterine pressure catheter (Assali et al. 1958; Assali et al. 1961; Greiss, 1965; Harbert, 1992). In addition, prolonged maternal pushing efforts (bearing down efforts) in the laboring ewe in late labor is associated with a further increase in intrauterine pressure and a decrease in uteroplacental blood flow (Greiss, 1965), similar to what has been described in the human (Barnett and Humenick, 1982, Janbu et al. 1985; Janbu and Nesheim, 1987).

1.6 Intrapartum Fetal Blood Gas Status and Acid Base Balance

1.6.1 Animal Data

In species such as the sheep and goat, indwelling catheters can be maintained in fetal vessels over prolonged periods so that samples can be collected from the undisturbed fetus in late gestation or during parturition (Meschia et al. 1967; Comline and Silver, 1972). However, there are very limited data on fetal blood gas and acid base changes during labor in animal species. In fetal lambs, there is a progressive decline in $P_{O_2}$ and pH and a rise in $P_{CO_2}$ during labor, with the greatest changes occurring in the 15 minutes prior to delivery. Fetal plasma lactate levels also rise (Comline and Silver, 1972; Stark et al. 1979; Stark et al. 1981; Rurak et al. 1987a). With continuous monitoring of fetal intravascular or
transcutaneous $\text{PO}_2$ in fetal sheep, it has also been shown that uterine contractions during labor produce a fall in fetal vascular or tissue $\text{PO}_2$ (Jansen et al. 1979b; Towell et al. 1985). In addition, blood samples taken from fetuses of sows during parturition demonstrated that fetal blood gas tensions, pH, glucose and lactic acid concentrations were stable until the last hour of labor with a fall in pH and $\text{PO}_2$ and mild hypercapnia in some fetuses (Randall, 1982). The changes in blood gas status and acid base balance have also been observed in the fetal rhesus monkey during labor (Morishima et al. 1975). Recently, experimental pulse oximetry devices have been calibrated using fetal lambs to provide rapid information about fetal oxygenation. Arterial oxygenation measurements obtained using pulse oximetry correlated well with simultaneous in vitro measurements over the range of 6 to 81% (Harris et al. 1993). However, they have not been used for estimating fetal oxygen saturation in animals during labor.

### 1.6.2 Human Data

Several approaches have been employed to evaluate human fetal blood gas status and acid-base balance during the peripartum period. These include measurement of blood gas status in blood obtained either from a segment of umbilical cord clamped at delivery or by ultrasound-guided cordocentesis or fetoscopy, which can gain access to fetal circulation (Hare and Ludomirsky, 1994). Also, fetal scalp blood may be analyzed either intermittently for both fetal blood gas and acid-base status in labor (Saling, 1968) or continuously with fetal scalp electrodes for acid-base determination only (Huch et al. 1977; Young et al. 1978). Technical difficulties, mainly relating to attachment of the
electrode to the fetal scalp, have inhibited the widespread acceptance of this latter method for human fetal monitoring in labor. Besides, the transcutaneous pH electrode does not provide data to differentiate between metabolic and respiratory components of the acidosis (Nickelsen and Weber, 1991). Currently, a new technique for intrapartum fetal oxygen saturation monitoring using pulse oximetry is under development and could be of potential use in assessing fetal well-being in utero during labor (Butterwegge, 1997; Dildy et al. 1997). However, this technique is susceptible to several types of physiological variations such as blood volume fraction, haematocrit and blood flow (Nijland et al. 1997) and there are still insufficient data to support its use as a replacement for fetal scalp capillary blood sampling (Johnson et al. 1994; Johnson et al. 1995).

Developed in the 1960's by Dr. Saling, fetal scalp sampling was the first method that allowed intrapartum evaluation of fetal oxygenation and acid-base status in humans. In his early studies, Saling (1968) found that fetal blood $O_2$ saturation falls gradually during labor, from $\sim 42\%$ in the early first stage to $\sim 30\%$ at the end of the second stage. The steepest fall in $O_2$ saturation occurred in the latter half of the second stage. There was also a progressive fall of pH and bicarbonate, accompanied by an accumulation of lactic acid. Fetal $P_o_2$ fell slightly while $P_co_2$ rose by $\sim 7$ mm Hg. Since then, studies by many other investigators have further demonstrated that as labor continues there is a progressive reduction in fetal pH, $P_o_2$, bicarbonate level and base excess and increase in $P_co_2$ (Huch and Huch, 1984). Also, the majority of studies have revealed a good correlation between scalp and central blood samples (Saling, 1980). Scalp samples have also been correlated with cord blood values and the condition of the infant at birth (Young et al. 1978).
Recently, experiments with the noninvasive measurement of fetal arterial oxygen saturation using pulse oximetry have also shown that fetal oxygen saturation decreases during normal labor, and drops after a uterine contraction, especially with oxytocin-induced labor (Dildy et al. 1994; Johnson et al. 1994; McNamara and Johnson, 1995).

As noted above, the normal fetus develops a mild acidosis during labor, with arterial pH falling from \( \sim 7.35 \) in the first stage of labor to \( \sim 7.25 \) at birth (Weisberg, 1982). Also, in normal term pregnancy, the blood lactate levels in both mother and fetus increase in labor, and reach their highest values at the time of vaginal delivery (Low et al. 1975a; Suidan et al. 1984). Lactate levels are highest in the umbilical artery (Lin et al. 1980; Eguiluz et al. 1983; Schneider et al. 1984). The umbilical arteriovenous lactate differences (UA-UV) are positive in both vigorous and depressed newborns (Piquard et al. 1991). These findings indicate that the increased lactate levels in human fetal blood at the time of vaginal delivery are primarily of fetal origin.

The very limited data obtained from human studies demonstrate fetuses with IUGR are at an increased risk of intrapartum fetal hypoxia and metabolic acidosis. Lin et al (1980) have studied cord blood lactate, pH, and blood gas values in IUGR and AGA infants at the time of delivery to compare differences in response to the stress of labor. Both maternal and fetal blood lactate levels increased with the progression of labor, fetal lactate being higher than maternal lactate. Also, with intrapartum fetal heart rate decelerations, IUGR fetuses demonstrated a significantly higher lactate level than AGA fetuses. When fetal pH was measured in a scalp blood sample at the beginning of the labor and in the
umbilical artery right after birth in both IUGR and normal fetuses, lower baseline pH, lower arterial blood pH at birth and a faster decrease of the pH during the labor were found in the IUGR group (Nieto et al. 1994).

In summary, as evidenced from both human and animal studies, even during normal labor, the normal fetus experiences a fall in pH, Po$_2$, O$_2$ saturation, bicarbonate level and base excess as well as an increase in Pco$_2$ and lactate concentrations. Thus, disturbances in fetal oxygen supply with a modest decrease in fetal oxygenation do occur during labor in the healthy fetus. On the other hand, labor appears to represent a more serious challenge to IUGR fetuses. They have an increased likelihood of metabolic acidosis and thus would tolerate labor less well as compared to normally grown fetuses.

1.7 Intrapartum Fetal Heart Rate Monitoring

In contrast to the situation in animal species, there are abundant data from human studies of fetal heart rate changes during labor. This is a consequence of the clinical use of fetal heart rate monitoring to assess fetal well being. Fetal heart rate monitoring for intrapartum surveillance is accomplished by two methods: intermittent auscultation with a stethoscope or a hand-held Doppler device, and continuous electronic monitoring.

It was initially thought that abnormal fetal heart rate responses during labor were evidence of a potential oxygen shortage. This led to development of electronic fetal heart rate monitoring (Hon, 1958; Hon and Quilligan, 1967; Hon, 1968), in the hope that this
technique could be used for the determination of fetal well-being and the early detection of inadequate fetal oxygenation during labor, so that measures could then be taken to avert long-term morbidity or mortality (Hon, 1968; Martin, 1978).

Since the development of this technique in 1958, there has been considerable debate over the actual benefits of its use. Reviews of the most recent controlled clinical trials of the effectiveness and safety of electronic fetal monitoring, as compared to intermittent auscultation, indicate that electronic fetal monitoring during labor and delivery offers little significant benefit to justify its routine use and has no measurable effect on the perinatal mortality and morbidity rate including cerebral palsy, and long-term neurological deficit. However, it is associated with a higher rate of Cesarean deliveries and operative vaginal deliveries because of erroneous indication of fetal distress, and this increases surgical risks to the mother (MacDonald et al. 1985; Prentice and Lind, 1987; Sandmire, 1990; Rosen and Dickinson, 1993; Vintzileos et al. 1993; Thacker et al. 1995; Nelson et al. 1996).

Also, there is still a lack of unequivocal terminology and definitions in the assessment of fetal heart rate recordings, and a significant interobserver variation in the assessment of fetal heart rate recordings (Donker et al. 1993; Strong, Jr. and Jarles, 1993; Cibils, 1996). Thus, the American College of Obstetricians and Gynecologists concluded that electronic fetal heart rate monitoring and intermittent auscultation are equivalent methods for intrapartum assessment (Sandmire, 1990). On the other hand, a few studies have reported benefit from the use of routine electronic fetal heart rate monitoring, including a decreased perinatal mortality due to fetal hypoxia (Vintzileos et al. 1993), a lower incidence of neurological complications within the monitored deliveries (Ellison et al. 1991), an
improved prediction about perinatal outcome in very low birth weight infants (Larson et al. 1989) and a reduction in neonatal seizures (Thacker et al. 1995).

In summary, substantial research has questioned the benefits of electronic fetal heart rate monitoring. Nevertheless, it is still often used during labor and delivery in North America. For instance, in Canada, most hospitals providing obstetric services have electronic fetal monitors and use them frequently (Davies et al. 1993). Thus, to improve its proper use and validity, further definitive research is required. Various attempts have to be made to standardize terminology and assessment of fetal heart rate recordings. Moreover, studies are needed to determine who benefits from this kind of surveillance and who does not. As suggested recently, a good understanding of fetal physiology is also necessary for accurately interpreting the information this technique can provide (Cibils, 1996). In addition, a computerized system for fetal heart rate analysis has been developed. The benefits of using this technique include improvement in record quality and saving of time. Furthermore, observer variation in visual analysis of fetal heart rate recordings would be avoided by this objective and accurate method (Dawes et al. 1995; Dawes et al. 1996).
1.8 Fetal and Perinatal Cardiovascular Function in the Sheep Fetus

1.8.1 Antepartum and Intrapartum Changes of Fetal Heart Rate and Blood Pressure

In the sheep fetus, mean arterial pressure increases steadily in late gestation by ~0.46 mmHg/day. This is partly accounted for by the increase in cardiac output during this period, but more importantly, to the fact that vascularity does not increase at the same rate as body size, hence peripheral vascular resistance rises (Boddy et al. 1974; Dawes, 1985; Kitanaka et al. 1989). On the other hand, fetal heart rate declines steadily in late gestation (0.67 beats/day), accompanied by a rise in stroke volume as the heart grows (Boddy et al. 1974; Dawes et al. 1980; Dawes, 1985; Blanco et al. 1988a; Kitanaka et al. 1989; Wakatsuki et al. 1992a). The fall in heart rate cannot simply be explained in terms of a baroreflex. Nor is the fall in heart rate due solely to increasing vagal tone, as vagotomy in late gestation does not produce an increase in heart rate to values seen in mid-gestation. The decline in fetal heart rate may depend on changes at the level of the sino-atrial node, but the mechanisms are not known (Dawes et al. 1980; Dawes, 1985; Blanco et al. 1988a; Kitanaka et al. 1989; Wakatsuki et al. 1992a). Also, heart rate variability in fetal sheep has been studied extensively and it has been shown that accelerations in the FHR are closely associated with skeletal muscle activity much like the human fetus, suggesting similar control mechanisms in the 2 species (Bocking, 1993). With advancing gestational age, both short- and long-term fetal heart rate variability in the sheep fetus gradually increases (Murotsuki et al. 1997).
During sheep parturition, there are increases in fetal arterial blood pressure, which are associated with uterine contractions. Moreover, associated with maternal abdominal contractions, there are very sharp increases in fetal blood pressure, probably due to the direct effect of the maternal bearing down efforts. Some bradycardia generally occurs under these conditions. When heart rates were measured during intervals between uterine contractions, a fall in heart rate was observed in all fetuses (n = 4) during parturition, but the time at which this occurred varied in the different individuals. The heart rate either fell gradually before birth or there was little change until a sudden a drop at delivery, depending upon position of the fetus and frequency of uterine contractions (Comline and Silver, 1972). Rurak et al. (1987a) reported that there was a definite tendency for fetal arterial pressure to increase during labor. However, no significant changes in heart rate were found.

In order to understand the mechanisms which regulate and control basal heart rate as well as heart rate variability in the fetus, a number of studies have been conducted in fetal sheep during the last third of gestation. A variety of techniques have been utilized experimentally to produce hypoxemia and/or acidemia in fetal animals, including umbilical cord compression, maternal hypoxemia, and uterine blood flow restrictions. The effects of acute reductions in fetal oxygen delivery on fetal heart rate and arterial blood pressure on the sheep fetus near term are well established, i.e., a rise in mean arterial pressure and a transient bradycardia usually develop in response to a decrease in blood oxygen levels (Yaffe et al. 1987; Block et al. 1990a; Giussani et al. 1993). These data indicate that both chemoreceptors and baroreceptors are present and active in the late gestation sheep fetus.
Fetal chemoreceptor and baroreceptor activities also appear to be influenced by gestational age and behavioral state. For instance, fetal baroreceptor activity increases during high-voltage slow activity and decreases with gestational age in the ovine fetus during late gestation (Wakatsuki et al. 1992b). Fetal sympathetic and parasympathetic systems also influence baseline fetal heart rate in these behavioral states and with gestational age. Fetal cardiac β-sympathetic and parasympathetic tone increases with gestational age in both low-voltage fast activity and high-voltage slow activity states, but with a greater elevation of β-sympathetic tone in high-voltage slow activity and in parasympathetic tone in low-voltage fast activity (Wakatsuki et al. 1992a). In addition, acute hypoxaemia in the sheep fetus gives rise to an increase in short-term fetal heart rate variability and it is only with severe acidosis that this variability decreases (Dalton et al. 1977). However, if the hypoxaemia is maintained for several hours to a day or more, these changes are reversed. Prolonged hypoxaemia with progressive acidaemia does not result in significant alterations in mean fetal heart rate (Rurak et al. 1990). Chronic hypoxaemia for up to 3 weeks in sheep fetuses does not produce long-term changes in fetal heart rate, occurrence of heart rate accelerations and decelerations, and mean arterial pressure (Bocking et al. 1988b; Bocking et al. 1989; Kitanaka et al. 1989).

1.8.2 Fetal Growth and Fetal Heart Rate and Blood Pressure Development

Recent data from Crowe et al. (1995) indicate an important association between fetal growth and blood pressure development, and also show that differences in prenatal growth
are associated with changes in cardiovascular control. There appear to be two distinct
groups in terms of blood pressure change in late gestation fetal sheep: those showing a rise
in mean arterial blood pressure over the 14 days observation time (PU group), and those
in which blood pressure did not increase or showed only a slight decrease (PD group).
PU group fetuses were proportionately larger than PD ones. In contrast to the PU group,
the PD group of fetuses had lower blood glucose concentration, arterial $O_2$ saturation,
$PO_2$, total haemoglobin, haematocrit and $O_2$ content, and higher lactate concentration, pH
and $PCO_2$. Both groups of fetuses were subjected to acute moderate isocapnic hypoxia
showed no significant differences in mean arterial blood pressure or fetal heart rate, as
compared with control fetuses. However, the PD group responded to hypoxia with a
greater increase in mean arterial blood pressure than the PU group.

Chronic placental insufficiency with long-term hypoxemia and intrauterine growth
restriction induced by long-term (21 days) embolization of the umbilical circulation in fetal
sheep is associated with a decrease in the number of fetal heart rate accelerations and
short- and long-term fetal heart rate variability. This is possibly due to a delay in the
normal maturational changes of the autonomic control of fetal heart rate in these fetuses
(Murotsuki et al. 1997). In contrast, when the effect of “acute on chronic” umbilical-
placental embolization of a shorter term (10 days) on fetal heart rate patterns was
examined in the near-term ovine fetus, the most consistent change in fetal heart rate
patterns related to the progressive metabolic acidosis was an 84% decrease in the absolute
acceleration frequency, but short-term fetal heart rate variability remained unaltered
(Gagnon et al. 1996). In the fetuses with mild growth restriction produced by maternal
hypovolemia, mean systolic and diastolic pressures as well as fetal heart rate were lower than the corresponding values in controls (Daniel et al. 1996). There were significant correlations between late systolic pressure and heart rate and fetal arterial PO\(_2\) and between fetal heart rate and blood pressure and birth weight/maternal weight.

1.8.3 Physiological Changes in the Fetal Circulation

Little quantitative information regarding the fetal and perinatal circulation in the human is available. Most current knowledge about the normal fetal and transitional circulation has been derived from studies of fetal and neonatal lambs (Rudolph, 1970), since the sheep fetus is one of the few animal models large enough for detailed cardiovascular studies.

There are a number of differences between the fetal and adult circulation, most of them being related to the fact, that during intrauterine life, the placenta serves as the site of gas exchange rather than the lungs. Therefore, the most important features of the fetal circulation are the relatively large proportion (about 45%) of combined ventricular output distributed to the umbilical cord and placenta and the small proportion distributed to the lungs (Dawes, 1968; Rudolph, 1985).

In the fetal circulatory system, the umbilical vein transports blood rich in O\(_2\) and nutrients from the placenta to the fetal body. The umbilical vein travels to the liver, where it joins the portal vein. About 45% the blood it carries passes into the liver. The other 55% of the blood enters the ductus venosus which bypasses the liver. The amount of oxygen delivered
to the right and left lobe of the liver is different, because while the right lobe receives almost all of the portal venous blood along with umbilical venous blood, the left liver lobe is almost exclusively supplied by the umbilical vein (Edelstone et al. 1978; Rudolph, 1985). The ductus venosus travels a short distance and joins the inferior vena cava, where the oxygenated blood from the placenta is mixed with the deoxygenated blood from the lower parts of the body. This mixture continues through the vena cava to the right atrium. Although it had been assumed that there is a reasonable admixture of poorly oxygenated abdominal inferior vena cava blood with highly oxygenated umbilical venous blood in the thoracic inferior vena cava, elegant cineangiographic studies by Barclay et al. (1944) suggested that blood from these sources streams selectively, so that ductus venous blood preferentially passes through the foramen ovale to the left atrium and ventricle and hence to the upper body organs. This was confirmed by Edelstone and Rudolph (1979), using radioisotope-labeled microspheres. The preferential streaming of umbilical venous blood to the upper body implies that any substance that enters the umbilical vein, for example, glucose and drugs administered to the mother, will be delivered to the heart and brain in higher concentrations than to the lower body (Rudolph et al. 1981, Tonn et al. 1996).

In the adult heart, blood flows from the right atrium to the right ventricle then through the pulmonary arteries to the lungs. In the fetus, however, the lungs are nonfunctional and the blood largely bypasses them. As the blood from the inferior vena cava enters the right atrium, a large proportion of it is shunted directly into the left atrium through the foramen ovale (Anderson et al. 1981). The rest of the fetal blood entering the right atrium, including a large proportion of the deoxygenated blood entering from the superior vena
Cava passes into the right ventricle and out through the pulmonary trunk. Only a small volume of blood enters the pulmonary circuit because the lungs are collapsed and their blood vessels have a high resistance to flow, but still enough blood reaches the lung tissue to sustain it (Rudolph and Heymann, 1968; Rudolph, 1984).

Most of the blood in the pulmonary trunk bypasses the lungs by entering the ductus arteriosus which connects the pulmonary trunk to the descending portion of the aortic arch. As a result of this connection, the blood with a relatively low O₂ concentration which is returning to the heart through the superior vena cava, bypasses the lungs. At the same time, the blood is prevented from entering the portion of the aorta that provides branches leading to the brain. The more highly oxygenated blood that enters the left atrium through the foramen ovale is mixed with a small amount of deoxygenated blood returning from the pulmonary veins. This mixture moves into the left ventricle and is pumped into the aorta. Some of it reaches the myocardium through the coronary arteries and some reaches the brain through the carotid and vertebral arteries. The blood carried by the descending aorta is partially oxygenated and partially deoxygenated. Some of it is carried into the branches of the aorta that lead to various parts of the lower regions of the body. The rest passes into the umbilical arteries, which branch from the internal iliac arteries and lead to the placenta, where the blood is reoxygenated. This fetal circulatory arrangement normally permits almost equal development of the two sides of the heart, which work in parallel during fetal life, but serially after birth (Dawes, 1968; Rudolph and Heymann, 1968; Rudolph, 1984; Rudolph, 1985).
1.8.4 Fetal Regional Blood Flow Changes over the Perinatal Period

Regional blood flow has been studied over the perinatal period using the radiolabeled microsphere technique in fetal sheep. Between 120 and 140 days gestation, regional blood flow increased in the adrenal glands, kidneys, gastrointestinal tract and liver. Blood flows to cerebral hemispheres and cerebellar tissues also increased dramatically. In contrast, blood flows to carcass tissues did not change over this gestational period (Bendeck and Langille, 1992). Since fetal head compression during normal labor would increase intracranial pressure, this effect on the cerebral and peripheral blood flow was also studied in utero by infusion of artificial cerebrospinal fluid into a lateral ventricle in ovine fetus. Cerebral blood flow at the highest intracranial pressure levels were not different from baseline values. However, at the highest intracranial pressure level, renal, gastrointestinal and skin blood flow decreased. Myocardial and adrenal blood flow doubled. Therefore, fetal lambs appear to be capable of sustaining cerebral perfusion by initiating profound visceral vasoconstriction in response to the increased intracranial pressure (Harris et al. 1989).

At birth, overall systemic perfusion is not changed from fetal levels, but specific tissues do exhibit quite substantial flow alterations. Myocardial blood flow changes reflect the perinatal changes in ventricular work and oxygen consumption (Fisher, 1984a; Smolich et al. 1992). Thus flow to the fetal right ventricular myocardium, reflecting the greater right ventricular output in the fetus, significantly exceeds that to the left in which the output is much less. After birth the right-left flow ratio is reversed as myocardial flow to the left
ventricle increases and flow to the right ventricle decreases. These myocardial flow changes are associated with the postnatal increase of aortic pressure and the decrease of pulmonary artery pressure, as well as the respective increase of ventricular outputs. The changes are gradual, much slower than the rapid changes in pulmonary artery pressure and arterial oxygenation that occur promptly with the onset of pulmonary ventilation, suggesting that there are significant alterations in myocardial oxygen metabolism in the perinatal period (Fisher, 1984a; Smolich et al. 1992). Pulmonary blood flow represents about 7% of fetal cardiac output. Shortly after birth, pulmonary vascular resistance and pulmonary arterial pressure fall rapidly, whereas pulmonary blood flow increases markedly and reaches systemic levels (Heymann, 1989).

Cerebral blood flow decreases promptly and substantially after birth (Szymonowicz et al. 1988; Richardson et al. 1989b; Bendeck and Langille, 1992). As cerebral metabolic rate is not changed at birth, this change is largely in response to increasing arterial oxygen levels and possibly to decreasing arterial Pco₂, each of which are powerful vasoconstriction influences in the cerebral circulation. Regionally within the brain there is a significant redistribution of flow after birth; notably, cortical flow increases relative to flow to the brainstem and cerebellum, perhaps reflecting enhanced cortical activity in the newborn (Szymonowicz et al. 1988; Richardson et al. 1989a; Richardson et al. 1989b).

Kidney blood flow, presumably reflecting a perinatal continuity of renal function, is transiently depressed immediately after birth, but recovers promptly to fetal levels. Similarly, spleen and thyroid flow are unchanged at birth. Adrenal flow decreases
progressively in inverse correlation to arterial oxygenation, whereas no significant decrease occurs in gastrointestinal blood flow. Tissues with significant thermoregulatory functions change substantially and rapidly at birth. Diaphragm flow, reflecting the high oxygen requirements of the newborn, and possibly increased work of breathing, increases significantly above the levels measured during fetal non-breathing periods and usually exceeds the flow measured during fetal breathing. Increased flow to brown fat and skeletal muscle and decreasing skin flow are explained by the need for increased metabolic heat production and thermal insulation required to sustain body temperature in the newborn. Muscle blood flow is also increased to support the postural needs of the awake lamb (Iwamoto et al. 1987; Teitel, 1988; Richardson et al. 1989b; Berger et al. 1990; Bendeck and Langille, 1992; Smolich et al. 1992).

Thus, variable blood flow changes over the perinatal period are evident. In some cases these changes reflect birth-related changes in either blood gases or functional activity whereas in others the changes simply reflect a continuum from the late fetal to the early neonatal period (Richardson et al. 1989b). Although fetal circulatory adaptations in the transitional period are of physiologic importance, the data discussed above are largely obtained from the prenatal and postnatal periods separately, and no information is available regarding fetal regional blood flows during parturition.
1.8.5 Fetal Cardiac Output Changes over the Perinatal Period

Fetal cardiac output and its distribution have been studied in animal models, particularly in chronically instrumented fetal lambs with the use of radiolabeled microspheres (Rudolph and Heymann, 1967; Lorijn et al. 1980; Iwamoto and Rudolph, 1981a; Jensen et al. 1991) or by electromagnetic flow transducers applied around the ascending aorta and the pulmonary trunk (Anderson et al. 1981; Kamitomo et al. 1995). Recently, estimates of fetal right ventricular cardiac output have been obtained in the sheep fetus using Doppler echocardiography (Shiraishi et al. 1993). In the term sheep fetus, approximately 70% of the combined ventricular output is directed toward the lower part of the body and placenta, 20% to the upper body, 7% to the lungs, and 3% to the coronary arteries. The superior vena cava flow, plus almost two-thirds of the inferior vena cava flow, and the coronary artery sinus flow enter the right atrium and ventricle. Thus, 60% of the combined ventricular output in the fetal sheep is ejected by the right ventricle and only 40% by the left (Anderson et al. 1981). In the human fetus, because the brain is larger, the relative distribution between right and left ventricular output is close to equality, being 53% and 47%, respectively (De Smedt et al. 1987). Combined ventricular output increases with fetal growth. However, proportions distributed to the various organs change as gestation progresses. The proportion of flow perfusing the placenta gradually decreases, whereas there is an increase in the percentage of flow going to the brain, lungs, and gastrointestinal tract (Rudolph, 1985).
Under normal physiological conditions, the mean values reported in the literature for combined ventricular output in fetal sheep *in utero* during late gestation range from 436 to 460 ml/min/kg with electromagnetic flow probes measurement (Anderson et al. 1981; Kamitomo et al. 1995) and from 480 to 554 ml/min/kg with radiolabeled microsphere measurement (Lorijn et al. 1980; Iwamoto and Rudolph, 1981a; Jensen et al. 1991). After birth, measurements in awake, resting neonatal lambs have yielded values for cardiac output of 300-425 ml/min/kg. The postnatal values have depended to a large extent on environmental temperature: the cooler the environment, the higher the output (Sidi et al. 1983). Since cardiac output represents the volume of blood flowing through the pulmonary and systemic circulation in series postnatally, the output of the two ventricles is about 600-850 ml/min/kg, a considerable increase compared with that of the fetus. The basis for this increase and the mechanisms responsible have not yet been fully defined.

Two studies in the sheep have determined the distribution of near term fetal cardiac output with the microsphere method (Teitel et al. 1987; Blanco et al. 1988b). Both demonstrated the major changes in blood flow to various organs during the establishment of the neonatal circulation. The study by Teitel et al. (1987) also emphasized changes during ventilation and cord occlusion, while the study by Blanco et al. (1988b) gave emphasis to the levels of oxygenation. Perhaps the most interesting and novel finding in these studies is that placental flow is greatly reduced with *in utero* ventilation and this is clearly dependent on blood oxygen tension. Ventilation, oxygenation, and umbilical cord occlusion are responsible for the transition from the fetal circulatory pattern to the circulation of the newborn. However, the increase in ventricular output at birth is not initiated by any of
these processes, so that other components of the perinatal process must be responsible (Teitel, 1988). The possibility has been raised that the increase in catecholamine concentration that normally occurs after birth may increase output. Catecholamines increase myocardial contractility and could improve cardiac performance postnatally. If this concept is correct, it might be expected that β-adrenoreceptor blockade would reduce cardiac output to the fetal levels. But this was not the experience of Klopfenstein and Rudolph (1978), who found that propranolol administration to lambs at varying periods after birth results in only a small decrease (about 10%) in output from resting levels. Thus, the mechanisms involved in the regulation of cardiac output and myocardial performance in the fetus and in the changes after birth are not yet defined.

In summary, the normal fetal cardiovascular system is characterized by three specialized shunts (ductus arteriosus, foramen ovale and ductus venosus) and the umbilical circulation. These features allow the fetus to oxygenate blood in the placenta and preferentially deliver the blood that is highest in oxygen content to the fetal upper body. This arrangement permits the heart and brain to receive the most oxygenated blood in the systemic circulation. A high pulmonary vascular resistance serves to divert blood flow away from the lungs to the rest of the body and thus maximize oxygen delivery to other fetal organs, and blood flow to the placenta.

The driving force for cardiovascular adaptation to birth is the dramatic fall in pulmonary vascular resistance as the newborn takes its first breath. At the same time, separation of the placental circulation, decreases inferior vena caval flow, in conjunction with increased
pulmonary blood flow and venous return to the left atrium, results in functional closure of the foramen ovale. Increasing oxygen tensions cause constriction of the ductus arteriosus. These actions functionally close the central cardiac shunts (Siassi, 1988). Cardiac output also rises at birth when the central circulation pattern switches to series flow through the ventricles and pulmonary and systemic circulation. With the alteration of circulatory patterns and the changes in arterial oxygen content at birth, blood flow to various organ systems is also affected. For those organs that do not have increased metabolic demands postnatally (e.g. brain), blood flow tends to decrease, due to the increased oxygen content of arterial blood. Those organs that do have increased metabolic demands placed upon them (e.g. skeletal muscle) demonstrate an increase in blood flow with the magnitude in flow reflecting the degree of oxygen demand.

1.9 Fetal and Perinatal Endocrine Function in the Sheep Fetus

1.9.1 Plasma Catecholamine Concentrations during the Perinatal Period

Plasma catecholamine concentrations are very low in chronically instrumented fetal sheep in late gestation, with noradrenaline levels ranging from 250 to 500 pg/ml, and adrenaline levels being less than 50 to 75 pg/ml (Buhler et al. 1978). In addition, the basal plasma levels of catecholamines decrease progressively in the non-stressed, chronically catheterized fetal sheep from 115 to 145 days gestation (Palmer et al. 1984a). The mechanisms for this decrease are not clear. However, during spontaneous labor, fetal sheep plasma catecholamines begin to rise in early labor and further increase in late labor
There are even greater increases in plasma catecholamine levels following delivery (Brown et al. 1983; Richet et al. 1985; Habib et al. 1991; Oyama et al. 1992) and umbilical cord cutting (Padbury et al. 1981; Padbury et al. 1985). For instance, the plasma levels of noradrenaline and adrenaline rise to 32,000 (range: 21,030 - 48,548) and 35,000 (15,747 - 76,699) pg/ml, respectively at 5 min after cord cutting and the elevations persist over 4 hr (Padbury et al. 1981; Padbury et al. 1985). There is also a striking reduction in the noradrenaline/adrenaline ratio at 5 and 15 min after umbilical cord cutting such that adrenaline becomes the predominant plasma catecholamine (Padbury et al. 1981; Padbury et al. 1985). Such a catecholamine surge at birth is similar to that observed in the human fetus (Lagercrantz and Bistoletti, 1977; Agata et al. 1995; Gulmezoglu et al. 1996). Moreover, noradrenaline and adrenaline concentrations as high as 80,000 pg/ml have been observed in fetal sheep during hypoxia (Cohen et al. 1982) and values as high as 160,000 pg/ml have been found in human fetal cord blood at birth (Nylund et al. 1979).

The marked increase in circulating catecholamine levels at birth may be due in part to a significant decrease in catecholamine clearance rate observed in newborn as compared to fetal sheep (Stein et al. 1993; Smolich et al. 1996). It is likely that removal of the placental contribution to whole body clearance accounts for much of the difference observed in fetal and newborn catecholamine clearance rates (Stein et al. 1993). In the newborn sheep, sulfoconjugation of circulating catecholamines does not occur rapidly during the marked increases of circulating catecholamines at birth (Oyama et al. 1992). The factors that regulate catecholamine secretion are incompletely understood.
Endogenous opiates may be involved, acting as partial catecholamine antagonists (Padbury et al. 1988), since opiate receptor blockade from birth markedly augments the neonatal sympathoadrenal response in the term newborn lamb (Padbury et al. 1987b).

It is not completely clear why large amounts of catecholamines are secreted at birth. Intermittent asphyxia during uterine contractions in labor is certainly important for the activation of the sympathoadrenal system. Thus, birth and its attendant hypoxia, represent the first major stress to which all mammals are exposed. Indeed, the magnitude of the rise in plasma catecholamine concentrations during parturition and in the immediate postnatal period far exceeds that seen in even the most extreme circumstances in adulthood (Slotkin and Seidler, 1988). It is clear however that such secretion of catecholamines plays an important role in several of the adaptations that characterize the transition from intra to extrauterine life including cardiovascular and metabolic events, and also several other adaptive processes such as fetal lung liquid reabsorption, surfactant release, and non-shivering thermogenesis (Lagercrantz and Slotkin, 1986; Padbury and Martinez, 1988).

### 1.9.2 Cardiovascular Effects of Catecholamines

During intrauterine asphyxia or the stress of labor, the sympathoadrenal system has an important role in maintaining cardiovascular homeostasis. The first major clue that catecholamines are important for fetal survival came from pioneering studies by Comline and Silver (1961), who found that in the fetal sheep the adrenal glands produce catecholamines in response to hypoxia, even prior to the innervation of the glands by the
sympathetic nerves. This was further confirmed by many other investigators. Hypoxia in the fetus is invariably associated with a rise in plasma catecholamines (Jones and Robinson, 1975; Cohen et al. 1982). Secondary to this sympathetic adrenergic activation, blood flow to the organs that are crucial to fetal survival, including the brain, heart and adrenals, is increased. As a result, oxygen delivery to these areas is maintained but at the expense of O$_2$ delivery to other areas of the body. Blood flow to the peripheral organs, such as the lungs, kidneys, gastrointestinal (GI) tract, carcass and skin is decreased (Rudolph et al. 1981; Jensen and Berger, 1991). However, in sympathectomized fetuses the redistribution of organ blood flow during hypoxemia and asphyxia is significantly different as compared with intact fetuses, in that blood flows to the GI tract and to the kidneys do not change. The percent cardiac output directed to the placenta is reduced, while that to the carcass is increased as compared with intact fetuses. Thus, in sympathectomized fetuses circulatory centralization is less effective in protecting the fetus against the adverse effects of asphyxia (Jensen and Berger, 1991). In addition, the response of intact fetuses to hypoxia is normally associated with a fall in heart rate and rise in blood pressure, but this does not happen to sympathectomized fetuses. Adrenal demedullation abolishes the rise in blood pressure during hypoxia, while chemical sympathectomy abolishes the fall in heart rate (Jones et al. 1988).

It is not clear whether these cardiovascular responses are due to local or reflex adrenal stimulation. $\alpha$-adrenergic and $\beta$-adrenergic mechanisms are of great importance during fetal responses to stress. $\alpha$-adrenergic mechanisms are important for maintaining arterial pressure and blood flow to the placenta, heart, brain, and adrenal by constricting the
splanchnic and pulmonary vascular beds, and β-adrenergic mechanisms are important primarily for maintaining heart rate and cardiac output. If the sympathetic response to hypoxemia is blocked by β-adrenoreceptor antagonists, the fall of fetal heart rate, cardiac output and umbilical blood flow is more pronounced. In addition, the increases in blood flow to the myocardium, brain, and adrenal gland usually achieved during hypoxemia are not achieved (Court et al. 1984). Blockade of α-adrenoreceptors during hypoxemia causes an increase in fetal heart rate and cardiac output, but arterial blood pressure and total vascular resistance fall. Blood flow to the heart, adrenals, gut and spleen is increased, while blood flow to the brain and carcass is maintained (Reuss et al. 1982). When both α- and β-adrenergic receptors are inhibited during hypoxemia, fetal demise usually occurs (Parer et al. 1978).

1.9.3 Metabolic Effects of Catecholamines

When acute hypoxemia is induced in fetal sheep, there is an increase in fetal hepatic glucose release from glycogen and a rise in fetal blood glucose concentration (Jones, 1977; Jones et al. 1983). The increase is associated with a fall in the plasma insulin concentration and a rise in plasma catecholamine and glucagon concentrations (Robinson et al. 1977b; Jones et al. 1983). Hypoxemia in the fetal sheep is also associated with a large rise in plasma lactate concentration, which is sustained long after the hypoxemia is terminated (Jacobs et al. 1988b; Bocking et al. 1992). Similarly, the infusion of catecholamines to fetal sheep increases the plasma concentrations of glucose and lactate (Jones and Ritchie, 1978; Palmer et al. 1984b; Apatu and Barnes, 1991). The rise in
glucose concentration is partially due to inhibition of insulin secretion and may be mediated by an α-adrenergic mechanism, whereas lactate formation is stimulated by a β-adrenergic mechanism (Jones and Ritchie, 1978; Jones et al. 1983). At birth there is an abrupt increase in plasma glucagon levels and a decrease in insulin secretion in newborn sheep (Grajwer et al. 1977). Also, plasma glucose levels rise immediately after birth (Padbury et al. 1981; Padbury et al. 1985). Thus, in the perinatal period there are dramatic changes in the metabolic status that appear to be coordinated mostly by alterations in perinatal plasma levels of insulin, glucagon and particularly catecholamines, which lead to mobilization of fuel from glycogen stores, activation of glucose synthesis and induction of gluconeogenic enzymes (Sperling et al. 1984; Ktorza et al. 1985; Apatu and Barnes, 1991; Jones, 1991). Moreover, increased plasma adrenaline concentrations at birth, together with increases in oxygen content and blood flow to brown adipose tissue and withdrawal of placental prostaglandins, evoke chemical thermogenesis in brown adipose tissue and increase free fatty acids and glycerol release, which are essential for neonatal adaptation as the newborn is exposed to an abrupt fall in environmental temperature after delivery (Padbury et al. 1987a; Power, 1989; Gunn et al. 1993; Gunn and Gluckman, 1995).

The cardiovascular and metabolic events around birth appear to be largely due to adrenaline, and not noradrenaline. Heart rate, blood pressure, cardiac output and contractility increase abruptly after cord cutting in control newborn sheep but do not increase in adrenalectomized animals (Padbury et al. 1987a). Chemical sympathectomy via 6-hydroxy-dopamine, which results in decreased noradrenaline secretion following
cord cutting, does not diminish the metabolic response observed after cord cutting, i.e., the rise in glucose (Agata et al. 1986). Fetal adrenalectomy, on the other hand, which abolishes the surge in adrenaline at parturition, results in blunting of the normal glucohomeostatic mechanism (Padbury et al. 1987a).

1.9.4 Plasma Arginine Vasopressin Concentrations during the Perinatal Period

Resting plasma arginine vasopressin (AVP) concentrations average about 2-5 pg/ml during late gestation (Stark et al. 1979; Kelly et al. 1983; Stark et al. 1985). Parturition in sheep is associated with a marked increase in fetal AVP secretion; but there is no concomitant maternal AVP secretion (Stark et al. 1979; Cummings et al. 1995). At the various stages of spontaneous labor, AVP increases variably but on average by about 30 fold above prelabor levels (Cummings et al. 1995) and after delivery reach peak levels in cord blood which range from 7.5-8,000 pg/ml (Stark et al. 1979). Also, during premature parturition (~130 days gestation) induced with adrenocorticotropin, fetal plasma AVP concentrations on average increase two fold in the early phases of labor and rise significantly in the active (20 fold) and expulsive phases (80 fold). Peak values are reached at delivery (290 fold) but decrease by 30 min after birth. Variation of AVP concentrations between animals is high, especially in late labor with a coefficient of variation (CV) of 88% (Stark et al. 1981). Similar to human fetal cord blood AVP levels measured at delivery, umbilical venous AVP concentrations in fetal lamb are less elevated than umbilical arterial samples (Hoppenstein et al. 1968; Hadeed et al. 1979), implying a fetal origin for the elevated plasma AVP. A careful analysis of cord blood AVP values
suggests that AVP levels reflect fetal hypoxic/asphyxial events in the intrapartum period (Stark et al. 1981). Following deliveries of sheep lambs by cesarean section, the plasma AVP levels are not significantly changed (Nakamura et al. 1987) or only minimally elevated (Leffler et al. 1985), supporting the view that the elevated AVP levels in vaginal deliveries may occur in response to hypoxia associated with labor. The surge of adrenaline associated with birth may also contribute to this effect (Ervin et al. 1989; Ervin et al. 1991).

1.9.5 Cardiovascular Effects of Arginine Vasopressin

AVP may also be important in the cardiovascular adaptations to hypoxaemia. Plasma AVP levels rise markedly in response to hypoxaemia (Rurak, 1978; Daniel et al. 1983), and the response is proportional to the degree and duration of reduced oxygenation (Daniel et al. 1983; Stark et al. 1984). AVP secretion is even more marked during asphyxial episodes where hypoxaemia is compounded by acidemia (Rurak, 1978; Daniel et al. 1983). Fetal cardiovascular effects following AVP infusion include redistribution of the cardiac output with a marked reduction of the proportion of cardiac output to the gastrointestinal and peripheral circulations and an increase in the percent of cardiac output to the umbilical-placental, myocardial, and cerebral circulations (Iwamoto et al. 1979; Rurak and Gruber, 1984), as well as increased peripheral vascular resistance and blood pressure and decreased fetal heart rate (Rurak and Gruber, 1984; Tomita et al. 1985). After infusion of AVP, there is also a rise in fetal oxygen delivery, due to the increase in umbilical blood flow (Rurak and Gruber, 1984) because AVP has no effect on umbilical-
placental outflow resistance (Paulick et al. 1991). Moreover, the fetal hypertension, bradycardia, gastrointestinal vasoconstriction, and brain vasodilation present during fetal hypoxemia are diminished when fetal AVP receptors are blocked (Perez et al. 1989). The stimulus for vasopressin release during hypoxia may be catecholamines, since adrenaline infusion in the fetal lamb is known to produce a rapid and significant increase in circulating AVP levels (Ervin et al. 1989; Ervin et al. 1991). However, recent data indicate that a hypoxia-induced rise in fetal adenosine levels also triggers vasopressin release (Koos et al. 1994a).

In summary, in response to hypoxia and asphyxia, fetal catecholamines play an important role in mediating the circulatory and metabolic changes. These include centralization of blood flow distribution, decrease in plasma insulin, increase in plasma glucagon and increase in hepatic glucose production. But there are also a number of other regulatory systems that are involved in the control of fetal cardiovascular and metabolic responses during the reduced oxygen delivery, including AVP and other hormones such as glucocorticoids (Challis et al. 1986; Challis et al. 1989), β-endorphins (Stark et al. 1982; Skillman and Clark, 1987), atrial natriuretic factor (Cheung and Brace, 1988) and the renin-angiotensin-aldosterone system (Iwamoto and Rudolph, 1981b). Thus, the endocrine reactions induced by the birth process are important regulators of a successful transition and essential for newborn adaptation to extrauterine life.
1.10 Breathing Movements in the Fetal Sheep

Fetal breathing movements (FBM) are vital for normal fetal lung development. FBM with a normal incidence and amplitude stimulate lung growth, possibly by stretching the pulmonary tissue (Kitterman, 1988). In contrast, inhibition of FBM is associated with pulmonary hypoplasia (Liggins, 1984). During fetal life, breathing movements occur episodically. They have been recorded in fetal lambs from as early as 40 days gestation (Dawes, 1984). From that age up to about 115 days, when the electrocortical activity becomes differentiated into low voltage and high voltage states, breathing is virtually continuous (Dawes et al. 1981). After this, rapid irregular FBM occur up to about 40 percent of the time and only during times of low-voltage electrocortical activity and electro-ocular activity characteristic of rapid eye movement sleep (Dawes, 1984). The physiologic mechanism responsible for the occurrence of fetal breathing only during low voltage electrocortical activity in the fetal sheep may be due to the development of descending inhibition of the respiratory pattern generator in association with the high voltage electrocortical state and non-rapid eye movement sleep (Walker, 1986). The precise location of these inhibitory inputs is unknown, but they probably lie above the pons because transection of the brainstem at the level of the upper pons dissociates respiratory activity and behavioural states (Dawes et al. 1983).

The presence of breathing movements in fetal sheep is not only dependent on the behavioral state but also on the fetal blood gas status. For instance, FBM are increased in response to a rise in arterial $P_{O_2}$ by hyperbaric oxygenation (Tiktinsky-Rupp et al. 1994).
On the other hand, acute fetal hypoxaemia inhibits FBM (Bissonnette et al. 1989; Koos et al. 1992; Giussani et al. 1993; Koos et al. 1994b). More severe hypoxaemia (e.g., reducing Po₂ from 16 to 11 mmHg) produces a cessation of FBM, accompanied by fetal gasping activity (Moore et al. 1989). The inhibition of FBM with hypoxaemia is thought to be a way in which the fetus can conserve oxygen for more vital functions, because FBM can increase fetal O₂ consumption by up to 30% (Rurak and Gruber, 1983a). This inhibitory effect is most likely triggered by a rise in central adenosine (Bissonnette et al. 1991; Koos et al. 1992; Koos et al. 1994b) or arginine vasopressin (AVP) concentrations as reported recently (Bessho et al. 1997). Fetal breathing responses to acute moderate hypoxemia in fetal sheep are also affected by gestational age, with a greater decrease in the incidence of FBM at 134-147 days than at 125-129 days of gestation (Akagi and Challis, 1990). However, during prolonged hypoxaemia (> 24 h), there is an initial inhibition of FBM, but after that, the incidence of fetal breathing increases over time and returns to control levels after 14-16h (Bocking et al. 1988a; Koos et al. 1988; Hooper et al. 1990). The mechanisms of this FBM adaptation are unknown, but may involve down-regulation of adenosine receptors. During fetal hypercapnia, the frequency, amplitude and incidence of fetal breathing movements increase significantly compared with isocapnic control (Kuipers et al. 1997). Also, hypercarbia can offset the respiratory inhibition of acute moderate hypoxia (Bissonnette et al. 1989). During mild hypocapnia, the opposite result is observed with a significant decrease in the overall incidence of FBM, the incidence of FBM during low voltage ECoG activity, and the mean duration of periods of breathing (Kuipers et al. 1994). FBM are also decreased following the exposure of
pregnant ewes to a high ambient temperature (e.g., 43°C for 8 h). Again, this is due to a fall in fetal Pco₂ produced by maternal hyperventilation (Walker and Davies, 1986).

Fetal glucose availability is an another important factor in determining the incidence of fetal breathing movements in utero. Fetal hypoglycemia induced by maternal fasting or insulin infusion is associated with the decreased FBM (Richardson et al. 1985; Fowden et al. 1989; Fowden et al. 1992). However, infusion of glucose alone does not affect FBM, despite a significant two fold increase in glucose concentrations (Murai et al. 1984). The reduced incidence of FBM that occurs during maternal fasting appears to be primarily caused by fetal hypoglycemia and does not directly involve changes in fetal plasma prostaglandin E₂ (PGE₂) concentrations (Fowden et al. 1992). In contrast, a reduction in the incidence of FBM by insulin infusion is associated with an increase in fetal plasma PGE₂ concentration (Fowden et al. 1989), which is a potent inhibitor of fetal breathing (see below). The mechanism whereby hypoglycemia lowers FBM may also involve a decrease in cerebral metabolism because of limited cerebral glucose and O₂ uptake (Richardson et al. 1985).

The prostaglandin system is known to have profound effects on fetal breathing movements (Thorburn, 1992). Of the various prostaglandins, PGE₂ is thought to be a physiological regulator of FBM in the sheep during late gestation. The incidence of FBM decreases with increasing gestational age, while fetal arterial concentrations of plasma PGE₂ increase significantly over the same period of gestation (Fowden et al. 1989). Also, a continuous PGE₂ infusions lead to a significant decrease in FBM (Kitterman et al. 1983; Savich et al.
1995; Hollingworth et al. 1996), whereas infusions of prostaglandin synthesis inhibitors result in an increase in the amplitude and incidence of breathing movements (Kitterman et al. 1979; Walker, 1990).

The incidence of FBM in sheep normally begins to decline 2-3 days prior to labor and diminishes considerably 24 to 36 hours before spontaneous parturition (Dawes, 1973; Berger et al. 1986). FBM are greatly reduced or virtually absent during labor (Dawes, 1973). The decline results from the fetus spending a greater proportion of time in apnoea as well as reducing the frequency of breathing within epochs of breathing (Berger et al. 1986). Thus, the diminution and/or cessation of fetal breathing movements is a normal physiologic event during term labor in sheep. Consequently, continuous monitoring of fetal breathing movements is helpful to predict impending spontaneous labor. Moreover, it has been proposed that, at least in human pregnancy, the absence of fetal breathing movements is a useful signal to differentiate true labor from false labor (Boylan et al. 1985) and that the continuation of normal episodic fetal breathing movements can be used to discriminate in suspected preterm labor between those women who will continue to term from those who will deliver within the next few days (Besinger et al. 1987).

The mechanisms behind the inhibition of fetal breathing with approaching labor and during labor are not fully understood. Experiments in sheep suggest that it may be related to the fall in placental progesterone output before labor (Parkes et al. 1988). It is most likely, however, that a rise in fetal plasma prostaglandin levels is involved, particularly during labor, since as discussed above, fetal administration of PGE₂ prior to labor arrests fetal
breathing movements, and since prostaglandin secretion in the fetal membranes, uterus and cervix is associated with the onset of normal labor (see section 1.2). It is a well-established fact that fetal behavioral patterns other than breathing continue episodically through labor, commonly until the second stage (Ruckebusch, 1972; Ruckebusch et al. 1977). It is therefore likely that the diminution or arrest of fetal breathing movements prior to the onset of labor is through a mechanism acting directly on the medulla rather than through the behavioural state cycle generator. This would fit with the known actions of prostaglandins (Kitterman, 1987). However, infusion of the prostaglandin synthesis inhibitors causes a significant increase in FBM only until 2 days before delivery. Although there are significant correlations between PGE$_2$ and FBM, both control and infusion groups showed similar decreases in FBM during the last 2 days before delivery. Thus, it appears that the rise in concentration of PGE$_2$ with approaching parturition may contribute to, but is not essential for, the decrease in FBM (Wallen et al. 1988), which suggests that some other factors may be involved. Nevertheless, recent data strongly indicate that the rapid decrease in circulating concentration of PGE$_2$ following delivery contributes to the onset of continuous breathing at birth (Adamson et al. 1991; Hollingworth et al. 1996).

1.11 Measurement of Regional Blood Flow with Microsphere Methods

1.11.1 Radioactive Microsphere Method

The radioactive microsphere technique was first introduced by Rudolph and Heymann in 1967 for measuring regional blood flow and cardiac output in fetal lambs in utero. They
demonstrated that plastic microspheres, labeled with gamma-emitting isotopes, were distributed in relation to blood flow (Rudolph and Heymann, 1967). After this, the technique was further improved and greatly simplified by the reference blood sample method (Makowski et al. 1968), in which blood is withdrawn at a known rate from an artery during the injection and circulation of microspheres. Since then, radioactive microspheres have been used extensively for quantification of systemic and regional organ blood flow in experimental animals, and the validity of their use is well documented (Buckberg et al. 1971; Heymann et al. 1977; Dole et al. 1982; Rurak et al. 1990b). The technique has become standard for studying regional blood flow to organs and distribution of cardiac output in fetal and adult physiology. The microsphere method applied to the chronically instrumented fetal sheep has contributed significantly to our understanding of fetal circulatory physiology and pathophysiology in utero (Rudolph and Heymann, 1967; Heymann et al. 1977).

Radioactive microspheres are non-biodegradable tracers or indicators, labeled with gamma-emitting radionuclides. They have a density of approximately 1.4, which is similar to blood. The size of microspheres commonly used is 15 μm in diameter. When injected into the blood stream, these tracers behave in a manner comparable to red blood cells. They are distributed to tissues in relation to flow to the tissue and are trapped in capillaries during their first passage through tissues; because of their size, they can not pass through the microcirculation. After experiments, the blood volume of each reference sample, and then withdrawal rate is measured. Also, the radioactivity of the reference sample and the radioactivity of the dissected organ or tissue are determined. The organ and tissue blood
flow rate is then calculated as the reference sample withdrawal rate multiplied by the ratio of organ radioactivity to arterial reference blood sample radioactivity. Fetal combined cardiac output can then be derived from the sum of all the organ and tissue blood flows. Since each batch of microspheres injected are labeled with different gamma emitting isotopes and each of these isotopes has its own appropriate gamma photon energy, the energy peak of each isotope from the photon spectrum can be identified using gamma spectrometry; also the amount of each isotope, which is proportional to the number of microspheres trapped in organ or tissues, can be quantified, thus allowing blood flow measurements in the same animal on several occasions (Rudolph and Heymann, 1967; Makowski et al. 1968; Heymann et al. 1977).

However, procedures employing radioactive microspheres have a number of serious disadvantages. These include restriction to specially licensed laboratories, and the short half-lives of some radioisotopes which requires that they be used soon after manufacture. There is also the inconvenience of prolonged storage of radioactive carcasses and waste before they can be disposed of, and the increasing difficulty in finding disposal sites due to increased regulatory restrictions. Most importantly, there is the potential of hazards to laboratory personnel as well as to the environment due to handling, processing, and disposal of radioactive material. Thus, the difficulties posed by the use of radioactive materials have prompted the quest for the use of methods that label plastic microspheres with different materials.
1.11.2 Non-Radioactive Microsphere Methods

In response to the practical and regulatory issues involved in the use of radionuclide-labeled microspheres, optical detection techniques involving several new types of microsphere have recently been developed. These include non-radioactive microspheres labeled with various colored dyes (Hale et al. 1988; Kowallik et al. 1991; Hakkinen et al. 1995), and x-ray fluorescence excitation of microspheres loaded with elements of high atomic number (Morita et al. 1990; Mori et al. 1992). In addition, a range of intensely fluorescent labeled latex microspheres specifically for regional blood flow determination are now commercially available (An et al. 1996). These non-radioactive microspheres are used in the same way to radioactive microspheres. However, instead of analyzing the samples for radioactive counts, the tissue samples are digested, the microspheres are isolated by filtration or centrifugation and then quantified by different methods, depending on which type of non-radioactive microspheres are used. Similarly, blood flow to any organ and tissue is calculated in an analogous manner as with the radioactive microsphere method (Glenny et al. 1993; Prinzen and Glenny, 1994).

With the colored microsphere method, the actual number of microspheres labeled with colored dye is counted in a haemocytometer using light microscopy (Hale et al. 1988), but this method requires substantial time for the tedious counting of individual microspheres. To circumvent the drawbacks, an alternative method has been developed in which the dyes can be extracted from microspheres and the blood flow is assessed as a reflection of the photometric absorption of the solution by using an absorption spectrophotometer.
(Kowallik et al. 1991). The major disadvantage of an x-ray fluorescent method is the need for sophisticated and relatively expensive equipment such as x-ray fluorescence spectrometer for x-ray excitation and fluorescence detection, which is not commercially available yet (Prinzen and Glenny, 1994). In addition, the x-ray method is hampered by leaching of the label from the microspheres over years (Morita et al. 1990). The isolated microspheres labeled with fluorescent dyes can also be counted in a haemocytometer with the use of a light microscope or a fluorescence activated cell sorter (Austin et al. 1993). With development of the fluorescent dye extraction techniques using chemical solvents, the fluorescence intensity, hence concentrations of each dye can be measured by using a fluorescent spectrophotometer (Glenny et al. 1993). Fluorescent colors are easily separated as each color is spectrally distinct from the others. Since the fluorescence emission of each dye is determined only during excitation at its own excitation wavelength, the fluorescent method is more sensitive and specific than the absorption method. In addition, fluorescent microspheres offer superior color separation and greater ease of measurement of regional blood flow compared to colored microspheres (Prinzen and Glenny, 1994). Furthermore, effective methods have been developed to extract the microspheres and fluorescent dyes they contain from tissue samples, including tissue digestion and filtration using filtration devices (Glenny et al. 1993). More recently a sedimentation method has been validated for dye extraction that does not require a filtration step (Van Oosterhout et al. 1995). Blood flow estimates have been obtained using fluorescent-labeled microspheres in some but not all tissues from dogs, pigs and rabbits (heart, skin, kidney, brain, spleen, skeletal muscle, gut, and lung), and compared with measurements obtained using radionuclide-labeled microspheres (Abel et al. 1993; Austin et al. 1993; Glenny et al. 1993; Chien et al. 1995; Van
The correlation between the flow estimates of the two methods was excellent.

In the published studies to date, the fluorescent dyes have been mostly measured with conventional cuvette-type recording spectrofluorometers. Use of automated fluorescence multi-well plate readers would allow for more rapid measurement of samples, and this would be a particular advantage when many or all tissues are studied, as is the case with most fetal sheep research that has employed radioactive microspheres (Rudolph and Heymann, 1967; Cohn et al. 1974; Court et al. 1984; Richardson et al. 1989b; Rurak et al. 1990b). Glenny et al. (1993) have described use of 96 well plate reader attached to a conventional fluorimeter via a top-down excitation and emission detection system, and found quenching of the fluorescent signals at higher microsphere concentrations. However, use of a stand-alone, automated multi-well plate reader does not appear to have been described. A new method is thus needed not only to quantitatively measure blood flow in all individual organs and tissues in fetal sheep by means of fluorescent microspheres but also to reduce the sample measurement time with an automated fluorescence microplate reader.

In summary, there is little question that microspheres are good indicators of whole body or organ-to-organ distribution of flow. However, like all the other methods used for studying the fetal circulation, the microsphere method has its own advantages and disadvantages. One of the primary advantages of the microsphere techniques is its ability to document blood flow to each of individual organ and tissue regions in the fetal body, thus giving more detailed information than electromagnetic or ultrasonic flow probes can.
provide. In addition, employment of different types of labels permits assessment of blood flow at various time points. Thus, serial flow measurements can be made in the same animal. The main disadvantages of the microsphere methods are that a limited number of observations can be made in a single animal and the measurements are not immediately available because tissues must be processed (Heymann et al. 1977; Prinzen and Glenny, 1994).

1.12 Rationale

Oxygen delivery to the fetus is a function of two variables, umbilical venous blood oxygen content and umbilical blood flow (Edelstone, 1984). During labor, several maternal and fetal conditions can directly or indirectly affect either of these two variables. Uteroplacental blood flow is intermittently diminished with uterine contractions during labor. Also, uterine contractions can cause a direct compression of the umbilical cord with restriction of the umbilical blood flow. Thus, the most common causes for fetal oxygen deprivation at labor are either insufficiency of uteroplacental blood flow due to uterine contractions, which in turn alters umbilical venous blood oxygen content, or insufficiency of umbilical blood flow caused by umbilical cord compression (Parer and Livingston, 1990). As a result, fetal distress may develop during labor and delivery. Therefore, labor and delivery is a time when there is a particular risk of a fall in fetal oxygen delivery.
Labor is stressful to all fetuses. Data from human (Saling, 1968; Huch and Huch, 1984; Dildy et al. 1994; Johnson et al. 1994; McNamara and Johnson, 1995) and sheep (Comline and Silver, 1972; Stark et al. 1979; Stark et al. 1981; Rurak et al. 1987a) studies have indicated that during normal labor, disturbances in fetal oxygen supply with a modest decrease in fetal oxygenation do occur even in the healthy fetus. Thus, the maintenance of adequate oxygenation is the major physiological requirement for the fetus in labor (Parer and Livingston, 1990). Fetuses with intrauterine growth restriction may be even more jeopardized by labor than those with normal growth (Lin et al. 1980; Lockwood and Weiner, 1986; Nieto et al. 1994; Neerhof, 1995), because they are often exposed to antepartum oxygen and nutrient deficiencies (Soothill et al. 1987; Economides and Nicolaides, 1989; Nicolaides et al. 1989).

During the last 3 decades, a number of biophysical and biochemical monitoring techniques have been developed in order to assess human fetal condition and identify compromise during labor. Although they have contributed to our understanding of fetal well being during this process, none of these methods can assist in the recognition of the degree of fetal oxygen delivery and oxygen consumption deficit. A fundamental problem in accurately describing human fetal oxygenation during labor and in identifying those maternal and fetal factors most important in compromising oxygenation, remains the inability to obtain more direct estimates of fetal oxygen supply and oxygen consumption (Rurak, 1982). This is because it is currently impossible to estimate human fetal oxygen delivery and consumption directly, due to ethical and technical difficulties.
A number of studies have been performed in the sheep fetus at late gestation to assess fetal cardiovascular and metabolic responses to reductions in umbilical blood flow (Itskovitz et al. 1983; Itskovitz et al. 1987) and in uterine blood flow (Wilkening and Meschia, 1983; Jensen et al. 1991). These experimental conditions, which result in fetal hypoxemia and asphyxia and largely determine fetal morbidity and mortality in human pregnancy; each have their clinical counterpart. As described above, cord compression during labor would reduce umbilical blood flow; and prolonged severe uterine contractions in labor would restrict uterine blood flow by increasing uterine vascular resistance and hence decrease oxygen delivery to the fetus. However, it is not known whether these experimental manipulations conducted in the antepartum period reflect real changes during spontaneous labor and delivery. Fetal oxygen delivery and consumption, fetal cardiac output and its distribution and regional blood flow have not been examined during spontaneous labor and delivery in any species.

Fetal growth restriction is associated with not only increased perinatal mortality and morbidity (Hagberg et al. 1989; Mir et al. 1989; Hagberg et al. 1996) but also an increased risk of adverse outcomes in adult life such as developing hypertension, ischaemic heart disease and diabetes (Barker and Fall, 1993; Barker, 1996a; Barker, 1996b). Similarly, restricted fetal growth in sheep results in poor perinatal outcome and reduced productivity (Barlow et al. 1987; Mellor, 1988). Improvement of fetal outcome requires knowledge of fetal physiologic responses to labor and delivery and the transition from fetal to post-natal life at this critical stage. However, there are limited data on the fetal cardiovascular and metabolic responses to spontaneous labor in both normally grown and growth restricted
fetuses. Because of the inaccessibility of the human fetus during labor, the use of chronically instrumented pregnant sheep to study fetal oxygen, cardiovascular and metabolic homeostasis during this process is a useful alternative.

During the course of this project, one group of animals (Group I) was observed to have a marked deterioration in metabolic and acid-base status during labor, whereas the other group (Group II) was not. Thus, we selected pH 7.15 at delivery as a cutoff value to classify our fetuses. Subsequently the Group I animals were found to be spontaneously growth restricted. This enabled several questions to be examined. These are: (1) What are fetal responses to labor in terms of cardiovascular, metabolic and endocrine changes, in both normally grown and undergrown fetuses? (2) Are these responses similar to, or different from, those observed during fetal hypoxemia or asphyxia during late gestation under various experimental conditions? (3) What are the fetal adaptive and compensatory mechanisms involved in these responses? (4) What are the implications of natural growth restriction of the fetus and placenta in terms of these responses?

1.13 Hypothesis and Overall Objective

The working hypothesis of this thesis was that cardiovascular, metabolic and endocrine responses to labor stress in the normally grown fetus are different from those in the growth restricted fetus. The difference of these responses between the normally grown and growth restricted fetuses is due to the different adaptive and compensatory mechanisms involved, because of a different margin of safety for the supply oxygen before
labor. To test this hypothesis, the overall objective of this study is to examine several aspects of fetal physiological change during spontaneous labor and at delivery in chronically instrumented pregnant sheep by firstly determining the cardiovascular, metabolic and endocrine responses, secondly establishing the possible mechanisms by which the fetuses adapt themselves in response to the stress of labor, and finally studying changes in fetal oxygen, glucose and lactate metabolism, fetal breathing movements as well as fetal heart rate and arterial blood pressure in the antepartum period in both normally grown and naturally occurring growth restricted fetuses.

1.14 Specific Objectives

1. To measure the following variables in normally grown and growth restricted fetuses at pre-labor and during spontaneous labor and delivery:
   a. fetal arterial and umbilical venous blood gas status, acid-base balance, oxygenation, glucose and lactate concentrations.
   b. fetal arterial blood pressure and heart rate.
   c. fetal regional blood flows, fetal combined ventricular output and its distribution.
   d. fetal oxygen delivery, extraction and consumption.
   e. umbilical lactate and glucose delivery, extraction and uptake.
   f. fetal plasma arginine vasopressin concentration.

2. To develop a method for measuring regional blood flow by means of fluorescent microspheres in all organs and tissues of the sheep fetus.
3. To validate the use of a column extraction procedure and double antibody radioimmunoassay method for measurement of arginine vasopressin in fetal sheep plasma.

4. To examine fetal arterial and umbilical venous blood gas, acid-base status as well as oxygen, lactate and glucose concentrations in both normally grown and growth restricted fetal lambs in the prenatal period; to investigate the changes in their time course and magnitude; and to compare these changes between the two group of fetuses.

5. To analyze antepartum and intrapartum fetal breathing movements.

6. To examine antepartum fetal heart rate and arterial blood pressure.

7. To quantify uterine contractions before and during labor and at delivery.
2. EXPERIMENTAL

2.1 Animal Preparation

The study was approved by the University of British Columbia Committee on Animal Care and conformed to the guidelines of the Canadian Council on Animal Care. Time dated pregnant sheep were obtained throughout the season from September to June of the year by breeding ewes (Dorset and Suffolk) at the south campus farm of The Department of Animal Science. Estrus synchronization was accomplished through intravaginal implantation of Veramix Sheep Sponges (Tuco Products Co., Orangeville, ON), which release a progestin (medroxyprogesterone acetate) to suppress spontaneous ovulation. Upon removal of the sponge 14 days later, ovulation induction was achieved by intramuscular injection of 500 I.U. pregnant mares' serum Gonadotropin (Ayrest Laboratories, Montreal, Que.). Ewes were then placed with a ram for 36-48 hours following gonadotropin injection to result in time-dated pregnancies. Pregnancy was confirmed by measuring maternal blood progesterone concentrations at approximately 19 days after the presumed day of ovulation, and later in pregnancy (about 50-100 days) by real-time ultrasound for visualization of the fetus and placenta. The sheep were transferred to the Research Center at least one week prior to surgery to allow the animals to become accustomed to the environment. They were housed indoors in the holding pen in the company of other sheep and received twice daily food and water ad libitum. Food was withheld during the 18 h immediately preceding surgery, but free access to water was allowed.
Pregnant sheep at 121-133 days gestation (term ~ 145 days) were operated on using aseptic surgical procedures, as previously described (Kwan et al. 1995a). On the day of surgery, the ewe received 6 mg atropine sulfate (Astra Pharmaceuticals Inc., Mississauga, ON) via the maternal jugular vein to control salivation. 10-15 min following injection, anesthesia was induced by an intravenous injection of 1g sodium pentothal (Abbott Laboratory, Montreal, Que.) and subsequently maintained by ventilation with 1.5 - 2.0% halothane (Ayerst Laboratory, Montreal, Que.) and 70% nitrous oxide in oxygen, delivered via an endotracheal tube. An intravenous infusion of 500 ml 5% dextrose solution (Baxter Canada, Toronto, ON) was administered to the ewe during surgery via a jugular vein. The uterus was exposed through a low midline laparotomy. Access to the fetal head and the hind quarters was gained through separate uterus incisions in areas free from placental cotyledons and major blood vessels. Polyvinyl or silicone rubber catheters (Silastic, Dow Chemical, Midland, MI) were implanted in a fetal femoral (FA) and carotid artery (CA), lateral tarsal vein (TV) (catheter i.d 0.64 mm and o.d. 1.19 mm), trachea and amniotic cavity (catheter i.d. 1.02 mm and o.d. 2.16 mm), as well as in the maternal femoral artery (MA) and vein (MV) (catheter i.d. 1.02 mm and o.d. 2.16 mm). In addition, a small, non-occlusive silicone rubber catheter was implanted in the common umbilical vein (UV) (catheter i.d. 0.51 mm and o.d. 0.94 mm). Amniotic fluid lost during surgery was replaced with sterile warm saline solution (Travenol Canada Inc., Mississauga, ON). The uterine and abdominal incisions were closed. All catheters were tunneled through the abdominal wall and exited on the right flank of the ewe. They were filled with heparin solution (0.9% NaCl solution containing 24 I.U./ml), plugged, and protected by a cloth pouch taped to the skin. In the case of twin or triplet pregnancies
only one fetus was surgically prepared. Fetal and maternal catheters were flushed daily with heparinized sterile saline as described above. Immediately after surgery, as well as once daily for the succeeding 3 days, antibiotics were administered to the ewe, fetus and amniotic cavity. The fetus received 250 mg ampicillin (Novopharm Ltd., Toronto, ON) and 10 mg gentamycin (Schering, Pointe Claire, Que.) intravenously, while the ewe received 250 mg ampicillin and 80 mg gentamycin intramuscularly. Also, 250 mg ampicillin and 20 mg gentamycin were administered to the amniotic cavity daily for the duration of preparation.

2.2 Experimental Protocol

2.2.1 Antepartum Period

Following surgery, the ewes were housed with companion sheep in open pens and offered food and water ad libitum. At least 3 days were allowed for recovery before commencing polygraph recording procedures. Following the recovery period, the ewes were moved to small monitoring pens adjacent to the housing pens and companion ewes. Fetal condition was monitored in the antepartum period by measuring fetal blood gases, acid-base balance and oxygenation, glucose and lactate concentrations on a daily basis, which started the day after surgery and continued until delivery. Such serial blood samples (~1.0 ml) were simultaneously collected from the fetal femoral artery and umbilical vein catheters and measured for fetal Po₂, Pco₂, pH, O₂ saturation and hemoglobin, glucose and lactate concentrations. Fetal arterial pressure and heart rate were continuously monitored and
daily mean values were calculated from the stored computer data (see section 2.3.1). Also, fetal breathing movements and gasping efforts were assessed from the polygraph recording of the tracheal pressure in the prenatal period and monitored until delivery.

2.2.2 Identification of the Imminent Onset of Parturition

In this study, the onset of labor was identified mainly by the switch from the pre-labor type uterine contractures to predominantly early-labor uterine contraction pattern. However, a major difficulty in this project was to precisely predict the time of onset of labor so that the pre-labor (i.e., control) measurements would be obtained at the appropriate time. The transition from pre-labor to early-labor type uterine activity only served as an indicator of the onset of parturition. It could not be used to predict in advance when the spontaneous onset of labor would exactly occur. For identification of imminent onset of parturition, we had to watch for other signals, such as diminution or cessation of fetal breathing movements (see section 1.10), the daily changes in fetal heart rate with gestation and maternal physical signs and behavioral changes, including enlargement and swelling of the vulva and/or a discharge of mucus from the vulva, ewe restlessness, frequent lying down and getting up, pawing at bedding and frequent urination (see section 1.4.1.1). The combination of all these factors gave us valuable information on the imminent onset of parturition.
2.2.3 Labor Stage Definition

In this study, the process of labor and delivery was partitioned into five stages: pre-labor (as a control), early-labor, mid-labor, late-labor and delivery, according to the pattern of uterine contractions, as assessed from the polygraph recording of intrauterine pressure (Figure 1).

Pre-labor is defined as uterine contractions (contractures) with low amplitude elevations in resting pressure (about 5 mm Hg pressure increase). This form of uterine activity usually lasts for about 5-10 min and occurs once every 20-60 minutes in individual sheep. Uterine contractures are present throughout late pregnancy in sheep as discussed in the previous section 1.3.1.1. Our pre-labor measurements were taken at 2-3 days before the anticipated time of parturition.

Early-labor refers to the time when simple coordinated contractile patterns appeared with elevations in intrauterine pressure of shorter duration and also with these cyclic changes being superimposed on contractures.

Mid-labor is the period when uterine activity was well established with the presence of well defined and vigorous uterine contractions. Cervical dilation was usually 3-4 cm by this time.
Figure 1. Representative polygraph recording of labor stages from one sheep. Signals are intrauterine pressure, measured from the amniotic catheter. Five uterine contraction patterns representative of the changes that occur at pre-labor, early-labor, mid-labor, late-labor and delivery are depicted.
Late-labor is the time when there was a fairly regular pattern of labor contractions, also accompanied by maternal abdominal pushing (bearing down efforts). The cervix was fully dilated at this stage.

Delivery is the stage when the fetal head was in the vagina (about 5-10 min prior to expulsion).

2.2.4 Sampling and Measurement Schedules

The following schematic diagram illustrates sampling and measurement schedules used in the present study.

<table>
<thead>
<tr>
<th>Pre-labor</th>
<th>Early-labor</th>
<th>Mid-labor</th>
<th>Late-labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Fetal blood gas status, oxygen content and acid-base balance.
2. Fetal blood glucose and lactate concentrations.
3. Fetal arterial blood pressure and heart rate.
4. Fetal regional blood flows.
5. Fetal cardiac output and its distribution, systemic and umbilical blood flow.
6. Fetal $O_2$ delivery, extraction and consumption.
7. Umbilical lactate and glucose delivery, extraction and uptake.
8. Fetal plasma arginine vasopressin and catecholamine concentrations.

At each of these five time points, we simultaneously collected blood samples (~1.5 ml) from the FA, CA and UV catheters. Measurements of $Po_2$, $Pco_2$, pH, base excess, bicarbonate, $O_2$ saturation and hemoglobin, glucose and lactate concentrations were obtained for each of these blood samples. The samples were then accompanied by
measurement of fetal arterial blood pressure and fetal heart rate, also fetal regional blood flows and fetal combined ventricular output and its distribution, as well as systemic and umbilical blood flow, using radionuclide-labeled microspheres or fluorescent-labeled microspheres. Fetal O\textsubscript{2} delivery, extraction and consumption were calculated (see section 2.7.2), as were umbilical lactate and glucose delivery, extraction and uptake (see section 2.7.3). The fetal FA blood samples (~1.5 ml) were also collected for measurement of arginine vasopressin and catecholamine concentrations. The volume of fetal blood removed via sampling was replaced by an equal volume of maternal blood after each sample period.

2.3 Fetal Monitoring and Analysis Procedures

2.3.1 Recording and Blood Sample Analysis

During the study period, fetal arterial, tracheal and amniotic pressures were measured continuously with disposable strain gauge pressure transducers (Model P23 Db or P23 ID, Spectramed Inc., Oxnard, CA). Fetal heart rate was measured from the arterial pulse via a cardiotachometer (Model 9875, Sensormedics, Anaheim, CA). All variables were recorded on a polygraph recorder (Beckman R612 recorder, Beckman Instruments Inc., Schiller Park, IL; Sensormedics R711, Sensormedics, Anaheim, CA or Gould TA4000 thermal array recorder, Gould Inc., Valley View, OH). The analog signals from heart rate, amniotic and arterial pressures were converted to digital form using an on-line computerized data acquisition system (Kwan et al. 1995a), which comprised an Apple IIe
computer system (Apple Computer Inc., Cupertino, CA) with an analog to digital conversion board (AI-13 Analog Input System, Daisi Electronics Inc., Newton Square, PA) and a time board (The clock®, Mountain computer Inc., Scotts Valley, CA). The digitized samples for each variable were averaged and displayed at 10 sec intervals. The 1 min averaged values were stored on floppy diskettes. Mean fetal arterial pressure was corrected for amniotic pressure by subtraction with the computer program. Fetal blood samples for blood gas analysis and glucose and lactate concentration measurements were collected into preheparinized blood gas syringes (Marquest Medical Products, Englewood, CO). Fetal blood pH, Po$_2$, Pco$_2$, base excess and bicarbonate were measured with an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy) with temperature corrected to 39.5°C for fetal blood samples and 39°C for maternal blood samples. Oxygen saturation and hemoglobin concentration were measured in triplicate with an OSM-2 Hemoximeter® (Radiometer, Copenhagen, Denmark). Blood glucose and lactate concentrations were measured in triplicate with a glucose/lactate 2306 STAT plus analyzer (YSI Inc., Yellow Springs, OH).

### 2.3.2 Analysis of Fetal Breathing Movements

Fetal breathing movements were defined as rapid irregular breathing movements (Dawes et al. 1972), and gasping was defined as episodes of slow relatively deep respiratory efforts with single negative pressure deflections (Lewis and Boylan, 1979). They were assessed from the tracheal pressure recorded on the polygraph chart and analyzed visually.
The minimum breath amplitude analyzed was 1 mm Hg and the minimum duration of breathing activity accepted as a breathing episode was 10 sec (Rurak et al. 1988).

In the present study, portions of the polygraph chart records could not be analyzed because of interruption of tracings, so that for the 77 days of data analyzed, the overall data lost was 13.9%. The remaining 86.1% of data was used to compute the incidence of breathing movements and gasping efforts. Data on the fetal breathing movements and gasping efforts were computed per day from the time of delivery of the fetus retrospectively for 7 days and expressed as days before delivery, with the day of delivery being day 0. Data was also estimated over 4 h periods on the last day of gestation (i.e., on the day 0). The overall incidence of fetal breathing movements on each day or over 4 h periods were expressed as percent of time by taking the time period (in minutes) that each breathing activity and gasping was observed and dividing it by the time period (in minutes) recorded. The percentage of time spent in breathing activity, nonbreathing and gasping were calculated using a computer program developed in our laboratory.

2.3.3 Quantitation of Uterine Contractions before and during Labor and Delivery

Uterine activity was measured by continuous recordings of intrauterine pressure via the amniotic catheter, which allow assessment of the frequency, duration and intensity of uterine contraction before and during labor and at delivery. The variables used to estimate the uterine activity included the frequency, average intensity, average duration, sum of intensity and sum of duration of the uterine contractions, Alexandria unit (El-Sahwi et al.
1967) and a modified Alexandria unit. These values were calculated from 30 min immediately before to 30 min after each microsphere injection at each labor stage. For calculation of the duration and amplitude (intensity) of each individual uterine contraction, these parameters were measured by hand with a measuring caliper from the original polygraph records. The duration of uterine contraction was measured as the distance between the onset and the end of the uterine contraction defined by El-Sahwi et al. (1967), while the amplitude of each uterine contraction was measured according to definitions by Braaksma et al. (1971), in which the intensity of uterine contraction was measured from baseline resting pressure rather than from zero.

The Alexandria unit was chosen to quantitate uterine contractions in this study because this method is considered to be superior to the more commonly used Montevideo unit (Caldeyro-Barcia et al. 1957) for measurement of uterine activity in human labor, in that it includes a measure of the duration of contraction in the calculation, thus more accurately expressing total uterine work performed in the process of labor. This unit is the product of the average intensity of uterine contraction times the average duration of contraction multiplied by the number of contractions observed during a 10 min period of recording time, thus giving the equation: the Alexandria unit = average amplitude (mmHg) x average duration (min) x average frequency (per 10 min). However, a problem with the Alexandria method is that the calculated unit during the last stage of labor does not reflect the final increase in total uterine work, which is known to occur at that time. This is because the average duration of the uterine contractions, one of the components involved in quantitation of uterine activity with the Alexandria unit, is decreased with the
progression of labor, thus affecting the overall product values calculated. To solve this problem, we modified the Alexandria unit by incorporating the sum of the intensity of uterine contractions and the sum of the duration of uterine contractions to indicate the total amount of uterine activity over a given period at each stage of labor. We also extended our calculation to 1 h instead of 10 min. The modified Alexandria unit is thus equal to the product of the sum intensity of uterine contraction (in mmHg) times the sum duration of uterine contraction (in minutes) multiplied by the contraction frequency per one hour period as following equation.

\[
\text{The modified Alexandria unit} = \text{sum amplitude (mmHg)} \times \text{sum duration (min)} \times \text{number of contractions (per hour)}.
\]

2.4 Fetal Regional Blood Flow Measurement

2.4.1 Microspheres

The method for measurement of regional blood flow in the fetus with radioactive microspheres has been described in detail before (Rurak et al. 1990b), and was followed for both radioactive and fluorescent microspheres in the current study. radioactive microspheres (15.5 ± 0.1 µm diameter) were obtained from DuPont NEN Research Products (Billerica, MA) and labeled with one of the following gamma emitting radionuclides: \(^{153}\text{Gd}, ^{51}\text{Cr}, ^{85}\text{Sr}, ^{95}\text{Nb}\) and \(^{46}\text{Sc}\). The physical characteristics of radionuclide-labelled microspheres we used are described in Table 1. Fluorescent-labeled polystyrene latex microspheres (FluoSpheres, diameter 15.5 ±
Table 1. Physical characteristics of radionuclide-labelled microspheres.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-Life (Days)</th>
<th>Photon Abundance (%)</th>
<th>Principal γ-ray Photon Energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gadolinium-153</td>
<td>242.0</td>
<td>50.6</td>
<td>97-103</td>
</tr>
<tr>
<td>Chromium-51</td>
<td>27.7</td>
<td>9.83</td>
<td>320</td>
</tr>
<tr>
<td>Strontium-85</td>
<td>64.8</td>
<td>98.0</td>
<td>514</td>
</tr>
<tr>
<td>Niobium-95</td>
<td>34.9</td>
<td>99.82</td>
<td>765</td>
</tr>
<tr>
<td>Scandium-46</td>
<td>83.8</td>
<td>99.984 &amp; 99.487</td>
<td>889 &amp; 1120</td>
</tr>
</tbody>
</table>
0.3 μm) were obtained from Molecular Probes, Inc. (Eugene, OR) and were stored in a refrigerator (4°C) away from light when not in use. These microspheres are now available with 10 different color labels (An et al. 1996). In the present study, only 5 were used: blue, yellow-green, orange, red and crimson. The excitation and emission maxima described by the manufacturer for the fluorescent dyes are given in Table 2.

2.4.2 Microsphere Injection

In order to assure a homogeneous suspension of microspheres, before every experiment the stock solution containing the radioactive or fluorescent labeled microspheres was manually shaken, placed in a sonicating water bath for at least 30 min, and then agitated on a vortex mixer (Vortex-Genie®, Fisher Scientific Instruments, Springfield, MA) for 1 min. An aliquot containing approximately $1.2 \times 10^6$ microspheres was removed from each of the stock vials just prior to injection. The microspheres were dispersed and mixed by drawing the solution back and forth through a sterile 26-gauge needle into a 1 or 3 ml plastic syringe. Following withdrawal of ~3ml of fluid from the fetal tarsal vein catheter, they were then injected via this catheter over 30 sec, followed by 3 ml of the initially withdrawn fluid and 5 ml of heparinized saline. The order of injection of the five microspheres was chosen randomly.

2.4.3 The Reference Blood Sample

Before each experiment, the reference sample withdrawal pump (Harvard Apparatus, Millis, Massachusetts) was accurately calibrated at the predetermined rate of withdrawal. The
Table 2. Excitation and emission wavelength peak and range of fluorescent-labeled microspheres, along with the optimal excitation and emission wavelengths in Cellosolve acetate given by the manufacturer (in parentheses), and the emission and excitation filter combinations used in the CytoFluor™ 2350 instrument.

<table>
<thead>
<tr>
<th>Color</th>
<th>EX peak (nm)</th>
<th>EX range (nm)</th>
<th>EX filter (nm)</th>
<th>EM peak (nm)</th>
<th>EM range (nm)</th>
<th>EM filter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>356 (360)</td>
<td>330-385</td>
<td>360/40</td>
<td>417 (420)</td>
<td>380-490</td>
<td>460/40</td>
</tr>
<tr>
<td>Yellow-green</td>
<td>497 (490)</td>
<td>435-515</td>
<td>485/20</td>
<td>505 (506)</td>
<td>480-570</td>
<td>530/25</td>
</tr>
<tr>
<td>Orange</td>
<td>533 (530)</td>
<td>470-560</td>
<td>530/25</td>
<td>547 (552)</td>
<td>520-600</td>
<td>560/20</td>
</tr>
<tr>
<td>Red</td>
<td>568 (565)</td>
<td>495-605</td>
<td>560/20</td>
<td>590 (598)</td>
<td>550-640</td>
<td>590/20</td>
</tr>
<tr>
<td>Crimson</td>
<td>610 (600)</td>
<td>500-645</td>
<td>590/20</td>
<td>618 (635)</td>
<td>575-660</td>
<td>645/40</td>
</tr>
</tbody>
</table>

EX = Excitation wavelength and EM = Emission wavelength. EX and EM filter represent CytoFluor™ 2350 standard filter sets with excitation or emission center wavelength/half bandwidth, respectively.
arterial reference samples were then withdrawn simultaneously from both the carotid and the femoral arterial catheters into 10 ml heparinized and greased glass syringes attached to a Harvard infusion-withdrawal pump, at a constant rate of 2.06 ml/min from 30 sec before microsphere injection to 2 min after the 5 ml saline flush. The blood in the glass syringe was then emptied into 4 individually labelled plastic gamma counting vials (radioactive microspheres) or a 50-ml graduated polypropylene conical centrifuge tube with a screwcap (fluorescent microspheres). Each syringe was then rinsed several times with saline into its corresponding vial or tube until blood was no longer visible in the syringe. After each reference sample withdrawal, the blood volume loss was replaced by an equal volume of maternal blood.

Reference blood flow was calculated as the difference between glass syringe weights pre- and post-withdrawal, corrected for blood density (1.024 g·ml⁻¹), divided by the collection time in min as follows.

Reference blood flow = difference between glass syringe weights pre- and post-withdrawal / (1.024 × collection time).

2.4.4 Sample Processing

After the last microsphere injection, a lethal dose of sodium pentobarbital (Euthanol, MTC Pharmaceuticals, Cambridge, ON, Canada) was given to the ewe. The fetus was removed from the uterus, towel-dried, and then weighed. All fetal organs and tissues were then
dissected, cleaned carefully, blotted dry and weighed. This included pituitary, spinal cord, cerebellum, medulla, pons, midbrain, frontal hemisphere, temporal hemisphere, right and left ventricle, interventricular septum, lung, nuchal muscle, fore limb skin, fore limb muscle (infraspinous), fore limb bone (humerus), diaphragm, omasum, abomasum, rumen, reticulum, spleen, kidney, brown fat, adrenal, small intestine, large intestine, liver, hind limb skin, hind limb bones (femur, tibia, metatarsal), hind limb muscle (biceps femoris) and placenta. The small and large intestines were separated from the mesentery, opened, and cleared of contents. Each tissue sample was cut into small pieces. The tissues from the animals which received radioactive microspheres were placed in aluminum foil dishes and carbonized in an oven at 350°C for several days. The carbonized tissues were then ground into a coarse powder and packed into plastic counting tubes and counted for 5 min on a Searle gamma counting system (Searle Analytical, Des Plaines, IL). Paired organs (kidneys, adrenals and cerebral hemispheres) were counted separately to verify complete mixing of the microspheres in the systemic arterial circulation.

The tissues obtained from animals which received fluorescent microspheres were processed using the sedimentation and dye extraction methods previously reported by others (Van Oosterhout et al. 1995), with the following modifications. The tissues were placed into pre-weighed 50-ml polypropylene centrifuge tubes (Elkay, Shrewsbury, MA). After weighing, the tissue samples were further minced finely with scissors inside the test tube. Tissue samples were digested by adding freshly prepared warm 4 M ethanolic KOH with 0.5 % Tween-80 (at least 3 ml/g tissue) so that the total volume was 50 ml. The tubes were then left at room temperature for 5 days, protected from the light and shaken periodically. On the last day of
tissue digestion, the tubes with brown fat and skin were placed in a warm water bath at 60°C for ~2 h. After these procedures, all the tissue samples were fully dissolved. They were then centrifuged at 1000g in a swinging-bucket rotor at 25°C for 20 min, following which the supernatant was carefully discarded by suction until < 1 ml remained above the sediment. The sediment was then resuspended in 50 ml deionized water, which was warmed in a 60°C water bath. The purpose of the 60°C temperature here and with the skin and brown fat digestion was to promote the removal of lipid from the samples. The tubes were centrifuged again, and the supernatant was discarded. Next, the sediment was resuspended in deionized water and centrifuged again, and in some cases, the washing step with deionized water was repeated if the supernatant was not clear. After the final centrifugation, the supernatant was removed carefully by hand using a Pasteur pipette, taking care not to either disturb or dry the pellet (~100 µl of supernatant left). Three ml of 2-ethoxyethyl acetate (Cellosolve acetate, Aldrich Chemical, Milwaukee, WI) was added to the pellet, and the tubes were vortexed and then allowed to stand for at least 4 h to extract the fluorescent dye from the microspheres. They were then vortexed again and centrifuged, leaving a clear dye-containing solution from which 200 µl aliquots were pipetted in triplicate into individual wells of a translucent, polypropylene 96 microwell plate (Nunc, Inter Med, well volume = 0.3ml, Canadian Life Technologies, Burlington, ON). Fluorescence was then determined. The reference blood samples were processed in the same manner as described above, except for those containing the blue microspheres. With these latter samples, at the final washing step, the supernatant was removed until ~10 ml of deionized water was left. Then nitrogen gas was bubbled through the solution for 10 min, since preliminary work indicated that in the absence of this step, there was interference with the blue fluorescent measurement, as described in the results section. Further
processing of these samples was not different from that of the other samples. After Cellosolve acetate was added, all samples were stored in the dark until ready for reading. All of the fluorescence measurements were made on all of the samples from a single animal on the same day.

Bone tissues required special treatment, since their digestion did not occur with ethanolic KOH and other methods of bone digestion that were tried were not successful. The bones were crushed with pincers within the test tubes. Six ml of Cellosolve acetate were then added, so that the bone tissues were completely immersed in the solvent. Over the next 5 days, the samples were periodically shaken. Thus, the fluorescent dye was extracted from the microspheres without tissue digestion. After centrifugation, 200 μl of the supernatant was transferred from each sample into individual wells of a 96 microwell plate. Fluorescence was then determined and corrected by a factor of 2 to account for the final dilution of the bone samples compared to the other tissues and reference samples.

2.4.5 Fluorescence Detection and Measurement

The absorption spectra of the five fluorescent microspheres dissolved in Cellosolve acetate were obtained by using an automated spectrophotometer (DU Series, Beckman Instruments, Inc., Fullerton, CA). The spectral absorbance curves thus established were used to determine the wavelengths at which the fluorescent microspheres have absorption maxima and minima. The emission spectra of the microspheres were determined using glass cuvettes with a spectrofluorometer (Model RF-540, Shimadzu Corporation, Kyoto, Japan). Each emission
spectrum was obtained by excitation at the optimal wavelength obtained from the absorption spectra.

The fluorescence of the extracted fluorophores in all samples and the solvent blanks was measured with an automated fluorescence multiwell plate reader (CytoFluor™ 2350 Fluorescence Measurement System, Millipore Corporation, Bedford, MA). The light source of the instrument was an M32-type halogen bulb (360-700 nm operating range), while the detection system employed a R928 Hamanatsu photomultiplier tube. Both excitation light and emission readings occurred from below the well (i.e. bottom to top orientation). Measurement of multiple fluorescent colors within a sample was accomplished by reading the sample multiple times with paired excitation and emission wavelength filters which were specific for each fluorophore. The filters were selected from the CytoFluor™ 2350 standard filter set to minimize spillover of fluorescence from one microsphere into the emission filter band of another, and are listed in Table 2. However, complete elimination of spillover was not possible with the filter set available and this required estimation of the extent of spillover, as described below. The scan speed of 2350 Microwell plate reader is 0.25 sec/well, or ~24 sec/plate. As each plate had to be read at least 5 times and as all tissues from a single animal required ~10 plates, the total run time for an animal was ~40 min. The computer acquired fluorescent readings were obtained with the standard CytoFluor™ software and analyzed using standard computer spreadsheet software.
2.4.6 Standard Curve and Relationship of Fluorescent Intensity and Microsphere Number

To determine the number of fluorescent microspheres in a sample, triplicate 200 μl aliquots of each fluorescent labeled microsphere were withdrawn, diluted to 10 ml with deionized water containing 0.25% Tween-80. The diluted microsphere specimens were thoroughly suspended, introduced into the counting chamber of a hemocytometer (American Optical, Buffalo, NY) and counted in 9 chambers (9 mm² in area, and 0.1 mm in depth) under a microscope (Nikon, TMS, Japan) using a × 4 objective with × 10 eyepieces. Each diluted microsphere suspension was counted in triplicate. The total number of microspheres in the solution was computed by the formula: Total microspheres = [Number of Microspheres counted / 0.9mm³] × 10³ × ml suspension. An aliquot of suspension, with known numbers of fluorescent microspheres, was then dissolved in 1 ml of solvent. The solvent remained in contact with the microspheres for at least 4 hours to ensure complete extraction of the dye. The fluorescence intensity of the supernatant was then analyzed in the CytoFluor™ 2350 microplate reader. The ratio of fluorescence intensity per number of microspheres (fluorescence units/per microsphere number) was then determined and used as the basis of establishing a standard curve for assessing microsphere numbers in tissue samples. The linearity of the CytoFluor™ 2350 instrument was tested by 3 repeated measurements of the fluorescence intensity of the microspheres in Cellosolve acetate in concentrations ranging from 80 to 20,000/ml. The assay precision was assessed by measuring the within-run and between-run variation of these samples, expressed as coefficient of variation. The stability of the dyes in solvent was
evaluated by dissolving fluorescent-labeled microspheres in Cellosolve acetate and reading the fluorescent intensities of each color batch every second day for 2 weeks.

2.4.7 Fluorescent Microsphere Recovery

Two hundred μl aliquots with known numbers of fluorescent microspheres labeled with the crimson fluorophore were dissolved in 3 ml Cellosolve acetate. These became the standards of 100% recovery (F_standard). Aliquots of the same microspheres were added to the blood and dissected tissue samples obtained from two fetuses in which no microspheres had been injected. The number of microspheres used to spike the blood and tissue samples and in the standards approximated those found in the tissue and blood samples derived from our previous radioactive microsphere studies, and ranged from 400 to 20,000. The blood and tissue samples were processed according to the sedimentation method as described above. Recovery (%) of microspheres from tissue or blood was calculated as $100 \times \frac{F_{\text{sample}}}{F_{\text{standard}}}$, where $F_{\text{sample}}$ was the fluorescence of the tissue and blood samples and $F_{\text{standard}}$ was the fluorescence of the standard.

2.4.8 Assessment and Correction of Spectral Overlap and Signal Quenching

With the relatively large bandpass values of the excitation and emission filters in the CytoFluor™ 2350 instrument (Table 2), it seemed possible that there could be spillover of the emitted light from one fluorophore into the emission band of another label. In addition, the emitted light of one fluorophore can be absorbed by another, if the excitation spectrum of the second label is close to the emission spectrum of the first dye, and this will result in quenching
of the first dye’s fluorescent signal (Prinzen and Glenny 1994). As an initial check for spillover
of a fluorescent signal into the emission spectra of adjacent colors, the fluorescent intensities of
each fluorophore dissolved in Cellosolve acetate were measured at each excitation/emission
pair listed in Table 2. A spillover matrix representing the signals from the specific fluorescent
color in each color band was thus determined, and the results indicated that spillover only
occurred for orange into yellow green, orange into red, red into orange and red into crimson.
Since both spillover and quenching are in part determined by the relative concentrations of the
fluorophore pairs, the effect of changes in these concentrations on the fluorescence reading of
the affected dye was examined for the following five sets of fluorescent-labeled microsphere
pairs. Set 1: red effects on crimson, Set 2: crimson effects on red, Set 3: orange effects on
yellow-green, Set 4: red effects on orange and Set 5: orange effects on red. For each of these
sets we prepared, in triplicate, 25 different combinations of concentration of the 2 probes. The
second color in each set was denoted as A, representing the affected fluorophore, whereas the
first one denoted as B, was the dye causing spillover and/or quenching (the interfering
fluorophore). For color A, 5 microsphere concentrations ranging from 500 to 20,000/ml were
employed, while 8 concentrations ranging from 0 to 40,000/ml were used for color B. The
microsphere combinations is shown in the following design, in which for each row, there was a
fixed concentration of A and increasing concentrations of B. The left most column in the design
had no B microspheres. To determine the fluorescence intensity of the added microsphere (B)
and hence the degree of enhanced fluorescence caused by overlap, the fluorescence intensity in
each block of this column (i.e. no added B microspheres) was subtracted from the fluorescence
reading of the other blocks in that row. Then, for each row in the design, the total fluorescence
intensity of both colors in the sample was plotted against the fluorescence intensity of the
Experimental design for spillover correction and signal quenching*.

<table>
<thead>
<tr>
<th>(A) 500</th>
<th>(A) 500</th>
<th>(A) 500</th>
<th>(A) 500</th>
<th>(A) 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B) 0</td>
<td>(B) 0</td>
<td>(B) 1000</td>
<td>(B) 2000</td>
<td>(B) 4000</td>
</tr>
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<td>(A) 1000</td>
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<td>(B) 500</td>
<td>(B) 5000</td>
<td>(B) 1000</td>
<td>(B) 2000</td>
<td>(B) 4000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) 20000</td>
<td>(A) 20000</td>
<td>(A) 20000</td>
<td>(A) 20000</td>
<td>(A) 20000</td>
</tr>
<tr>
<td>(B) 0</td>
<td>(B) 5000</td>
<td>(B) 10000</td>
<td>(B) 20000</td>
<td>(B) 40000</td>
</tr>
</tbody>
</table>

* In each cell, A represents the microsphere concentration of the affected color and B represents the microsphere concentration of the interfering color.

added color. The % spillover of the fluorescence emission signal of the adjacent color was calculated using the following formula: \( S = \frac{\alpha}{\beta} \times 100\% \), where \( \alpha \) is crossover fluorescence intensity of the specific color in its adjacent window, \( \beta \) is the fluorescence intensity of the specific color in its own window and \( S \) is the spillover expressed as a percentage. To validate these spillover corrections, regression analysis was used to compare the fluorescence intensity of samples with each of the colors alone with samples containing all 5 labels together, before and after spillover correction. Five concentrations of each microspheres (500-20,000) were examined in this analysis. Quenching was also assessed by regression analysis of the
fluorescence intensity in the emission band of label A against the microsphere concentration of label B.

2.4.9 Determination of Blood Flow

Regional blood flows were computed with the same formula for both radioactive and fluorescent microspheres. The values for fluorescent microspheres were calculated after subtracting fluorescent background, endogenous fluorescence (for blue only) and making corrections for spectral spillover, while for radioactive microspheres, background, decay and spillover corrections occurred for all isotopes. Organ blood flow was calculated as reference sample withdrawal rate (ml/min) × [I_{organ} /I_{reference}], where I = fluorescent intensity or gamma counts. Fetal organ blood flow was further divided by the tissue weight and normalized to 100 g. Flow results are thus expressed as milliliters per minute per 100 gram (ml/min/100 g). For the fetal organs and tissues supplied by the ascending aorta, the carotid arterial reference sample was used, whereas for those supplied by the descending aorta, the femoral arterial reference sample was used (Heymann et al, 1977). Lung blood flow was also estimated using the femoral arterial reference sample, since Rosenberg et al (1984) found this to be an acceptable approach.

2.5 Estimate of Fetal Combined Cardiac Output

The most common method of measuring fetal combined cardiac output with microspheres involves either injecting two differently labeled microspheres simultaneously at two
injection sites plus two withdrawal sites or using only one injection site plus two withdrawal sites but requiring a further surgical intervention with catheterization of the pulmonary artery (Rudolph and Heymann, 1980). In addition, the whole carcass (including the skin, bone and muscle) of animals containing radioactive microspheres must be minced and then incinerated in aluminum foil at 350°C to reduce the total volume (Faber and Green, 1972). After that, foil and ashes have to be thoroughly homogenized in a blender and a small aliquot taken for counting carcass radioactivity. These procedures were very impractical in the present study and therefore not used.

Our procedure to estimate fetal cardiac output is as follows. At postmortem, fetal organs and tissues were dissected and weighed. The weight of the remaining large part of the carcass (skin, bone and muscle) was also measured. Percentages of fetal body weight accounted for by the skin, bone and muscle were obtained from the data on the body proportions of fetal lambs (Wallace, 1948). From these data, we calculated the total skin, bone and muscle weight, respectively. As blood flow to skin, bone and muscle samples was measured in ml/min/100 g tissue by the radioactive or fluorescent microsphere method, total skin, bone and muscle flows were then derived from these corresponding values. Fetal combined ventricular output was then calculated as the sum of all the individual organ and tissue blood flows plus placental blood flow.
2.6 Hormone Measurement

2.6.1 Sample Preparation

Fetal FA blood samples were transferred immediately into chilled test tubes for determinations of either catecholamines or AVP. Since catecholamines are generally present in biological fluids at low concentration, are susceptible to oxidation, and unstable at basic pH, the sample collection assumes considerable importance, particularly with plasma. Thus, for plasma catecholamine assay, blood samples (1.0 ml) were placed into test tubes, which contain an anticoagulant, EGTA (Ethylene glycol -bis- (B-amino ethyl ether) N, N, N, N-tetra acetic acid) and an antioxidant, glutathione (Amersham Corporation, UK). These substances were added to the tubes in a solution (pH 6.0-7.4) containing 90 mg/ml EGTA and 60 mg/ml glutathione. Twenty microlitres of this solution are needed for each ml of blood to be collected. For plasma AVP assay, blood samples (~1.5 ml) were collected into EDTA-containing tubes.

The test tube were slowly turned in the hand a few times to ensure adequate mixing of the blood with the reagent solution but under no circumstances are the samples shaken vigorously. The test tube was then placed in an ice bath and immediately centrifuged at 760g for 20 min at 4°C. The plasma obtained was transferred immediately to a clean test tube, which is then tightly sealed (for catecholamine assay plasma) and stored in an upright position at -70°C. Fetal catecholamine concentrations will be measured in plasma samples.
collected in this study in collaboration with a research group at the University of Western Ontario (Dr. Lawrance Fraher).

2.6.2 Measurement of Plasma Arginine Vasopressin Concentrations by Radioimmunoassay

2.6.2.1 Extraction of Plasma Samples

Small columns packed with octadecasilyl-silica (ODS-silica, C18 sep-paks, INCSTAR Corporation, Stillwater, MN) were used. Each Sep-Pak column was attached to a polypropylene syringe and washed with 5 ml methanol followed by 20 ml deionized H$_2$O. The plasma was acidified with 1M HCL (100µl/ml) loaded into a syringe and pushed slowly, over a period of 1 minute, through the column. The column was then rinsed with 20 ml 4% (v/v) acetic acid. 3 ml of methanol were pushed into the column to displace any residual fluid from the previous wash, and left in contact with the ODS-silica for 3 minutes. Then there was passage of another 1 ml methanol to insure complete elution of the peptide. The two volumes of effluent were combined and dried under nitrogen. The sample was then reconstituted in 0.5 ml assay diluent. Aliquots of 0.2 ml (equivalent to 0.4 ml of sample if 1 ml is extracted) were assayed in duplicate.
2.6.2.2 Radioimmunoassay

Immunoreactive arginine vasopressin was measured in 1 ml fetal plasma, using a double antibody radioimmunoassay method (INCSTAR Corporation, Stillwater, MN). A standard curve of arginine vasopressin was obtained by making serial dilutions of purified arginine vasopressin, starting at a concentration of 80 pg/ml, with 1% BSA-borate buffer. Also included in the assay were duplicates of total activity tubes, non-specific binding tubes and zero standard tubes. Control samples containing purified arginine vasopressin provided by INCSTAR were included at the beginning, middle and the end of the assay. The percentage of arginine vasopressin recovered from the extraction step was calculated by determining the ratio of the amount of $^{125}$I arginine vasopressin obtained from extraction of 100µl $^{125}$I arginine vasopressin and 900 µl of maternal plasma to 100 µl of unextracted $^{125}$I arginine vasopressin.

The sample and primary antibody, which was raised in guinea pig against arginine vasopressin, was incubated for 18-24h at 2-8°C. The $^{125}$I vasopressin tracer was then added and incubated for 18-24h at 2-8°C. Phase separation was completed in 15-25 minutes at 20-25°C with a pre-precipitated complex of second antibody, which is an anti-guinea pig IgG raised in goats, plus carrier and polyethylene glycol in a single pipetting step. After centrifugation, the supernatant was aspirated by vacuum. The pellet was then counted for 5 min on a Searle gamma counting system (Searle Analytical, Des Plaines, IL).
Assay validations and performance tests included: (1) a parallel assay study in which the linear portions of the concentration-response curves are compared for control (AVP standard) and test solutions (fetal sheep plasma extract); (2) extraction recovery and (3) assay reproducibility.

2.7 Data Calculation

2.7.1 Calculation of Oxygen Content

Oxygen content is calculated using the formula below.

\[
\text{Oxygen content (mmol/L)} = \text{Hb concentration (g %)} \times \text{O}_2 \text{ saturation (\%)} \times 0.0621(\text{mM})
\]

Where Hb, is hemoglobin. The amount of oxygen carried by 1 gram of hemoglobin in mM is derived from the following: 1 mole of Hb = 64458 g, 1 mole of Hb carries 4 moles (4000mM) of O\textsubscript{2}. Thus, the amount of oxygen carried by 1 gram of Hb is 4000/64458 = 0.0621 mM. The dissolved oxygen in fetal blood was considered negligible in this calculation because of the low vascular Po\textsubscript{2} in the fetus.

2.7.2 Calculation of Fetal Oxygen Delivery and Consumption

Fetal O\textsubscript{2} delivery was calculated as the product of the rate of umbilical blood flow and the oxygen content in umbilical venous blood. Fetal oxygen consumption was calculated as
the product of umbilical blood flow and the umbilical veno-arterial difference in oxygen content. Fetal oxygen extraction is the ratio between fetal oxygen consumption and oxygen delivery.

2.7.3 Calculation of Substrate Delivery, Uptake and Extraction

By using calculations analogous to fetal oxygen delivery, consumption and extraction, glucose or lactate delivery to the fetus, glucose or lactate uptake by the fetus and extraction of glucose or lactate by the fetus are determined in the same way as those of fetal oxygenation as described above.

2.7.4 Calculation of Substrate Oxygen Quotients

This is the fraction of oxygen consumed to completely oxidize a substrate. It is calculated by dividing venoarterial concentration differences for glucose or lactate by the venoarterial concentration differences for oxygen. This quotient is multiplied by 6 for glucose or 3 for lactate.

2.7.5 Calculations of Radioimmunoassay Results

In trying to find an optimal method for the calculation of results for AVP, several methods of expressing the data resulting from radioimmunoassay (RIA) were compared.
(1) Bound counts versus Log concentration

In this method, the raw bound counts are plotted against the logarithm of the standard AVP concentration. Bound counts are maximal in the absence of unlabeled AVP and fall toward zero when the concentration of unlabeled AVP is increased. The shape of the resulting curve is sigmoidal. The advantage of this method of data representation is its simplicity; however, its disadvantage is that it makes comparisons between assays for quality control and validation test very difficult.

(2) B/Bo versus Log concentration

This calculation method is used by INCSTAR (Vasopressin RIA kit, Instruction Manual, INCSTAR Corporation, Stillwater, Minnesota, USA). It normalizes the standard curve of each assay to facilitate interassay comparisons, but preserves the sigmoidal shape of the response curve inherent in the raw count data; so it is still not possible to perform parallel-line comparisons for assay validation.

(3) Logit B/Bo versus Log concentration

This type of data representation is involved in data transformation - Logit transformation (Rodbard and Lewald, 1970). The following response variable was computed for each assay tube:
where \( B/Bo \) is as defined as follows:

\[
\frac{B}{B_o} = \frac{(B'' - NSB)}{(B''o - NSB)}
\]

where \( B'' \) is the number of labeled AVP-antibody complex counts (cpm), \( B''o \) is the number of labeled AVP-antibody complex counts in the absence of unlabeled AVP (cpm), NSB is the nonspecific binding (cpm).

The Logit response variable is plotted on a linear scale against the AVP standard concentration on a logarithmic scale. As a result, this logarithmic transformation of the normalized bound counts results in a linear response curve. Thus, a straight line can be fit to these data and the standard curve can be completely characterized by the resulting slope and intercept. This provides a basis for quantitative interassay comparison and assay validation. Therefore, this method was used in calculating values of unknowns in our AVP assay. The estimated AVP concentrations are interpolated from the standard curve regression line:

\[
\text{Logit}(B/Bo) = M \cdot \log_{10}(AVP) + C
\]

or

\[
\log_{10}(AVP) = \frac{1}{M} \cdot \left( \text{Logit}(B/Bo) - C \right)
\]
or

\[ AVP = \text{Anti log} \left( \frac{1}{M} \right) \text{Logit} \left( \frac{B}{B_0} \right) - C \]

where \([AVP]\) is the estimated concentration, \(M\) is the slope of the standard curve regression line, and \(C\) is the intercept of the standard curve regression line.

### 2.8 Units of Experimental Measurement

- Gestational age (days).
- Fetal and maternal weight (kg).
- Fetal organ weight (gm/kg fetal body weight).
- Placental weight (g).
- Duration of labor (d or h or min).
- Fetal blood gas status, oxygen content and acid-base balance:
  - \(\text{pH} (-\log H^+, \text{dimensionless number})\), \(\text{Po}_2\) (mmHg), \(\text{Pco}_2\) (mmHg), \(\text{BE}\) (mEq/L), \(\text{HCO}_3^-\) (mEq/L), \(\text{Tco}_2\) (mmol/L), [Hb] (g%), \(\text{O}_2\) saturation (%), \(\text{O}_2\) content (mmol/L), lactate (mmol/L), glucose (mmol/L).
- Fetal heart rate (beats/min).
- Fetal arterial pressure (mmHg).
- Fetal rate pressure product (beats/min \(\times\) mmHg).
- Fetal organ/tissue blood flows (ml/min/100g of wet tissue weight).
- Umbilical placental blood flow (ml/min/kg fetal body weight or ml/min).
- Fetal cardiac output (ml/min/kg fetal body weight or ml/min).
- Distribution of fetal cardiac output (%).
- Fetal plasma arginine vasopressin concentrations (pg/ml).
- Fetal oxygen delivery, fetal oxygen consumption (μmol/min/kg of fetal body weight).
- Fetal oxygen extraction (%).
- Umbilical glucose delivery, umbilical glucose uptake (μmol/min/kg of fetal body weight).
- Umbilical lactate delivery, umbilical lactate uptake (μmol/min/kg of fetal body weight).
- Umbilical glucose and lactate extraction (%).
- Substrate oxygen quotients: glucose/O₂ and lactate/O₂ (dimensionless values).
- Uterine contractions: frequency (contractions/hr), intensity (mmHg), duration (min), modified Alexandria unit (kilounit).
- Fetal breathing movements (% time breathing).

### 2.9 Statistical Analysis

A commercial software program NCSS (Number Cruncher Statistical System) on an IBM computer system (Copyright © by Dr. Jerry L. Hintze, & NCSS, Kaysville, UT) was used for statistical analysis. The data were analyzed for statistical significance by means of an analysis of variance (ANOVA) or T-tests, depending upon the data obtained. Comparisons of the mean values of a given variable between the two groups were done by three way analysis of variance for repeated measures (ANOVA). Comparison of the
mean value of a given variable within the same group were done by two way analysis of variance for repeated measures. The advantage of two way and three way ANOVA is the changes with time could be determined separately from the inter-animal variability. If the resulting F values were statistically significant (p<0.05), differences between individual means were determined with Fisher's least significant difference test for multiple comparisons. Comparisons of the mean values of a given variable between the two groups at any particular prenatal period and pre-labor stage were done with use of the unpaired t test. The regression line was analyzed by the least-squares fitting method. The standard error of the estimate (SEE) was calculated for these regression equations as a measure of random error (Westgard and Hunt, 1973). Correlation coefficients were determined by the Pearson method. The differences in blood flow values between the paired organs were analyzed by the paired t test. The duplicate variability was expressed as the absolute value of the difference of the blood flow values determined by the 2 fluorescent-labeled microspheres divided by each arithmetic mean × 100. The coefficient of variation (SD/mean) was used to describe the variability of repeated measurements, where SD is standard deviation. For comparison of the blood flow estimates obtained in the 4 animals which received radioactive microspheres (R) with the fluorescent microsphere estimates (F) in the 3 fetuses at pre-labor stage, the following formula was used for each tissue and organ: (mean flow\textsubscript{F} - mean flow\textsubscript{R})/SD\textsubscript{R}. All values are presented as mean value ± SEM (standard error of the mean), unless otherwise specified. A p value < 0.05 is considered statistically significant.
3. Results

3.1 Validation of an Automated Fluorescent Microsphere Method for Measurement of Regional Blood Flow in the Fetal Sheep

3.1.1 Analytical Evaluation

Figure 2 shows the spectral absorption and emission spectra of the 5 different fluorescent-labeled microspheres in Cellosolve acetate. The absorption wavelength peak and range of these 5 labels determined by the spectrophotometer are shown in Table 2. Also presented in Table 2 are the emission wavelength peak and range of the labels, which were collected at the specific absorption wavelength peaks and determined by the spectrofluorometer. The optimal absorption and emission wavelengths for blue, yellow-green, orange, red and crimson were 356/417, 497/505, 533/547, 568/590 and 610/618 nm, respectively. These values are in accordance with the published data for each fluorescent dye (Glenny et al. 1993). The excitation and emission filter combinations used for each microsphere are also given in Table 2.

The CytoFluor™ 2350 instrument has sensitivity settings which enabled us to adjust the voltage on the photomultiplier tube for a scan. Settings range from 1 to 10, with 10 giving the most sensitive readings. For our studies, the gain settings were 6-7 for blue, 3-4 for yellow-green, 3-4 for orange, 3-4 for red and 2-3 for crimson. We studied the relationship between fluorescent intensity and the number of microspheres per sample. The range of microsphere concentration examined encompassed the range we have encountered in our previous studies on regional
Figure 2. Fluorescent absorbance and emission spectra. Upper panel shows spectral absorbance curves of fluorescent-labeled microspheres in Cellosolve acetate, representing composite graph of individual absorbance spectra of 5 colors measured with the spectrophotometer. Lower panel shows emission spectra of fluorescent-labeled microspheres in Cellosolve acetate, representing composite graph of individual emission spectra of 5 colors excited at peak wavelengths derived from their absorbance spectra. Colors are (left to right) blue, yellow-green, orange, red and crimson.
blood flow in fetal lambs using radioactive microspheres. Figure 3 shows the fluorescence was linear over a broad range of microsphere numbers examined (80-20000). Crimson appeared to have the greatest sensitivity because it has steepest slope. Correlation coefficients for the 5 fluorescent labels were 0.999 (all p < 0.001), and the standard error of the estimate (SEE) ranged from 9.08 to 56.97.

The within-run precision, expressed as the coefficient of variation (CV) was evaluated by replicate measurement of each color batch with eight samples containing low to high concentrations of fluorescent-labeled microspheres in Cellosolve acetate (160-20000/ml). The overall CV was 3.39 ± 1.10%. The between-run precision was obtained by analyzing control preparations with 2,500 microspheres/ml (dissolved in Cellosolve acetate) of each color in each assay. The overall CV was 4.54 ± 1.10%. The samples assayed for the stability of each color in Cellosolve acetate were stored at 4°C in the dark after each scanning, and run with 7 consecutive scans 2 days apart. The fluorescent dyes under these conditions were stable, with no demonstrable loss of fluorescent activity.

There was no measurable intrinsic fluorescence in control tissues and the blood in the yellow-green, orange, red and crimson fluorescence settings that were used. However, at the fixed wavelengths of excitation and emission (360/460) for the blue color, we observed intrinsic color in the control tissues, with the intensity ranging from 50 to 500 units. This intrinsic blue color was not found in Cellosolve acetate alone. The tissue blue fluorescence obtained for flow determination was thus corrected for those values derived from corresponding blank tissues and organs. In addition, extracted blood reference samples exhibited significant color in the
Figure 3. Relationship between the fluorescent intensity and the number of fluorescent microspheres per sample. Samples were excited and read at the following wavelengths (nm): blue, 360/460; yellow-green, 485/530; orange, 530/560; red, 560/590 and crimson, 590/645. The sensitivity settings were 6 for blue (○) and 3 for yellow-green (●), orange (□), red (■) and crimson (♦). Upper panel: range of microsphere numbers (80-20000/ml), Lower panel: range of microsphere numbers (80-2500/ml).
blue band (~2,600 units), which interfered with the fluorescence reading for the blue dye. This interference was eliminated by bubbling N₂ through the final wash solution. In blood spiked with 1,500 blue microspheres, the observed fluorescence was then similar to that from the control standards (362 ± 12 vs. 397 ± 13 units).

### 3.1.2 Recovery

The procedure using tissue digestion, microsphere sedimentation and dye extraction in the same test tube resulted in minimal loss of microspheres. When fluorescence intensities from the standards were compared with those from the blood and 14 tissue extracts of samples spiked with the crimson fluorescent microspheres, the recovery was 94.3 ± 2.5% (Table 3). The mean difference from 100% was 5.7 ± 1.0% and this was significantly different from 0 (p < 0.001). However, over the range of microsphere numbers in the spiked samples (400 - 20000), there was not a significant relationship between the number of microspheres and % recovery (r = 0.4332, p > 0.1).

### 3.1.3 Spectral Overlap and Signal Quenching

As noted in the section 2.4.8, the fluorescence overlap matrix data showed that significant spillover into adjacent color windows occurred with orange into yellow-green, orange into red, and red into crimson. There was also a slight spillover of red into orange. There was no spillover of crimson into red; however there was the possibility of quenching of the red signal by the crimson fluorophore (Prinzen and Glenny 1994). Although the fixed excitation and
Table 3. Recovery of the fluorescent-labeled microspheres added to the blood and tissue samples.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Microspheres Added</th>
<th>Fluorescence Intensity</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td>Brain</td>
<td>20000</td>
<td>6511</td>
<td>6072</td>
</tr>
<tr>
<td>Heart</td>
<td>20000</td>
<td>6505</td>
<td>6409</td>
</tr>
<tr>
<td>Lung</td>
<td>2000</td>
<td>656</td>
<td>640</td>
</tr>
<tr>
<td>Stomach</td>
<td>5000</td>
<td>1571</td>
<td>1502</td>
</tr>
<tr>
<td>S. intestine</td>
<td>10000</td>
<td>3122</td>
<td>2959</td>
</tr>
<tr>
<td>L. intestine</td>
<td>8000</td>
<td>2641</td>
<td>2539</td>
</tr>
<tr>
<td>Liver</td>
<td>400</td>
<td>142</td>
<td>128</td>
</tr>
<tr>
<td>Spleen</td>
<td>10000</td>
<td>3285</td>
<td>3114</td>
</tr>
<tr>
<td>Kidney</td>
<td>15000</td>
<td>4788</td>
<td>4633</td>
</tr>
<tr>
<td>Adrenal</td>
<td>20000</td>
<td>6499</td>
<td>6009</td>
</tr>
<tr>
<td>Brown fat</td>
<td>1500</td>
<td>501</td>
<td>480</td>
</tr>
<tr>
<td>Skin</td>
<td>1000</td>
<td>321</td>
<td>295</td>
</tr>
<tr>
<td>Muscle</td>
<td>800</td>
<td>268</td>
<td>242</td>
</tr>
<tr>
<td>Bone</td>
<td>600</td>
<td>205</td>
<td>188</td>
</tr>
<tr>
<td>Blood</td>
<td>1000</td>
<td>342</td>
<td>324</td>
</tr>
</tbody>
</table>

Data are average of 2 experiments. S., Small; and L., Large.
emission filters employed had a relatively wide bandwidth, the spillover of different fluorescent dyes into windows beyond the immediately adjacent colors was < 0.7% in every case. Thus, correction for spillover was only made between adjacent colors as described in the section 2.4.8.

For the 4 sets of paired fluorescent-labeled microspheres where spillover was apparent, linear regression was performed to compare the total fluorescence intensity of both colors (A and B) in the samples to the fluorescence intensity of the serially added color (B). The data obtained for 1 of 4 sets (red into crimson) are illustrated in Figure 4. Irrespective of the amount of A label, the fluorescence was consistently affected by the B label at all concentrations tested. These results suggest that there is a constant degree of fluorescence overlap, depending upon the interfering color concentration, and moreover, that any quenching present did not introduce any non-linearity into the relationship between fluorescence of B and fluorescence in the A window. For the crimson (B)-red (A) microsphere pair, the slopes of the curves for the relationship between the total fluorescence intensity of both colors (A and B) and the concentration of the serially added color (B) averaged \(6.72 \times 10^{-4} \pm 3.53 \times 10^{-4}\) and was not significantly different from 0, indicating the absence of both spillover and quenching effects of crimson on red. The fluorescence overlap values averaged \(9.1 \pm 0.5\%\) for the red signal in the crimson window, \(4.0 \pm 0.1\%\) for the orange signal in the yellow-green, \(8.1 \pm 0.4\%\) for the orange in the red windows, and \(1.0 \pm 0.1\%\) for red into orange. No spillover correction was made for the latter microsphere pair. As illustrated in Figure 5, application of the spillover correction factors resulted in a much tighter correlation between the expected and measured fluorescence obtained for each microsphere when all 5 microspheres were present together.
Figure 4. Relationship between the total fluorescence intensity of red and crimson fluorophores against the fluorescence intensity of the added color (red) only during serial addition of the red fluorophore to samples containing fixed amounts of the crimson microsphere. The numbers at the top of the figure give the concentration of crimson microspheres/ml for each curve. The sensitivity setting of the Cytofluor™ instrument was 2.

3.1.4 Regional Blood Flow Measurements

The overall CV for the blood flows estimated from the triplicate wells for each tissue was 2.78 ± 1.52%. The ratio of blood flows (mean ± SD) between right and left kidneys and that between paired cerebral hemispheres (frontal and temporal hemisphere) for the 3 animals which received fluorescent microspheres was 1.03 ± 0.12 and 1.02 ± 0.06, respectively. There was no significant difference in blood flow values between the paired organs (p > 0.5), and the duplicate variability was 5.65 ± 2.09 %, thus indicating uniformity of distribution of the fluorescent-labeled microspheres.
Figure 5. Comparison of the fluorescence of samples with single a color alone with those of samples obtained by mixing the 5 different fluorescent microspheres together, with and without spillover correction (calculated and observed). Lines a and b represent the regression line obtained before (a) and after (b) spillover correction. The regression equation for line a is $y = 1.078 \times \text{(expected fluorescence)} + 66.887; r = 0.995$, and for line b is $y = 0.993 \times \text{(expected fluorescence)} + 2.072; r = 0.999$. OR-1 and OR-2 are fluorescence readings at the red window before and after correcting spillover of orange into red; RC-1 and RC-2 are fluorescence readings at the crimson band before and after correcting spillover of red into crimson; and OY-1 and OY-2 are fluorescence readings at the yellow-green window before and after spillover correction of orange into yellow-green.

Tables 4 and 5 give the mean fetal organ and tissue blood flow estimates obtained from the 4 fetuses which received radioactive microspheres, and the 3 animals which received fluorescent microspheres. In the latter, flow measurements to upper body structures were only available for 2 of the fetuses, due to failure of the carotid arterial catheter in the third animal. A total of 41 flow estimates were examined in each group, with mean tissue flows ranging from 3 to 472 ml/min/100g. The number of radioactive or fluorescent microspheres in each sample was >400,
Table 4. Comparison between radioactive and fluorescent microsphere methods for measurement of fetal upper body organ and tissue blood flows.

<table>
<thead>
<tr>
<th>Organ and tissue</th>
<th>Radioactive</th>
<th>Fluorescent</th>
<th>SD unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>271 ± 46</td>
<td>276</td>
<td>0.11</td>
</tr>
<tr>
<td>Front. Hemisphere</td>
<td>212 ± 45</td>
<td>260</td>
<td>1.07</td>
</tr>
<tr>
<td>Temp. Hemisphere</td>
<td>197 ± 54</td>
<td>247</td>
<td>0.93</td>
</tr>
<tr>
<td>Midbrain</td>
<td>344 ± 105</td>
<td>397</td>
<td>0.50</td>
</tr>
<tr>
<td>Pons</td>
<td>378 ± 120</td>
<td>406</td>
<td>0.23</td>
</tr>
<tr>
<td>Medulla</td>
<td>337 ± 86</td>
<td>297</td>
<td>0.47</td>
</tr>
<tr>
<td>Pituitary</td>
<td>125 ± 43</td>
<td>116</td>
<td>0.21</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>291 ± 49</td>
<td>222</td>
<td>1.41</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>127 ± 32</td>
<td>110</td>
<td>0.53</td>
</tr>
<tr>
<td>Heart</td>
<td>378 ± 124</td>
<td>222</td>
<td>1.26</td>
</tr>
<tr>
<td>R. ventricle</td>
<td>472 ± 229</td>
<td>284</td>
<td>0.82</td>
</tr>
<tr>
<td>L. ventricle</td>
<td>358 ± 153</td>
<td>210</td>
<td>0.97</td>
</tr>
<tr>
<td>Ventricles</td>
<td>415 ± 190</td>
<td>247</td>
<td>0.88</td>
</tr>
<tr>
<td>Septum</td>
<td>348 ± 115</td>
<td>188</td>
<td>1.39</td>
</tr>
<tr>
<td>Lungs</td>
<td>79 ± 50</td>
<td>69</td>
<td>0.20</td>
</tr>
<tr>
<td>Forelimb skin</td>
<td>20 ± 5</td>
<td>35</td>
<td>3.00</td>
</tr>
<tr>
<td>Nuchal muscle</td>
<td>13 ± 5</td>
<td>9</td>
<td>0.80</td>
</tr>
<tr>
<td>Forelimb muscle</td>
<td>11 ± 4</td>
<td>8</td>
<td>0.75</td>
</tr>
<tr>
<td>Humerus</td>
<td>19 ± 8</td>
<td>24</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values are mean ± SD for fetal organ and tissue blood flows, which are expressed as mL·min⁻¹·100 g⁻¹. n = 4 for radioactive microsphere (R) and 2 for fluorescent microsphere method (F). SD unit = (mean flow_R - mean flow_F)/SD_R. Front., Frontal; Temp., Temporal; R., Right; L., Left; and Septum, interventricular septum.

except for the pituitary and diaphragm, where the numbers were ~100 and 250, respectively.

In general the measurements obtained in the 2 groups were similar. The difference between the fluorescent and radioactive microsphere estimates divided by the SD of the radioactive estimate was <1 for 36 (88%) of the tissues, between 1 and 2 for 4 (10%) and >2 for only 1 tissue (2%), forelimb skin. In the 1 animal in which duplicate fluorescent microsphere flow estimates were obtained within 7 min, the correlation coefficient for duplicate measurements was 0.997.
Table 5. Comparison between radioactive and fluorescent microsphere methods for measurement of fetal lower body organ and tissue, systemic and umbilical blood flows and fetal combined cardiac output.

<table>
<thead>
<tr>
<th>Organ and tissue</th>
<th>Radioactive</th>
<th>Fluorescent</th>
<th>SD unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm</td>
<td>21 ± 12</td>
<td>16 ± 8</td>
<td>0.42</td>
</tr>
<tr>
<td>Stomach</td>
<td>79 ± 42</td>
<td>86 ± 19</td>
<td>0.17</td>
</tr>
<tr>
<td>Reticulum</td>
<td>52 ± 30</td>
<td>49 ± 27</td>
<td>0.10</td>
</tr>
<tr>
<td>Rumen</td>
<td>35 ± 26</td>
<td>29 ± 16</td>
<td>0.23</td>
</tr>
<tr>
<td>Omasum</td>
<td>97 ± 49</td>
<td>107 ± 20</td>
<td>0.20</td>
</tr>
<tr>
<td>Abomasum</td>
<td>131 ± 72</td>
<td>160 ± 26</td>
<td>0.40</td>
</tr>
<tr>
<td>Small intestine</td>
<td>153 ± 62</td>
<td>101 ± 14</td>
<td>0.84</td>
</tr>
<tr>
<td>Large intestine</td>
<td>97 ± 46</td>
<td>71 ± 23</td>
<td>0.57</td>
</tr>
<tr>
<td>Liver</td>
<td>3 ± 2</td>
<td>4 ± 1</td>
<td>0.50</td>
</tr>
<tr>
<td>Spleen</td>
<td>213 ± 144</td>
<td>160 ± 37</td>
<td>0.37</td>
</tr>
<tr>
<td>Kidney</td>
<td>247 ± 66</td>
<td>216 ± 15</td>
<td>0.47</td>
</tr>
<tr>
<td>Adrenal</td>
<td>389 ± 144</td>
<td>420 ± 51</td>
<td>0.22</td>
</tr>
<tr>
<td>Brown fat</td>
<td>107 ± 63</td>
<td>112 ± 10</td>
<td>0.08</td>
</tr>
<tr>
<td>Skin, total</td>
<td>22 ± 10</td>
<td>31 ± 5</td>
<td>0.90</td>
</tr>
<tr>
<td>Hindlimb skin</td>
<td>24 ± 6</td>
<td>27 ± 3</td>
<td>0.50</td>
</tr>
<tr>
<td>Muscle, total</td>
<td>14 ± 9</td>
<td>9 ± 4</td>
<td>0.56</td>
</tr>
<tr>
<td>Hindlimb muscle</td>
<td>17 ± 11</td>
<td>9 ± 4</td>
<td>0.73</td>
</tr>
<tr>
<td>Bone, total</td>
<td>22 ± 9</td>
<td>24 ± 3</td>
<td>0.22</td>
</tr>
<tr>
<td>Femur</td>
<td>24 ± 11</td>
<td>27 ± 3</td>
<td>0.27</td>
</tr>
<tr>
<td>Tibia</td>
<td>26</td>
<td>24 ± 2</td>
<td></td>
</tr>
<tr>
<td>Metatarsal</td>
<td>20</td>
<td>23 ± 3</td>
<td></td>
</tr>
<tr>
<td>Fetal body</td>
<td>320 ± 77</td>
<td>260</td>
<td>0.78</td>
</tr>
<tr>
<td>Umbilical</td>
<td>185 ± 47</td>
<td>217</td>
<td>0.68</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>503 ± 133</td>
<td>477</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are mean ± SD for fetal organ and tissue blood flows, which are expressed as mL-min⁻¹·100 g⁻¹. Fetal body and umbilical blood flows and combined cardiac output are mL-min⁻¹·kg fetal wt⁻¹. n = 4 for radioactive microsphere (R) except for tibia and metatarsal where n = 1; and n = 3 for fluorescent microsphere method (F) except for fetal body and umbilical blood flows and combined cardiac output where n = 2. SD unit = (mean flow_F - mean flow_R)/SD_R.

(p < 0.001), and the SEE was 9.55 ml/min/100g. The duplicate variability was 10.60 ± 2.16%.
3.2 Validation and Performance Tests on Measurement of Plasma Arginine Vasopressin Concentrations

3.2.1 Assay Validation Test

RIA is based on an interaction between a radioisotope-labeled compound and an antibody directed against it that can be inhibited by a similar but unlabeled compound. The ligand studied in our RIA is fetal lamb plasma AVP, while labeled ligand provided by INSCSTAR is human AVP and consequently the antibody is prepared with guinea pig anti-human AVP serum. Thus, a validation test is required to examine the immunologic reactivity between the antibody and ligands. One way to approach this problem is to run a parallel assay study in which the linear portions of the concentration-response curves are compared for control (AVP standard) and test solutions (fetal lamb plasma extract). The underlying assumption is that the antibody is completely discriminating for particular molecules, regardless of whichever species of AVP used. The similarity of immunologic behaviors will produce parallel concentration-response curves.

The curves in Figure 6 are plotted as logit B/Bo vs. log dose for better comparison of slopes. The curve generated by serial dilution of extracted plasma obtained from a fetal lamb during late-labor was parallel to the human AVP standard. This confirms the radioimmunological identity of the extraction of fetal lamb plasma with AVP.
3.2.2 Assay Performance Test

The specificity characteristics, reported by INCSTAR, show cross-reactivities of 100% with arginine vasopressin, 600% with lysine vasopressin, less than 0.01% with oxytocin and 0.14% with vasotocin. The mean assay sensitivity is $2.0 \pm 0.1$ pg/ml. The overall mean percentage of arginine vasopressin recovered from the extraction step in our hands is $74.10 \pm 3.93\%$ (Mean ± SD; n = 16). The slopes of standard curve in each assay were compared for run-to-run reproducibility. The mean slope was -2.74 with an inter-assay coefficient of variation of 7.02% (n = 6).
3.3 Outcome of Experiments and General Information on Experimental Animals

From April 1992 to April 1996, a total of 33 animals were surgically prepared for the labor study. Of these, only 12 animals were successfully studied. Eleven fetuses died in utero and were not studied (E971Y, E314Y, E2216, E0145, E158X, E55W, E142X, E2174, E4218, E4130, E0101). Six were prematurely delivered (E4107, E3221, E4136, E112Z, E4146, E4230). There were two maternal deaths several days after surgery (E239Z, E1224). In the remaining two fetuses the results were not suitable for analysis because the control (pre-labor) data were not available (E1142, E1119). Thus, the success rate with our labor study is 36%. Detailed data on individual animals with successful labor studies are given in Table 6 and the corresponding maternal data are shown in Table 7. In one of the sheep, labor was induced by the infusion of ACTH to the fetus; in all the other animals, the onset of labor was spontaneous. In 5 of the animals, 1-3 experiments were performed in the antepartum period (see Table 6). These included maternal or fetal drug administration with serial blood sampling from the ewe and fetus, or short term (60 min) maternal hypoxemia. Fetal blood sampled during these experiments was replaced with an equal volume of maternal blood. In the present study, no data obtained from these animals during the experiments and for 12h afterwards were included.

The original primary aim of the thesis was to measure cardiovascular, respiratory, metabolic and endocrine parameters during labor and delivery in normal, healthy fetal lambs. However, once the study was underway, it became obvious that 2 groups were being studied, one (Group II) in which there was a minimal deterioration in acid-base status during labor and a second (Group I) in which profound and progressive metabolic acidemia occurred (see section 3.6.3).
Table 6. Data on individual animals with successful labor studies.

<table>
<thead>
<tr>
<th>Ewe #</th>
<th>Group</th>
<th>GA at surgery</th>
<th>GA at birth</th>
<th>Date at birth</th>
<th># of fetuses</th>
<th>Sex of Op fetus</th>
<th>Op FBW (kg)</th>
<th>Up FBW (kg)</th>
<th>Up FBW (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E968</td>
<td>I</td>
<td>124</td>
<td>140</td>
<td>92-05-08</td>
<td>3</td>
<td>F</td>
<td>2.177</td>
<td>1.953^M</td>
<td>2.850^F</td>
</tr>
<tr>
<td>E499*</td>
<td>I</td>
<td>121</td>
<td>140</td>
<td>92-11-08</td>
<td>3</td>
<td>M</td>
<td>2.496</td>
<td>2.347^F</td>
<td>3.607^F</td>
</tr>
<tr>
<td>E144†*</td>
<td>I</td>
<td>123</td>
<td>136</td>
<td>93-02-08</td>
<td>2</td>
<td>M</td>
<td>2.247</td>
<td>2.650^F</td>
<td>-</td>
</tr>
<tr>
<td>E1118*</td>
<td>I</td>
<td>123</td>
<td>138</td>
<td>93-02-24</td>
<td>2</td>
<td>F</td>
<td>2.646</td>
<td>2.892^M</td>
<td>-</td>
</tr>
<tr>
<td>E1250*</td>
<td>I</td>
<td>125</td>
<td>142</td>
<td>93-04-11</td>
<td>2</td>
<td>M</td>
<td>3.122</td>
<td>3.840^M</td>
<td>-</td>
</tr>
<tr>
<td>E1102</td>
<td>I</td>
<td>124</td>
<td>142</td>
<td>95-03-31</td>
<td>3</td>
<td>M</td>
<td>3.071</td>
<td>2.673^F</td>
<td>3.502^F</td>
</tr>
<tr>
<td>E2101*</td>
<td>II</td>
<td>127</td>
<td>141</td>
<td>93-12-09</td>
<td>1</td>
<td>F</td>
<td>2.927</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4102</td>
<td>II</td>
<td>131</td>
<td>142</td>
<td>95-03-31</td>
<td>2</td>
<td>M</td>
<td>4.188</td>
<td>3.950^M</td>
<td>-</td>
</tr>
<tr>
<td>E0151</td>
<td>II</td>
<td>132</td>
<td>145</td>
<td>95-05-01</td>
<td>1</td>
<td>M</td>
<td>4.144</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E2202</td>
<td>II</td>
<td>133</td>
<td>143</td>
<td>95-05-27</td>
<td>2</td>
<td>M</td>
<td>3.710</td>
<td>3.872^F</td>
<td>-</td>
</tr>
<tr>
<td>E212</td>
<td>II</td>
<td>127</td>
<td>140</td>
<td>95-12-20</td>
<td>1</td>
<td>F</td>
<td>3.467</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E0160</td>
<td>II</td>
<td>130</td>
<td>142</td>
<td>96-04-02</td>
<td>2</td>
<td>F</td>
<td>5.089</td>
<td>4.467^F</td>
<td>-</td>
</tr>
</tbody>
</table>

† ACTH induced labor. GA, gestational age (days); # of fetuses, number of fetuses; Op FBW, birth weight of operated fetus; Up FBW, birth weight of unoperated fetus; ^M sex of the fetus, male; ^F sex of the fetus, female.

* Animals subjected to other experiments as follows:
E499, diphenhydramine fetal infusion at 127 days gestation and valproic acid fetal bolus injection at 130 days gestation;
E144, metoclopramide fetal infusion with hypoxemia experiments at 128 days gestation;
E1118, valproic acid maternal bolus injection at 130 days gestation, labetalol maternal infusion at 133 days gestation and valproic acid fetal bolus injection at 136 days gestation;
E1250, progesterone control experiments at 130 days gestation, progesterone with hypoxemia experiments at 132 days gestation and valproic acid maternal infusion at 136 days gestation;
E2101, diphenhydramine fetal infusion at 131 days gestation and valproic acid fetal bolus injection at 135 days gestation.
<table>
<thead>
<tr>
<th>Ewe #</th>
<th>Group</th>
<th>Breeding season</th>
<th>Age (year)</th>
<th>BW (kg)</th>
<th>BW at birth (kg)</th>
<th># of fetuses</th>
<th>Parity</th>
<th>Ram name</th>
</tr>
</thead>
<tbody>
<tr>
<td>E968a</td>
<td>I</td>
<td>1991-92</td>
<td>2.1</td>
<td>90.35</td>
<td>4.086</td>
<td>twins</td>
<td>2</td>
<td>Luke</td>
</tr>
<tr>
<td>E499d</td>
<td>I</td>
<td>1992-93</td>
<td>6.6</td>
<td>81.27</td>
<td>5.448</td>
<td>singleton</td>
<td>6</td>
<td>Luke</td>
</tr>
<tr>
<td>E144a</td>
<td>I</td>
<td>1992-93</td>
<td>2.5</td>
<td>87.17</td>
<td>3.632</td>
<td>twins</td>
<td>1</td>
<td>Simon</td>
</tr>
<tr>
<td>E1118a</td>
<td>I</td>
<td>1992-93</td>
<td>1.6</td>
<td>87.62</td>
<td>4.540</td>
<td>twins</td>
<td>1</td>
<td>Luke</td>
</tr>
<tr>
<td>E1250d</td>
<td>I</td>
<td>1992-93</td>
<td>1.0</td>
<td>74.00</td>
<td>5.902</td>
<td>singleton</td>
<td>1</td>
<td>Luke</td>
</tr>
<tr>
<td>E1102a</td>
<td>I</td>
<td>1994-95</td>
<td>3.7</td>
<td>98.52</td>
<td>2.724</td>
<td>twins</td>
<td>3</td>
<td>Henry</td>
</tr>
<tr>
<td>E2101a</td>
<td>II</td>
<td>1993-94</td>
<td>1.5</td>
<td>81.72</td>
<td>3.178</td>
<td>twins</td>
<td>1</td>
<td>Luke</td>
</tr>
<tr>
<td>E4102d</td>
<td>II</td>
<td>1994-95</td>
<td>0.7</td>
<td>67.19</td>
<td>4.540</td>
<td>twins</td>
<td>1</td>
<td>Henry</td>
</tr>
<tr>
<td>E0151a</td>
<td>II</td>
<td>1994-95</td>
<td>4.7</td>
<td>78.54</td>
<td>4.086</td>
<td>twins</td>
<td>3</td>
<td>Henry</td>
</tr>
<tr>
<td>E2202a</td>
<td>II</td>
<td>1994-95</td>
<td>2.2</td>
<td>93.07</td>
<td>2.724</td>
<td>twins</td>
<td>2</td>
<td>Henry</td>
</tr>
<tr>
<td>E212d</td>
<td>II</td>
<td>1995-96</td>
<td>6.7</td>
<td>62.20</td>
<td>3.178</td>
<td>triplets</td>
<td>5</td>
<td>Henry</td>
</tr>
<tr>
<td>E0160a</td>
<td>II</td>
<td>1995-96</td>
<td>5.6</td>
<td>108.96</td>
<td>2.270</td>
<td>triplets</td>
<td>4</td>
<td>Henry</td>
</tr>
</tbody>
</table>

* Breed = Suffolk; ‡ Breed = Dorset.

Age, maternal age at conception; BW, maternal body weight before surgery; BW at birth, birth weight of ewe; # of fetuses, number of fetuses at birth.
Once the animal experimentation was completed, it was found that these 2 groups were also
distinguishable on the basis of birthweight, with Group I being growth restricted and Group II
normally grown (see section 3.4). This then led to a retrospective examination of the fetal
blood gas and metabolite measurements and the recordings of fetal biophysical parameters that
were routinely collected in the antepartum period (see section 3.5) and not initially planned for
inclusion in the thesis. Thus in conducting the study we went backwards in time from the end
of pregnancy to examine prenatal factors, including fetal growth. However, in writing the
thesis it seems sensible to proceed "forward in time" by presenting the fetal growth data first,
then the antepartum results, and finishing with the measurements obtained during labor and
delivery.

In Table 8, the study animals are listed with their gestational age, pregnancy and sex,
respectively. The mean gestational age at delivery was shorter in Group I than in Group II
(139.7 ± 0.9 vs. 142.2 ± 0.7 days), but this did not reach statistical significance. The
gestational age at surgery was significantly less in Group I than Group II (123.3 ± 0.6 vs. 130.0
± 1.0). In Group I, 3 of the ewes carried twins and 3 had triplets, whereas in Group II, 3
carried singletons and 3 had twins. Maternal data are presented in Table 9. There were no
significant differences between two groups in maternal age at conception, maternal body
weight, birth weight of ewes and parity. However, the average number of fetuses at birth was
significantly less in Group I.
Table 8. General information for Group I and Group II fetuses.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at birth (days)</td>
<td>139.7 ± 0.9</td>
<td>142.2 ± 0.7</td>
</tr>
<tr>
<td>Gestational age at surgery (days)</td>
<td>123.3 ± 0.6*</td>
<td>130.0 ± 1.0</td>
</tr>
<tr>
<td>Interval from surgery to birth (days)</td>
<td>16.3 ± 0.9*</td>
<td>12.2 ± 0.6</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Twin</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Triplet</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fetal body weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>3.513 ± 0.352</td>
<td></td>
</tr>
<tr>
<td>Twin</td>
<td>2.671 ± 0.253*</td>
<td>4.329 ± 0.404</td>
</tr>
<tr>
<td>Triplet</td>
<td>2.581 ± 0.262</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* p < 0.05 compared with Group II.

Table 9. Maternal information for Group I and Group II animals.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at conception (years)</td>
<td>2.9 ± 0.8</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>Maternal body weight (kg)</td>
<td>86.49 ± 3.39</td>
<td>81.95 ± 7.01</td>
</tr>
<tr>
<td>Birth weight of ewe (kg)</td>
<td>4.389 ± 0.479</td>
<td>3.329 ± 0.345</td>
</tr>
<tr>
<td>Number of fetuses at birth</td>
<td>1.7 ± 0.2*</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Parity</td>
<td>2.3 ± 0.8</td>
<td>2.7 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* p < 0.05 compared with Group II.
3.4 Fetal Body Weight, Placental and Fetal Organ Weight Data

The fetal weight at birth is presented in Table 10. In Group I, fetal weight was significantly less than in Group II (2.627 ± 0.164 vs. 3.921 ± 0.301 kg, P < 0.005). The lower birth weight also occurred in the unoperated fetuses in Group I compared to those in Group II (2.924 ± 0.206 vs. 4.096 ± 0.187 kg, P < 0.05). As shown in the Table 10, the weight of operated fetuses in Group I twins were also significantly smaller than the Group II twins (2.671 ± 0.253 vs. 4.329 ± 0.404 kg, P < 0.05). In addition, fetal weight-to-maternal weight ratio was also significantly lower in Group I than in Group II (3.07 ± 0.26 vs. 4.89 ± 0.41; P < 0.005), calculated as fetal birth weight divided by maternal weight x 100.

Figure 7 plots the weights of all fetuses in Groups I and II as a function of the number of fetuses in each pregnancy, together with 95% confidence intervals as the horizontal bar for each group of animals. The Figure shows that the 95% confidence intervals for twin and triplet gestations overlap each other in Group I, as it does for single and twin gestations in Group II. There were no significant differences in fetal weight between twin and triplet pregnancies in Group I. This was also the case for single and twin pregnancies in Group II. Thus, overall there was no obvious relationship between the number of fetuses and fetal weight. Also, there is no within litter difference in weights of litter mates for the twins in either group. (Group I twins: operated vs. unoperated 2.671 ± 0.253 vs. 3.127 ± 0.363, P = 0.368; Group II twins: 4.329 ± 0.404 vs. 4.096 ± 0.187, P = 0.640).
Table 10. Birth weight of operated and unoperated fetuses.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operated fetuses (kg)</td>
<td>2.627 ± 0.164* (6)</td>
<td>3.921 ± 0.301 (6)</td>
</tr>
<tr>
<td>Unoperated fetuses (kg)</td>
<td>2.924 ± 0.206* (9)</td>
<td>4.096 ± 0.187 (3)</td>
</tr>
<tr>
<td>Operated + unoperated (kg)</td>
<td>2.805 ± 0.141* (15)</td>
<td>3.979 ± 0.204 (9)</td>
</tr>
<tr>
<td>Operated twins (kg)</td>
<td>2.671 ± 0.253* (3)</td>
<td>4.329 ± 0.404 (3)</td>
</tr>
<tr>
<td>Unoperated twins (kg)</td>
<td>3.127 ± 0.363 (3)</td>
<td>4.096 ± 0.187 (3)</td>
</tr>
<tr>
<td>Operated + unoperated twins (kg)</td>
<td>2.900 ± 0.223* (6)</td>
<td>4.213 ± 0.206 (6)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. The number in parenthesis are the number of animals.
* p < 0.05 compared with Group II.

Table 11 gives the mean values for fetal tissue/organ weights and placental weight and cotyledon number for the 2 groups. Note that the n value for some organs in Group I is less than 6 because the weight of the entire organ was not obtained in some animals. In Group I there was a significant reduction in both total placental weight (156.21 ± 13.23 vs. 299.57 ± 30.46 g, P < 0.005) and average individual cotyledon weight (4.11 ± 0.16 vs. 5.62 ± 0.20 g, P < 0.0001), compared with Group II. There was no significant difference in number of cotyledons between the two groups. Moreover, in Group I, fetal weight was not as markedly reduced as placental weight (33 vs. 48 %), which resulted in a significant decrease in the placental weight/fetal body weight ratio (5.93 ± 0.25 vs. 7.70 ± 0.63 %, P < 0.05).
Figure 7. Individual fetal birth weight of both operated and unoperated fetuses in Group I and Group II. Each group is divided into two subgroup, based upon single, twin or triplet pregnancy, which is labeled on the abscissa. Also are shown 95% confidence intervals as the horizontal bar for each group of animals.

After the fetal organ weights were normalized for fetal weight, the fetal brain and adrenal weights in Group I were significantly larger than in Group II (17.58 ± 0.94 vs. 14.08 ± 0.87 gm/kg body weight; 0.32 ± 0.02 vs. 0.19 ± 0.02 gm/kg, respectively). The fetal heart weight in Group I tended to be larger, but due to the limited number (n = 3) it did not reach statistical significance from that of Group II. In contrast, fetal spleen, liver, stomach, kidney and lung weights tended to be smaller in Group I than in Group II. As a consequence, the brain to liver weight ratio is significantly higher in Group I fetuses than Group II (0.59 ± 0.07 vs. 0.44 ± 0.03, P < 0.05).
Table 11. Fetal weight at birth and individual organ and placental weight at postmortem examination.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group I / II (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal body weight (kg)</td>
<td>2.627 ± 0.164†</td>
<td>3.921 ± 0.301</td>
<td>67</td>
</tr>
<tr>
<td>Fetal organ weight (gm/kg body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>17.58 ± 0.94*</td>
<td>14.08 ± 0.87</td>
<td>125</td>
</tr>
<tr>
<td>Heart</td>
<td>9.21 ± 0.27</td>
<td>8.73 ± 0.31</td>
<td>105</td>
</tr>
<tr>
<td>Lungs</td>
<td>24.48 ± 1.08</td>
<td>25.08 ± 2.25</td>
<td>98</td>
</tr>
<tr>
<td>Stomach</td>
<td>10.27 ± 0.59</td>
<td>11.35 ± 0.47</td>
<td>90</td>
</tr>
<tr>
<td>Liver</td>
<td>28.67 ± 2.48</td>
<td>32.29 ± 2.71</td>
<td>89</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.47 ± 0.11</td>
<td>1.87 ± 0.28</td>
<td>79</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.32 ± 0.02‡</td>
<td>0.19 ± 0.02</td>
<td>168</td>
</tr>
<tr>
<td>Kidneys</td>
<td>8.49 ± 0.39</td>
<td>9.05 ± 0.87</td>
<td>94</td>
</tr>
<tr>
<td>Brain/liver weight ratio</td>
<td>0.59 ± 0.07*</td>
<td>0.44 ± 0.03</td>
<td>134</td>
</tr>
<tr>
<td>Number of cotyledons</td>
<td>38 ± 4</td>
<td>53 ± 10</td>
<td>72</td>
</tr>
<tr>
<td>Singleton</td>
<td></td>
<td>59 ± 22</td>
<td></td>
</tr>
<tr>
<td>Twin</td>
<td>42 ± 6</td>
<td>48 ± 2</td>
<td>88</td>
</tr>
<tr>
<td>Triplet</td>
<td>34 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental weight (gm)</td>
<td>156.21 ± 13.23†</td>
<td>299.57 ± 30.46</td>
<td>52</td>
</tr>
<tr>
<td>Singleton</td>
<td></td>
<td>260.86 ± 11.00</td>
<td></td>
</tr>
<tr>
<td>Twin</td>
<td>145.37 ± 13.82</td>
<td>338.28 ± 54.94</td>
<td>43</td>
</tr>
<tr>
<td>Triplet</td>
<td>167.04 ± 23.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental weight/fetal body weight (%)</td>
<td>5.93 ± 0.25*</td>
<td>7.70 ± 0.63</td>
<td>77</td>
</tr>
<tr>
<td>Individual cotyledon weight (gm)</td>
<td>4.11 ± 0.16§</td>
<td>5.62 ± 0.20</td>
<td>73</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Compared with Group II, * p < 0.05; † p < 0.005; ‡ p < 0.001; § p < 0.0001; The number in parenthesis are the number of animals or organs and tissues.
Also the mean values of placental weight and cotyledon numbers for the singletons, twins and triplets in each group are presented in Table 11. The mean placental weight in Group II twins was well above that in Group I twins (338 ± 55 vs. 145 ± 14 g), but numbers (n = 3) were too small for significant difference. The total placental weights were variable. In Group I twins the placental weight ranged from 120.18 to 167.80 g and in triplets from 131.32 to 212.15 g; while in Group II singletons the weight of cotyledons ranged from 240.29 to 277.9 g and in twins from 245.82 to 435.94 g. However, there was no overlap of placental weights between Group I and II animals. The number of cotyledons was similar in both groups of twins. Thus, placental weight is a better index of placental size than the cotyledon number.

Figures 8 and 9 show the relationship of placental to fetal weight and to the placental weight/fetal body weight ratio. There is a positive correlation between fetal and placental weight (r = 0.844, P < 0.001). The placental weight/fetal body weight ratio is also positively related to placental weight (r = 0.829, P < 0.001). Figure 10 illustrates that fetal liver weight is also significantly correlated with placental weight (r = 0.739, P < 0.05). In contrast, fetal brain weight does not correlate with placental weight (Figure 11; r = 0.398).

The frequency distributions of individual cotyledon weights for Group I and II are presented in Figure 12 as histograms. In both groups, cotyledon weight was skewed to the right. The cotyledon weight in Group I deviated significantly from a normal distribution, whereas this was not the case for Group II. This was evidenced by a test for normality of the distribution of cotyledon weight. The test value was 1.137 for Group I (P < 0.05) and 0.963 for Group II.
Figure 8. Relation of fetal weight to placental weight \((y = 1446.5 + 8.018x, r = 0.844, P < 0.001)\). Group I fetuses, (○); Group II fetuses, (●).

Figure 9. Relation of placental/fetal weight ratio (%) to placental weight \((y = 3.86 + 0.01x, r = 0.829, P < 0.001)\). Group I fetuses, (○); Group II fetuses, (●).
Figure 10. Relation of fetal liver weight to placental weight ($y = 54.39 + 0.22x$, $r = 0.739$, $P < 0.05$). Group I fetuses, (○); Group II fetuses, (●).

Figure 11. Relation of fetal brain weight to placental weight ($y = 47.49 + 0.02x$, $r = 0.398$). Group I fetuses, (○); Group II fetuses, (●).
Figure 12. Histogram showing the frequency distribution of individual cotyledon weight. Group I fetuses, (upper panel); Group II fetuses, (lower panel).
(P > 0.05). The major difference between the two groups was the absence of larger weight 
cotyledons in Group I.

3.5 Fetal Condition in the Antepartum Period

As described in the section 2.2.1, we routinely monitored fetal condition in the antepartum 
period by measuring fetal blood gases, acid-base balance and oxygenation on a daily basis, 
which started on the day after surgery and carried through until the onset of labor. Such serial 
blood samples were simultaneously collected from the fetal femoral artery and umbilical vein 
catheters and measured for fetal Po₂, Pco₂, pH, O₂ saturation and hemoglobin, glucose and 
lactate concentrations. Data presented here represent our results obtained throughout the last 
10 days of gestation prior to the onset of labor, but not including the data taken on the 3-5 days 
immediately after surgery. In addition, the blood flow measurements and the estimates of fetal 
oxygen delivery and consumption and umbilical glucose and lactate fluxes obtained in the pre-
labor stage of the labor protocol (i.e. 2-3 days prior to labor onset) in the 2 groups are 
compared. And there are also the results on fetal heart rate, arterial pressure and breathing 
activity obtained from the continuous polygraph recordings of these variables.

3.5.1 Fetal Blood Gas, Acid-Base Status, Oxygen, Glucose and Lactate Metabolism

The antepartum values of fetal descending aortic blood gases, oxygen content and acid-base 
balance are summarized in Table 12 and the umbilical venous data in Table 13. Data obtained 
over the last 10 days of gestation before the onset of labor were pooled and expressed as mean
Table 12. Prenatal values of FA blood gases, oxygen content and acid-base balance.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=60)</th>
<th>Group II (n=51)</th>
<th>T value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descending aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.326 ± 0.005</td>
<td>7.331 ± 0.002</td>
<td>1.266</td>
<td>NS</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>49.4 ± 0.4</td>
<td>48.7 ± 0.4</td>
<td>1.051</td>
<td>NS</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>18.0 ± 0.4</td>
<td>20.0 ± 0.3</td>
<td>3.677</td>
<td>0.0004</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>0.3 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>3.905</td>
<td>0.0002</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>25.4 ± 0.3</td>
<td>27.1 ± 0.2</td>
<td>5.675</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>26.7 ± 0.3</td>
<td>28.5 ± 0.2</td>
<td>5.708</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.0 ± 0.1</td>
<td>11.3 ± 0.2</td>
<td>1.475</td>
<td>NS</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>38.8 ± 1.2</td>
<td>42.8 ± 1.0</td>
<td>2.582</td>
<td>0.0112</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>2.56 ± 0.09</td>
<td>2.89 ± 0.08</td>
<td>2.872</td>
<td>0.0049</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.18 ± 0.06</td>
<td>0.94 ± 0.02</td>
<td>3.680</td>
<td>0.0005</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.49 ± 0.02</td>
<td>0.73 ± 0.02</td>
<td>7.230</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
Table 13. Prenatal values of UV blood gases, oxygen content and acid-base balance.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=45)</th>
<th>Group II (n=21)</th>
<th>T value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Umbilical vein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.352 ± 0.007</td>
<td>7.385 ± 0.005</td>
<td>4.111</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>44.5 ± 0.5</td>
<td>42.9 ± 0.6</td>
<td>1.994</td>
<td>0.0505</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>28.4 ± 0.8</td>
<td>36.9 ± 1.4</td>
<td>5.471</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>0.2 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>3.116</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>24.4 ± 0.3</td>
<td>25.5 ± 0.3</td>
<td>2.420</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>25.6 ± 0.3</td>
<td>26.6 ± 0.2</td>
<td>2.383</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>10.9 ± 0.2</td>
<td>11.4 ± 0.3</td>
<td>1.609</td>
<td>NS</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>63.4 ± 1.4</td>
<td>81.5 ± 1.4</td>
<td>9.235</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>4.13 ± 0.12</td>
<td>5.57 ± 0.15</td>
<td>7.286</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.33 ± 0.08</td>
<td>1.21 ± 0.03</td>
<td>1.310</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.60 ± 0.02</td>
<td>0.82 ± 0.05</td>
<td>4.746</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
values. The mean fetal arterial $\text{PO}_2$, base excess, bicarbonate ($\text{HCO}_3$), $\text{O}_2$ saturation and $\text{O}_2$ content were all significantly lower in Group I than in Group II. Fetal blood glucose levels were also significantly lower in Group I, while blood lactate levels was significantly higher. Data obtained from umbilical vein samples were essentially the same as those for descending aorta, with the exception that the umbilical venous (UV) pH value was significantly lower in Group I as compared to Group II, whereas there was no significant difference in UV lactate concentrations. The umbilical venoarterial differences (UV-FA) for oxygen, glucose and lactate concentration are presented in Table 14. In comparison to Group II, Group I fetuses had significantly smaller UV-FA oxygen content difference, but significantly larger UV-FA glucose concentration difference.

Figure 13 presents the individual values for UV-FA $\text{O}_2$ content difference over the antepartum period for the two groups. Although the two groups had similar slopes, the Y intercept values were significantly different with a much higher value for Group II ($P < 0.0001$). Assuming the umbilical blood flows over the prenatal period were similar to those measured from the pre-labor stage (see section 3.5.4), these results would indicate that oxygen consumption in Group I fetuses was less than in Group II throughout the last 10 days of gestation.

The change in fetal oxygen extraction in Group II was independent of the time prior to labor ($r = 0.241$), whereas fetal oxygen extraction in Group I progressively increased as term approached ($r = 0.479$, $P < 0.001$). On the day before the onset of labor, the fractional extraction reached a similar mean value to that in Group II (Figure 14).
Table 14. Fetal oxygenation and substrate concentrations in the antepartum period.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV O₂ content (mmol/L)</td>
<td>4.13 ± 0.12§</td>
<td>5.57 ± 0.15</td>
</tr>
<tr>
<td>FA O₂ content (mmol/L)</td>
<td>2.56 ± 0.09†</td>
<td>2.89 ± 0.08</td>
</tr>
<tr>
<td>UV-FA O₂ content (mmol/L)</td>
<td>1.58 ± 0.05§</td>
<td>2.45 ± 0.08</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV concentration (mmol/L)</td>
<td>0.60 ± 0.02§</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>FA concentration (mmol/L)</td>
<td>0.49 ± 0.02§</td>
<td>0.73 ± 0.02</td>
</tr>
<tr>
<td>UV-FA concentration (mmol/L)</td>
<td>0.11 ± 0.01*</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV concentration (mmol/L)</td>
<td>1.33 ± 0.08</td>
<td>1.21 ± 0.03</td>
</tr>
<tr>
<td>FA concentration (mmol/L)</td>
<td>1.18 ± 0.06‡</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>UV-FA concentration (mmol/L)</td>
<td>0.13 ± 0.08</td>
<td>0.18 ± 0.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
Group I: n= 45 (UV) and 60 (FA) except for glucose and lactate where n=50 (FA); Group II: n= 21 (UV) and 51 (FA).
UV, umbilical vein; FA, femoral artery; UV-FA, umbilical venoarterial difference.
Compared with Group II, * p < 0.01; † p < 0.005; ‡ p < 0.001; § p < 0.0001.
Figure 13. Comparison of changes with time in UV-FA oxygen content differences (Cvo₂ - CaO₂) over the last 10 days of gestation before the onset of labor between Group I and II fetuses. Data were obtained on the daily basis from the time of the onset of labor retrospectively for 10 days and expressed as days prior to onset of labor, with the day of onset of labor being day 0. Lines a and b represent the regression line for Group I and II, respectively. The regression equation for a is y = 1.72 + 0.03x, r = 0.228, and for b is y = 2.55 + 0.02x, r = 0.149). Group I fetuses, (O); Group II fetuses, (●).

The UV-FA glucose concentration difference tended to be initially higher in Group I than in Group II, but to decrease with advancing gestation (Figure 15). Again, assuming that umbilical blood flow over the last 10 days was unchanged from the pre-labor measurement, the initially higher UV-FA glucose difference in Group I could compensate for the reduced flow to augment fetal glucose uptake. As illustrated in Figure 16, a lower UV glucose concentration was associated with a higher umbilical glucose extraction and this was especially observed in the Group I fetuses. This is analogous to the maintenance of fetal oxygen consumption by increased oxygen extraction. In Group II, the UV-FA glucose difference tended to increase...
Figure 14. Comparison of changes with the time in fetal oxygen extraction over the last 10 days of gestation before the onset of labor between Group I and II fetuses. Data are obtained on the daily basis from the time of the onset of labor retrospectively for 10 days and expressed as days prior to onset of labor, with the day of onset of labor being day 0. Lines a and b represent the regression line for Group I and II, respectively. The regression equation for a is $y = 47.41 + 1.57x$, $r = 0.479$, $P < 0.001$; and for b is $y = 46.38 + 0.48x$, $r = 0.241$, $P > 0.2$). Group I fetuses, (O); Group II fetuses, (●).

with advancing gestation suggesting an increasing rate of fetal glucose uptake (Figure 15). The changes in the UV-FA lactate concentration difference with advancing gestation are illustrated in Figure 17. As with the glucose difference, there was a trend for a decrease in the UV-FA lactate difference in Group I and a trend for an opposite change in Group II. This suggests that, as with fetal glucose uptake, the uptake of lactate from the placenta would be lower in Group I compared to Group II towards end of gestation.
Figure 15. Comparison of changes with the time in UV-FA glucose concentration differences over the last 10 days of gestation before the onset of labor between Group I and II fetuses. Data were obtained on the daily basis from the time of the onset of labor retrospectively for 10 days and expressed as days prior to onset of labor, with the day of onset of labor being day 0. Lines a and b represent the regression line for Group I and II, respectively. The regression equation for a is $y = 0.11 - 0.002x$, $r = -0.101$, and for b is $y = 0.09 + 0.002x$, $r = 0.168$). Group I fetuses, (O); Group II fetuses, (●).

Table 15 indicates the changes in blood gases, oxygen content, lactate and glucose concentrations over the last 10 days of gestation before labor by listing the correlation coefficients of each variable against time. In Group I fetuses, the UV and FA blood $O_2$ saturation ($r = -0.44; P < 0.005$ and $r = -0.45; P < 0.001$) and $O_2$ content ($r = -0.42; P < 0.005$ and $r = -0.38; P < 0.005$) decreased significantly, while FA blood lactate concentrations significantly increased ($r = 0.33; P < 0.05$). In Group II, the only variable which was significantly related to time was the UV glucose concentration, which significantly increased ($r = 0.45; P < 0.05$).
Figure 16. Relationship between percentage of glucose extracted by the fetus to UV glucose concentrations over the last 10 days of gestation before the onset of labor. The logarithmic function is shown with an equation: \( y = 9.23 - 17.47 \ln(x) \), \( r = -0.572 \), \( P < 0.001 \). Group I fetuses, (○); Group II fetuses, (●).

Table 16 lists the data comparing the slopes of the regression lines of the two groups for blood gases, oxygen content and acid-base balance obtained during the antepartum period. The mean slopes for UV \( \text{P}O_2 \), \( \text{O}_2 \) saturation and glucose in Group I were significantly lower as compared to Group II, while the mean slope values of the regression lines for FA \( \text{O}_2 \) saturation and \( \text{O}_2 \) content were also significantly lower than those in Group II. Thus, throughout the last 10 days of pregnancy, fetal \( \text{O}_2 \) saturation and \( \text{O}_2 \) content in Group I decreased more rapidly than in Group II.

The lower oxygenation observed in Group I fetuses appears to be related to the lower placental
Figure 17. Comparison of changes with the time in UV-FA lactate concentration differences over the last 10 days of gestation before the onset of labor between Group I and II fetuses. Data were obtained on the daily basis from the time of the onset of labor retrospectively for 10 days and expressed as days prior to onset of labor, with the day of onset of labor being day 0. Lines a and b represent the regression line for Group I and II, respectively. The regression equation for a is \( y = 0.09 - 0.005x \), \( r = -0.027 \), and for b is \( y = 0.21 + 0.006x \), \( r = 0.369 \). Group I fetuses, (○); Group II fetuses, (●).

Figure 18 demonstrates that there is a significant correlation between umbilical venous oxygen saturation and total cotyledon weight \( (r = 0.684, P < 0.05) \), thus suggesting that placenta weight was associated with its ability to supply oxygen to the fetus.

### 3.5.2 Fetal Heart Rate and Arterial Pressure

Figures 19 and 20 illustrate the mean 24 h values for fetal arterial pressure and heart rate, respectively, in the two groups over the last 7 (Group I) or 8 (Group II) days of gestation,
Table 15. Correlation analysis for changes of blood gases, oxygen content, lactate and glucose concentrations with time over last 10 days prior to labor.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td></td>
<td>r</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Umbilical vein (UV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{O_2}$ (mmHg)</td>
<td>-0.07</td>
<td>NS</td>
<td></td>
<td>0.41</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>$P_{CO_2}$ (mmHg)</td>
<td>0.16</td>
<td>NS</td>
<td></td>
<td>0.13</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>O$_2$ saturation (%)</td>
<td>-0.44</td>
<td>&lt;0.005</td>
<td></td>
<td>0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>O$_2$ content (mmol/L)</td>
<td>-0.42</td>
<td>&lt;0.005</td>
<td></td>
<td>-0.07</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0.24</td>
<td>NS</td>
<td></td>
<td>0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.03</td>
<td>NS</td>
<td></td>
<td>0.45</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Descending aorta (FA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{O_2}$ (mmHg)</td>
<td>-0.17</td>
<td>NS</td>
<td></td>
<td>0.16</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>$P_{CO_2}$ (mmHg)</td>
<td>0.12</td>
<td>NS</td>
<td></td>
<td>0.00</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>O$_2$ saturation (%)</td>
<td>-0.45</td>
<td>&lt;0.001</td>
<td></td>
<td>-0.09</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>O$_2$ content (mmol/L)</td>
<td>-0.38</td>
<td>&lt;0.005</td>
<td></td>
<td>-0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0.33</td>
<td>&lt;0.05</td>
<td></td>
<td>-0.00</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.02</td>
<td>NS</td>
<td></td>
<td>-0.12</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Group I: n=45 (UV) and 60 (FA);
Group II: n=21 (UV) and 51 (FA).
Table 16. Comparison between two slopes of regression lines on prenatal blood gases, oxygen content and acid-base balance.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td><strong>Umbilical vein (UV)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po2 (mmHg)</td>
<td>27.6 ± 1.8</td>
<td>-0.15 ± 0.30*</td>
</tr>
<tr>
<td>PcO2 (mmHg)</td>
<td>45.5 ± 1.0</td>
<td>0.18 ± 0.16</td>
</tr>
<tr>
<td>O2 saturation (%)</td>
<td>55.7 ± 2.7</td>
<td>-1.44 ± 0.44*</td>
</tr>
<tr>
<td>O2 content (mmol/L)</td>
<td>3.53 ± 0.23</td>
<td>-0.11 ± 0.04</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.57 ± 0.18</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.59 ± 0.05</td>
<td>-0.002 ± 0.01*</td>
</tr>
<tr>
<td><strong>Descending aorta (FA)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po2 (mmHg)</td>
<td>17.2 ± 0.9</td>
<td>-0.18 ± 0.10</td>
</tr>
<tr>
<td>PcO2 (mmHg)</td>
<td>50.2 ± 1.0</td>
<td>0.15 ± 0.16</td>
</tr>
<tr>
<td>O2 saturation (%)</td>
<td>31.0 ± 2.3</td>
<td>-1.42 ± 0.37*</td>
</tr>
<tr>
<td>O2 content (mmol/L)</td>
<td>2.06 ± 0.18</td>
<td>-0.09 ± 0.03*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.47 ± 0.13</td>
<td>0.05 ± 0.02*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.49 ± 0.05</td>
<td>-0.001 ± 0.01</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with Group II.

Group I: n=45 (UV) and 60 (FA);
Group II: n=21 (UV) and 51 (FA).
Figure 18. Relation of UV O₂ saturation (%) to placental weight \( y = 48.6 + 0.09x, r = 0.684, P < 0.05 \) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (○); Group II fetuses, (●).

ending with the day of labor. For Group I, recordings were obtained over 3-9 days in the individual animals, and in 5 of these, at least 7 consecutive days of recordings were obtained. The figures thus represent the results from these 5 sheep; the data from the remaining animal (E499), which was obtained over only the last 3 days of pregnancy, is not included. For Group II, recordings were obtained over 8-9 days, and in all animals at least 8 days were collected. Thus in Figures 19 and 20, the results are from all animals in Group II.

In both groups arterial pressure increased over the recording period with the highest value being on the day of labor. However, the daily rate of pressure increase in Group II \( (1.8 \pm 0.3 \text{ mm Hg/d}) \) was significantly greater than that in Group I \( (0.9 \pm 0.1 \text{ mm Hg/d}) \). As a
Figure 19. Daily averages of fetal arterial pressure over the last 7 (Group I, n = 5) or 8 (Group II, n = 6) days of gestation with day 0 being the day of labor and delivery. The asterisks above some data points indicate a significant difference from the day -6 (Group I) or day -7 (Group II) value. The regression lines were calculated from the mean values. For Group I the equation is: \( Y = 0.9X + 58.7, \ r = 0.9615, \ p < 0.001. \) For Group II, the equation is: \( Y = 1.8X + 65.0, \ r = 0.9388, \ p < 0.001. \)
Figure 20. Daily averages of fetal heart rate over the last 7 (Group I, n = 5) or 8 (Group II, n = 6) days of gestation with day 0 being the day of labor and delivery. The asterisks above some Group II data points indicate a significant difference from the day -7 value. The regression line for group II was calculated from the mean values over day -7 to day -2, with the equation: \( Y = -4.4X + 126.2, \) \( r = 0.9668, \) \( p<0.002 \).
consequence arterial pressure on the day prior to labor onset was significantly higher in Group II than in Group I (67.2 ± 2.5 vs. 58.4 ± 3.0 mm Hg). Moreover, in the former, pressure increased significantly from day -1 to the day of labor (mean increase = 4.4 ± 1.3 mm Hg), whereas this did not occur with Group I. As illustrated in Figure 20, there were also differences in the antepartum fetal heart rate changes in the 2 groups. In Group II, heart rate fell progressively and significantly from day -7 to day -2, with the mean decrease being 4.4 ± 0.6 bpm/d. However, there then was a significant rise in heart rate on the day prior to labor onset (8.0 ± 2.5 bpm), with no further change on the day of labor. In Group I, there was no antepartum decrease in heart rate. However, there was a tendency for the rate to rise on day -1, as in Group II. When the results from E499 were included, the rate increased in 5 of the 6 Group I fetuses and the mean increase (7.5 ± 5.2 bpm) was similar to that in Group II. However, because of inter-animal variability, the change did not reach statistical significance. Nonetheless, in both Groups the change in heart rate from day -2 to day -1 was found to be a useful predictor of the approximate time (i.e. within ~24-48 h) of labor onset in individual animals.

3.5.3 Fetal Organ and Tissue Blood Flow

Blood flow to the fetal brain, heart and adrenals in the pre-labor period is shown in Table 17. Although a higher mean value in blood flow in Group I was observed in the pituitary, spinal cord, cerebellum, right ventricle, left ventricle, interventricular septum, total heart, but not in the adrenal glands and the other regions within the brain, none of these values attained statistical significance. Moreover, blood flow to the other organs and tissues at
Table 17. Pre-labor blood flow to the fetal brain, heart and adrenal.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>131 ± 33</td>
<td>122 ± 14</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>137 ± 30</td>
<td>123 ± 10</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>303 ± 48</td>
<td>268 ± 22</td>
</tr>
<tr>
<td>Medulla</td>
<td>311 ± 64</td>
<td>320 ± 30</td>
</tr>
<tr>
<td>Pons</td>
<td>334 ± 58</td>
<td>394 ± 40</td>
</tr>
<tr>
<td>Midbrain</td>
<td>299 ± 58</td>
<td>368 ± 37</td>
</tr>
<tr>
<td>Frontal hemisphere</td>
<td>216 ± 40</td>
<td>228 ± 18</td>
</tr>
<tr>
<td>Temporal hemisphere</td>
<td>210 ± 36</td>
<td>217 ± 22</td>
</tr>
<tr>
<td>Total brain</td>
<td>242 ± 41</td>
<td>265 ± 21</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>569 ± 156</td>
<td>410 ± 85</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>386 ± 92</td>
<td>309 ± 59</td>
</tr>
<tr>
<td>Ventrices</td>
<td>478 ± 122</td>
<td>359 ± 72</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td>542 ± 138</td>
<td>288 ± 53</td>
</tr>
<tr>
<td>Total heart</td>
<td>480 ± 113</td>
<td>304 ± 48</td>
</tr>
<tr>
<td>Adrenals</td>
<td>371 ± 63</td>
<td>400 ± 48</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.

Pre-labor was comparable in both groups and showed no significant differences (Data not shown).

3.5.4 Fetal Combined Cardiac Output, Systemic and Umbilical Blood Flows

The pre-labor data on fetal combined cardiac output, systemic and umbilical blood flows are presented in Table 18. Prior to labor, fetal combined ventricular output and blood flow to the fetal body, normalized for the fetal body weight (ml/min/kg), was lower in Group I than in Group II, but this was not significant. However, there was a significantly lower umbilical blood flow in Group I, expressed on a weight-specific basis (ml/min/kg).
Table 18. Pre-labor fetal combined cardiac output, systemic & umbilical blood flow.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined ventricular output (ml/min/kg)</td>
<td>372 ± 47</td>
<td>495 ± 42</td>
</tr>
<tr>
<td>Systemic blood flow (ml/min/kg)</td>
<td>253 ± 39</td>
<td>300 ± 28</td>
</tr>
<tr>
<td>Umbilical blood flow (ml/min/kg)</td>
<td>119 ± 13*</td>
<td>194 ± 19</td>
</tr>
<tr>
<td>Abs. combined cardiac output (ml/min)**</td>
<td>956 ± 172*</td>
<td>1785 ± 216</td>
</tr>
<tr>
<td>Abs. systemic blood flow (ml/min)</td>
<td>654 ± 132*</td>
<td>1076 ± 123</td>
</tr>
<tr>
<td>Abs. umbilical blood flow (ml/min)</td>
<td>302 ± 48*</td>
<td>708 ± 106</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. ** Abs. = absolute values.
* p < 0.05 compared with Group II.

When fetal combined cardiac output, systemic and umbilical blood flows are expressed as absolute flow values (ml/min), they were all significantly lower in Group I compared with Group II. In Figure 21, placental blood flow in ml/min/kg is plotted against total cotyledon weight for both groups together. Umbilical blood flow was significantly correlated with placental weight (r = 0.878; P < 0.001). This correlation becomes stronger when umbilical blood flow is expressed as absolute flow values in ml/min (Figure 22, r = 0.915; P < 0.001). Fetal combined ventricular output was also significantly correlated with total cotyledon weight (Figure 23, r = 0.765; P < 0.005). Again, a closer relationship was found between absolute cardiac output and placental weight (Figure 24, r = 0.920, P < 0.001).

Data on the percentage distribution of fetal cardiac output prior to the onset of labor are given in Table 19. The percentage of fetal cardiac output distributed to the placenta was lower in Group I than Group II (33.12 ± 3.14 vs. 39.16 ± 2.17 %), but this was not significantly
Figure 21. Relation of umbilical blood flow (ml/min/kg) to placental weight ($y = 37.9 + 0.52x$, $r = 0.878$, $P < 0.001$) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (○); Group II fetuses, (●).

Figure 22. Relation of absolute umbilical blood flow (ml/min) to placental weight ($y = 138.8 + 2.83x$, $r = 0.915$, $P < 0.001$) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (○); Group II fetuses, (●).
Figure 23. Relation of fetal combined ventricular output (ml/min/kg) to the placental weight \( y = 202.9 + 1.01x, r = 0.765, P < 0.005 \) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (o); Group II fetuses, (●).

Figure 24. Relation of absolute fetal combined ventricular output (ml/min) to placental weight \( y = -50.07 + 6.23x, r = 0.920, P < 0.001 \) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (o); Group II fetuses, (●).
### Table 19. Pre-labor % distribution of fetal combined cardiac output.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=6)</th>
<th>Group II (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic blood flow</td>
<td>66.88 ± 3.14</td>
<td>60.84 ± 2.17</td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>33.12 ± 3.14</td>
<td>39.16 ± 2.17</td>
</tr>
<tr>
<td>Brain</td>
<td>9.77 ± 0.97</td>
<td>9.27 ± 0.94</td>
</tr>
<tr>
<td>Heart</td>
<td>6.92 ± 2.31</td>
<td>5.86 ± 0.80</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.17 ± 1.10</td>
<td>4.69 ± 0.86</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.44 ± 0.18</td>
<td>1.70 ± 0.26</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3.76 ± 0.13</td>
<td>3.96 ± 0.73</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.72 ± 0.05</td>
<td>0.69 ± 0.25</td>
</tr>
<tr>
<td>Liver</td>
<td>0.20 ± 0.07</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.58 ± 0.43</td>
<td>1.21 ± 0.38</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.30 ± 0.05*</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Kidneys</td>
<td>5.61 ± 0.49</td>
<td>4.81 ± 0.70</td>
</tr>
<tr>
<td>Brown fat</td>
<td>1.01 ± 0.16</td>
<td>0.97 ± 0.28</td>
</tr>
<tr>
<td>Skin</td>
<td>9.56 ± 1.93</td>
<td>8.73 ± 0.88</td>
</tr>
<tr>
<td>Bones</td>
<td>9.03 ± 1.48</td>
<td>7.19 ± 0.59</td>
</tr>
<tr>
<td>Muscles</td>
<td>14.21 ± 1.93</td>
<td>12.06 ± 2.95</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* p < 0.05 compared with Group II.

different. The proportion of combined ventricular output directed to the brain, heart, adrenals, small intestine, large intestine, spleen, kidneys, skin, bone and muscle was higher in Group I, whereas the percentage of fetal cardiac output perfusing the lungs, stomach and liver was lower. However, none of these differences were statistically significant, except for the fraction of fetal cardiac output delivered to the adrenal glands, which was significantly greater than that in Group II (0.30 ± 0.05 vs. 0.16 ± 0.01 %).
3.5.5 Fetal Oxygen Delivery and Consumption

Data on fetal oxygen delivery, extraction and consumption prior to the onset of labor are presented in Table 20. Both fetal oxygen delivery and consumption were significantly lower in Group I as compared to Group II, while fetal oxygen extraction was similar in the two groups. The umbilical venous and arterial oxygen content difference (Cvo2-Cao2) was also significantly lower in Group I, as was the umbilical venous oxygen content (Cvo2).

Figure 25 shows that fetal O2 delivery was significantly correlated with total cotyledon weight (r = 0.820, P < 0.01). There was also a significant correlation between fetal O2 consumption and placental weight (Figure 26, r = 0.879, P < 0.002). In contrast, fetal O2 extraction did not correlate with placental weight (Figure 27, r = 0.037). Figure 28 demonstrates the relationship between placental weight and the margin of safety for oxygen supply to the fetus at the pre-labor stage. The margin of safety is defined as the difference between fetal substrate delivery and substrate uptake (Owens et al. 1987a; Owens et al. 1987b). As placental weight decreased, the margin of safety for oxygen supply to the fetus became markedly narrowed.

3.5.6 Umbilical Glucose and Lactate Delivery and Uptake

Table 21 shows umbilical lactate and glucose delivery, extraction and uptake prior to the onset of labor. Umbilical lactate uptake was significantly less in Group I compared with Group II (12 ± 6 vs. 36 ± 6 mmol/min/kg). On the other hand, umbilical glucose uptake
Table 20. Pre-labor fetal oxygen delivery, consumption and extraction.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=5)</th>
<th>Group II (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen delivery (µmol/min/kg)</td>
<td>476 ± 61*</td>
<td>1128 ± 178</td>
</tr>
<tr>
<td>Oxygen Consumption (µmol/min/kg)</td>
<td>213 ± 20*</td>
<td>513 ± 86</td>
</tr>
<tr>
<td>Oxygen extraction (%)</td>
<td>46.40 ± 5.09</td>
<td>45.16 ± 2.40</td>
</tr>
<tr>
<td>Cvo2-Cao2 (mmol/L)a</td>
<td>1.81 ± 0.15*</td>
<td>2.50 ± 0.17</td>
</tr>
<tr>
<td>Cvo2 (mmol/L)b</td>
<td>3.97 ± 0.25*</td>
<td>5.59 ± 0.48</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. a The umbilical venous and arterial oxygen content difference; and b the umbilical venous oxygen content. * p < 0.05 compared with Group II.

was similar in both groups (16 ± 3 vs. 19 ± 5 µmol/min/kg), although umbilical glucose delivery was significantly decreased in Group I (71 ± 12 vs. 168 ± 35 mmol/min/kg). The extraction of glucose across the umbilical circulation was significantly higher in Group I than in Group II (24.95 ± 4.92 vs. 10.94 ± 1.40 %). Analysis of the substrate oxygen quotients revealed that glucose could account for 46% of oxidative metabolism in Group I and only 21% in Group II fetuses. This difference appears largely due to a much lower oxygen uptake in Group I than in Group II (Table 20). Data on lactate oxygen quotients indicated that at this stage, lactate account for 16% of total fetal oxidative metabolic rate for Group I and 22% for Group II.

Figure 29 presents the relationship between placental weight and the margin of safety for glucose supply to the fetus at the pre-labor stage. Due to the increased glucose extraction by the fetus from umbilical circulation in Group I, umbilical glucose uptake was maintained. The consequence, however, is a reduced margin of safety for glucose supply.
Figure 25. Relation of fetal oxygen delivery (μmol/min/kg) to placental weight ($y = -16.5 + 3.35x$, $r = 0.820, P < 0.01$) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (○); Group II fetuses, (●).

Figure 26. Relation of fetal oxygen consumption (μmol/min/kg) to placental weight ($y = -39.8 + 1.65x$, $r = 0.879, P < 0.002$) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (○); Group II fetuses, (●).
Figure 27. Relation of fetal oxygen extraction (%) to placental weight \((y = 45.12 + 0.003x, r = 0.037)\) at the pre-labor stage (i.e., 2-3 days prior to labor onset). Group I fetuses, (O); Group II fetuses, (●).

to the fetus as placental weight declined.

3.5.7 Fetal Plasma Arginine Vasopressin Concentrations

At the time of the pre-labor measurements, fetal plasma arginine vasopressin concentrations were significantly higher in Group I as compared to Group II \((11.7 \pm 1.4 \text{ vs. } 4.2 \pm 0.8 \text{ pg/ml, } P < 0.05)\).
Figure 28. Relationship between placental weight and the margin of safety for oxygen supply to the fetus at the pre-labor stage. The margin of safety is the difference between fetal oxygen delivery (dashed line, \( y = -16.5 + 3.35x, r = 0.820, P < 0.01 \)) and oxygen consumption (solid line, \( y = -39.8 + 1.65x, r = 0.879, P < 0.002 \)). Group I fetuses, open symbols (○, oxygen delivery; □, oxygen consumption); Group II fetuses, closed symbols (●, oxygen delivery; ■, oxygen consumption).

3.5.8 Fetal Breathing Activity

Table 22 illustrates fetal breathing movements (FBM), fetal nonbreathing movements and fetal gasping activities over the last 7 days of gestation, expressed as the percentage of time spent on each day. In both groups, breathing incidence decreased towards term, becoming significant 2 or 3 days before delivery. The decrease in the percentage of time spent breathing from day -5 to day -1 before delivery was 3.7 ± 0.3%/d for Group I and 5.5% ± 0.8%/d for Group II (Group I: \( Y = 3.7X + 11.4, r = -0.9893, P < 0.002 \); and Group II: \( Y = 5.6X + 13.9, r = \)
Table 21. Pre-labor umbilical lactate and glucose delivery, extraction and uptake.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=5)</th>
<th>Group II (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (μmol/min/kg)</td>
<td>229 ± 80</td>
<td>253 ± 45</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>7.40 ± 3.15</td>
<td>14.79 ± 1.64</td>
</tr>
<tr>
<td>Uptake (μmol/min/kg)</td>
<td>12 ± 6*</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.11 ± 0.05</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Lactate / O₂</td>
<td>0.16 ± 0.07</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (μmol/min/kg)</td>
<td>71 ± 12*</td>
<td>168 ± 35</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>24.95 ± 4.92*</td>
<td>10.94 ± 1.40</td>
</tr>
<tr>
<td>Uptake (μmol/min/kg)</td>
<td>16 ± 3</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.14 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Glucose / O₂</td>
<td>0.46 ± 0.07*</td>
<td>0.21 ± 0.04</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* p < 0.05 compared with Group II.

-0.9725, P < 0.01). The mean rates of decline in FBM in the 2 groups were not significantly different. However, there was a significant difference between the 2 groups in terms of the overall incidence of FBM over the last 7 days of gestation (19.7 ± 2.0 vs. 28.0 ± 1.8%, P < 0.02) and the overall incidence of breathing movements over the last day of gestation (1.4 ± 0.9 vs. 5.8 ± 0.8, P < 0.005). Fetal breathing movements were consistently lower in Group I than Group II over each of the last 7 days of gestation (Table 22). Conversely, the incidence of fetal gasping activity was consistently higher, although the difference between the 2 groups did not reach statistical significance (P = 0.10). The prepartum decline in FBM was found to be another useful criteria for the prediction of time of labor onset.
Figure 29. Relationship between placental weight and the margin of safety for glucose supply to the fetus at the pre-labor stage. The margin of safety is the difference between umbilical glucose delivery (dashed line, $y = -2.33 + 0.50x$, $r = 0.732$, $P < 0.05$) and umbilical glucose uptake (solid line, $y = 14.87 + 0.01x$, $r = 0.144$). Group I fetuses, open symbols (○, umbilical glucose delivery; □, umbilical glucose uptake); Group II fetuses, closed symbols (●, umbilical glucose delivery; ■, umbilical glucose uptake).

3.6 Fetal Condition during Labor and Delivery

3.6.1 Duration of Labor and Sampling Times

Table 23 presents duration of labor and the sampling times in relation to the time of delivery in Groups I and II. The total duration of labor was similar in Groups I and II (17.9 ± 1.3 vs. 15.1 ± 1.5 h), as were the times at which measurements were taken. Measurements were made at the following five stages in Group I and Group II, respectively (numbers in parentheses give
Table 22. Fetal breathing movements before and during labor and delivery.

<table>
<thead>
<tr>
<th></th>
<th>-6</th>
<th>-5</th>
<th>-4</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
<td>(day)</td>
</tr>
<tr>
<td>Group I (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Time breathing</td>
<td>23.6 ± 4.3</td>
<td>29.4 ± 3.1</td>
<td>27.5 ± 3.0</td>
<td>21.6 ± 3.9*</td>
<td>18.6 ± 1.4†</td>
<td>15.5 ± 2.0‡</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>% Time nonbreathing</td>
<td>71.8 ± 4.0</td>
<td>66.2 ± 2.3</td>
<td>68.7 ± 2.9</td>
<td>75.6 ± 3.6†</td>
<td>76.3 ± 1.9†</td>
<td>80.3 ± 2.2‡</td>
<td>97.2 ± 0.9</td>
</tr>
<tr>
<td>% Time gasping</td>
<td>4.5 ± 1.5</td>
<td>4.4 ± 1.4</td>
<td>3.8 ± 0.8§</td>
<td>2.8 ± 0.7§</td>
<td>5.1 ± 1.9</td>
<td>4.2 ± 0.8</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>Group II (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Time breathing</td>
<td>36.6 ± 3.1</td>
<td>39.5 ± 3.3</td>
<td>37.1 ± 1.8</td>
<td>33.5 ± 4.2</td>
<td>25.8 ± 3.3‡</td>
<td>17.9 ± 1.3‡</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>% Time nonbreathing</td>
<td>61.6 ± 3.0</td>
<td>59.0 ± 3.3</td>
<td>61.1 ± 1.1</td>
<td>64.3 ± 4.0</td>
<td>72.4 ± 3.4‡</td>
<td>80.0 ± 1.8‡</td>
<td>93.2 ± 1.2</td>
</tr>
<tr>
<td>% Time gasping</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>1.9 ± 0.7</td>
<td>2.2 ± 0.9</td>
<td>1.8 ± 0.4</td>
<td>2.1 ± 1.1</td>
<td>0.9 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Data on the incidence of fetal breathing movements are computed per day (over 24 h) from the time of delivery of the fetus retrospectively for 7 days and expressed as days before delivery, with the day of delivery being day 0. The overall incidence of fetal breathing movements on each day are expressed as percent of time spent breathing on each day.

* P <0.05 vs. -5 day.
† P <0.05 vs. -5 and -4 day.
‡ P <0.05 vs. -6, -5 and -4 day.
§ P <0.05 vs. -2 day.
|| P <0.05 vs. all previous days.
Table 23. Duration of labor & time of measurement in relation to delivery.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of labor (h)</td>
<td>17.9 ± 1.3</td>
<td>15.1 ± 1.5</td>
</tr>
<tr>
<td>Pre-labor (d)</td>
<td>-2.2 ± 0.5</td>
<td>-2.9 ± 0.4</td>
</tr>
<tr>
<td>Early labor (h)</td>
<td>-10.8 ± 1.2</td>
<td>-9.7 ± 1.2</td>
</tr>
<tr>
<td>Mid labor (h)</td>
<td>-5.6 ± 0.9</td>
<td>-5.0 ± 1.0</td>
</tr>
<tr>
<td>Late labor (h)</td>
<td>-1.5 ± 0.5</td>
<td>-1.9 ± 0.5</td>
</tr>
<tr>
<td>Delivery (min)</td>
<td>-6.3 ± 0.6</td>
<td>-7.2 ± 1.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

The mean time prior to delivery: pre-labor (-2.2 ± 0.5 vs. -2.9 ± 0.4 d), early labor (-10.8 ± 1.2 vs. -9.7 ± 1.2 h), mid-labor (-5.6 ± 0.9 vs. -5.0 ± 1.0 h), late labor (-1.5 ± 0.5 vs. -1.9 ± 0.5 h) and delivery (-6.3 ± 0.6 vs. -7.2 ± 1.0 min). Note that much of the pre-labor data has been presented in the antepartum comparisons between Groups I and II (see section 3.5). The data will be used again in this section as the control values for the results obtained once labor commenced. In 2 of the Group I sheep (E968 and E144), measurements at delivery were not obtained as the fetuses were delivered before the samples could be collected.

3.6.2 Uterine Activity

The general patterns of uterine activity that were present at the sampling times prior to and
during labor are illustrated in Figure 1 and were discussed earlier (see section 2.2.3). Table 24 lists the mean values of the various quantitative measures of uterine activity in Group I and II ewes before and during labor and delivery. These include the frequency, average intensity, average duration, sum of intensity and sum of duration of uterine contraction, Alexandria unit and the modified Alexandria unit. A steady and significant increase in uterine activity occurred in both groups with the progress of labor. However, there were no statistically significant difference in the mean values for any of the parameters between the two groups. In Figures 30 and 31, the mean values for uterine contraction frequency and intensity, and the modified Alexandria unit during labor for Groups I and II, respectively, are plotted against the time before delivery. For all 3 variables, there was a very gradual rise through most of labor, with most of the increase occurring in approximately the last 2 h, and the pattern of change in the 2 groups was virtually identical.

3.6.3 Fetal Acid-Base and Blood Gas Variables

As discussed in the section 3.3, the initial division of the study animals into 2 groups was done on the basis of the degree of fetal metabolic acidemia that developed during labor. At the outset, a cutoff value of 7.15 was chosen for fetal arterial pH at delivery, as in several large series human studies, a cord blood arterial pH of < 7.15 was used as a significance value outside the "normal" range to define fetal acidosis (Ramin et al. 1989; Thorp et al. 1989; Vintzileos et al. 1992). The mean values for fetal FA blood gases, acid-base balance and glucose and lactate concentrations for Groups I and II are given in Tables 25 and 26, respectively, while the pH and lactate results for the individual animals in each group are
Table 24. Uterine contraction data before and during labor and delivery.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (per h)</td>
<td>1.7 ± 0.2</td>
<td>7.5 ± 0.8*</td>
<td>15.7 ± 2.3†</td>
<td>25.5 ± 3.7‡</td>
<td>40.4 ± 3.2§</td>
</tr>
<tr>
<td>Average intensity (mmHg)</td>
<td>5.0 ± 0.4</td>
<td>5.4 ± 0.5</td>
<td>7.0 ± 0.8</td>
<td>8.8 ± 0.8†</td>
<td>14.4 ± 3.4§</td>
</tr>
<tr>
<td>Average duration (min)</td>
<td>7.6 ± 0.5</td>
<td>3.4 ± 0.4*</td>
<td>2.2 ± 0.3†</td>
<td>1.3 ± 0.2†</td>
<td>0.8 ± 0.1‡</td>
</tr>
<tr>
<td>Sum intensity (mmHg)</td>
<td>8.7 ± 1.5</td>
<td>41.8 ± 8.0*</td>
<td>117.4 ± 31.4†</td>
<td>234.5 ± 47.5†</td>
<td>611.3 ± 185.9§</td>
</tr>
<tr>
<td>Sum duration (min)</td>
<td>12.8 ± 2.0</td>
<td>25.8 ± 4.0*</td>
<td>31.7 ± 2.4*</td>
<td>30.2 ± 2.2*</td>
<td>31.4 ± 3.9*</td>
</tr>
<tr>
<td>Alexandria unit</td>
<td>65.8 ± 11.5</td>
<td>144.4 ± 32.1*</td>
<td>220.5 ± 29.3†</td>
<td>259.3 ± 14.7†</td>
<td>399.4 ± 56.6§</td>
</tr>
<tr>
<td>Modified Alexandria unit</td>
<td>0.2 ± 0.1</td>
<td>10.1 ± 4.0*</td>
<td>67.6 ± 26.7†</td>
<td>193.1 ± 63.4‡</td>
<td>723.4 ± 238.0§</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (per h)</td>
<td>1.7 ± 0.2</td>
<td>7.8 ± 0.9*</td>
<td>14.8 ± 1.5†</td>
<td>27.0 ± 3.5‡</td>
<td>44.1 ± 4.0§</td>
</tr>
<tr>
<td>Average intensity (mmHg)</td>
<td>5.5 ± 0.5</td>
<td>5.5 ± 0.6</td>
<td>7.3 ± 0.4</td>
<td>9.9 ± 0.7†</td>
<td>17.7 ± 3.8§</td>
</tr>
<tr>
<td>Average duration (min)</td>
<td>7.5 ± 0.7</td>
<td>3.2 ± 0.1*</td>
<td>2.1 ± 0.1†</td>
<td>1.2 ± 0.1†</td>
<td>0.7 ± 0.0‡</td>
</tr>
<tr>
<td>Sum intensity (mmHg)</td>
<td>8.8 ± 0.9</td>
<td>42.7 ± 6.7*</td>
<td>107.4 ± 11.9†</td>
<td>269.5 ± 42.9†</td>
<td>781.1 ± 176.7§</td>
</tr>
<tr>
<td>Sum duration (min)</td>
<td>13.2 ± 2.5</td>
<td>25.3 ± 3.5*</td>
<td>30.2 ± 2.8*</td>
<td>30.1 ± 1.5*</td>
<td>28.6 ± 2.8*</td>
</tr>
<tr>
<td>Alexandria unit</td>
<td>68.1 ± 11.9</td>
<td>139.7 ± 28.7*</td>
<td>217.9 ± 21.9†</td>
<td>297.9 ± 28.8†</td>
<td>498.7 ± 105.3§</td>
</tr>
<tr>
<td>Modified Alexandria unit</td>
<td>0.2 ± 0.1</td>
<td>10.3 ± 3.9*</td>
<td>54.1 ± 15.5†</td>
<td>251.0 ± 81.3‡</td>
<td>1064.1 ± 297.3§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Modified Alexandria unit = kilounit.

*  P<0.05 vs pre labor.
†  P<0.05 vs pre and early labor.
‡  P<0.05 vs pre, early and mid labor.
§  P<0.05 vs pre, early, mid and late labor.
Figure 30. Mean values (± SEM) of uterine contraction frequency and intensity and modified Alexandria unit for Group I ewes at the various sampling times during labor. For statistical significance, see Table 24.
Figure 31. Mean values (± SEM) of uterine contraction frequency and intensity and modified Alexandria unit for Group II ewes at the various sampling times during labor. For statistical significance, see Table 24.
Table 25. Fetal FA blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descending aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.323 ± 0.018</td>
<td>7.239 ± 0.042</td>
<td>7.127 ± 0.058†</td>
<td>7.002 ± 0.051‡</td>
<td>6.876 ± 0.095§</td>
</tr>
<tr>
<td>Pco₂ (mmHg)</td>
<td>49.9 ± 1.8</td>
<td>49.4 ± 1.6</td>
<td>51.4 ± 1.5</td>
<td>52.6 ± 2.1†</td>
<td>54.4 ± 3.7‡</td>
</tr>
<tr>
<td>Po₂ (mmHg)</td>
<td>17.8 ± 1.1</td>
<td>15.0 ± 0.9*</td>
<td>16.3 ± 0.8</td>
<td>18.8 ± 1.5**</td>
<td>22.0 ± 1.2§</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>0.3 ± 0.8</td>
<td>-5.2 ± 2.2*</td>
<td>-10.8 ± 3.1†</td>
<td>-17.1 ± 2.2‡</td>
<td>-22.3 ± 3.5§</td>
</tr>
<tr>
<td>HCO₃ (mEq/L)</td>
<td>25.5 ± 0.6</td>
<td>21.0 ± 1.6*</td>
<td>17.3 ± 2.3†</td>
<td>13.0 ± 1.4‡</td>
<td>10.1 ± 1.7‡</td>
</tr>
<tr>
<td>Tco₂ (mmol/L)</td>
<td>26.8 ± 0.6</td>
<td>22.4 ± 1.6*</td>
<td>18.7 ± 2.3†</td>
<td>14.4 ± 1.4‡</td>
<td>11.6 ± 1.6‡</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.2 ± 0.2</td>
<td>11.2 ± 0.5</td>
<td>11.3 ± 0.6</td>
<td>11.0 ± 0.5</td>
<td>10.5 ± 0.4</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>33.4 ± 4.0</td>
<td>20.9 ± 2.0*</td>
<td>19.6 ± 2.2*</td>
<td>18.4 ± 2.2*</td>
<td>19.5 ± 3.2*</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>2.23 ± 0.26</td>
<td>1.40 ± 0.13*</td>
<td>1.33 ± 0.17*</td>
<td>1.24 ± 0.17*</td>
<td>1.24 ± 0.23*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.71 ± 0.50</td>
<td>5.12 ± 0.44*</td>
<td>8.09 ± 0.83†</td>
<td>12.67 ± 1.29‡</td>
<td>16.71 ± 2.78§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.45 ± 0.07</td>
<td>0.67 ± 0.10</td>
<td>0.80 ± 0.17*</td>
<td>0.91 ± 0.17*</td>
<td>0.92 ± 0.19*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre labor.
† P <0.05 vs pre and early labor.
‡ P <0.05 vs pre, early and mid labor.
§ P <0.05 vs pre, early, mid and late labor.
** P <0.05 vs early and mid labor.

n = 6 for pre, early, mid and late labor measurements, and n = 4 for delivery measurements.
Except for lactate and glucose where n = 5 for pre, early, mid and late labor measurements, and n = 4 for delivery measurements.
Table 26. Fetal FA blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descending aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.333 ± 0.006</td>
<td>7.323 ± 0.008</td>
<td>7.302 ± 0.010</td>
<td>7.278 ± 0.009†</td>
<td>7.215 ± 0.019§</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>51.4 ± 1.3</td>
<td>54.4 ± 1.2*</td>
<td>54.3 ± 1.4*</td>
<td>55.3 ± 1.1*</td>
<td>57.5 ± 1.8§</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>19.7 ± 0.8</td>
<td>16.8 ± 0.4*</td>
<td>16.2 ± 1.1*</td>
<td>15.0 ± 0.9*</td>
<td>14.8 ± 1.0†</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>1.6 ± 0.6</td>
<td>2.1 ± 0.6</td>
<td>0.6 ± 0.5</td>
<td>-0.8 ± 0.7†</td>
<td>-3.4 ± 1.2§</td>
</tr>
<tr>
<td>HCO₃ (mEq/L)</td>
<td>26.9 ± 0.7</td>
<td>27.8 ± 0.6</td>
<td>26.4 ± 0.5</td>
<td>26.3 ± 0.9</td>
<td>22.9 ± 1.0§</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>28.3 ± 0.8</td>
<td>29.3 ± 0.6</td>
<td>27.9 ± 0.5</td>
<td>26.9 ± 0.6**</td>
<td>24.5 ± 1.0§</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.6 ± 0.6</td>
<td>12.4 ± 0.6</td>
<td>12.1 ± 0.5</td>
<td>12.2 ± 0.6</td>
<td>11.8 ± 0.4</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>42.2 ± 1.6</td>
<td>29.2 ± 0.8*</td>
<td>26.5 ± 2.5*</td>
<td>18.9 ± 1.2‡</td>
<td>17.1 ± 1.2‡</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>2.94 ± 0.24</td>
<td>2.17 ± 0.12*</td>
<td>1.89 ± 0.16*</td>
<td>1.38 ± 0.10‡</td>
<td>1.21 ± 0.10‡</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.03 ± 0.07</td>
<td>1.29 ± 0.11</td>
<td>1.88 ± 0.22</td>
<td>3.43 ± 0.42‡</td>
<td>5.65 ± 1.11§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.69 ± 0.07</td>
<td>0.89 ± 0.11</td>
<td>0.95 ± 0.13</td>
<td>1.13 ± 0.11*</td>
<td>1.59 ± 0.21§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

* P < 0.05 vs pre labor.
† P < 0.05 vs pre and early labor.
‡ P < 0.05 vs pre, early and mid labor.
§ P < 0.05 vs pre, early, mid and late labor.
** P < 0.05 vs early labor.

n = 6 for pre, early, mid, late labor and delivery measurements.
illustrated in figures 32 and 33. In Group I, fetal FA pH fell progressively and markedly during labor. In the 4 fetuses sampled at delivery, the value ranged from 6.671 to 7.112, while in the remaining 2 animals even the late labor values (6.827 and 7.114) were below the 7.15 cutoff. In the Group II animals, there was also a decrease in fetal FA pH during labor, but the magnitude of the fall was less than in Group I, and at delivery the values ranged from 7.153 to 7.260. There was a more striking difference in the changes in FA lactate level between the 2 groups. This was obvious even in early labor, where the Group I values ranged from 3.55 to 5.83 mM and those in Group II from 0.92 to 1.59 mM. At delivery, the values in the 2 Groups were more markedly different, with the mean values for Groups I and II being 16.71 ± 2.78 and 5.65 ± 1.11 mM, respectively, with the rise in lactate in Group II occurring largely in late labor and at delivery.

As shown in Table 25, the Group I FA base excess decreased from 0.3 ± 0.8 to -22.0 ± 1.2 mEq/L during labor and there was a corresponding fall in fetal bicarbonate level 25.5 ± 0.6 to 10.1 ± 1.7 mEq/L at delivery. These data indicate that the acidemia that developed during labor was predominantly metabolic in origin, which is consistent with the marked rise in lactate concentration. Following the onset of labor, fetal arterial $P_{O_2}$ initially decreased significantly, but thereafter increased beyond the pre-labor value in concert with the decrease in pH. Thus, the severe fetal acidemia produced a higher fetal arterial $P_{O_2}$, likely as a result of a marked rightward shift in the hemoglobin-$O_2$ dissociation curve. Fetal $P_{CO_2}$ in Group I was progressively increased during the course of labor. In addition, a marked decrease in fetal arterial $O_2$ saturation and content occurred, likely as a result of a Bohr shift in hemoglobin
Figure 32. Individual Group I values for fetal FA pH (upper panel) and lactate concentration (lower panel) during labor and delivery.
Figure 33. Individual Group II values for fetal FA pH (upper panel) and lactate concentration (lower panel) during labor and delivery.
oxygen dissociation curve. There was also a significant increase in fetal glucose concentration during labor and delivery (0.45 ± 0.07 at pre-labor vs. 0.92 ± 0.19 mmol/L at delivery).

As shown in Table 26, the lesser reduction in fetal FA pH in Group II animals was accompanied by smaller falls in base excess (1.6 ± 0.6 to -4.3 ± 1.2 mEq/L) and bicarbonate concentration (26.9 ± 0.7 to 22.9 ± 1.0 mEq/L) in comparison to Group I. Nonetheless, modest metabolic acidemia did occur in the Group II fetuses. There was also a progressive reduction in fetal FA \( P_O2 \) and an increase in fetal \( P_C02 \), as well as a decrease in fetal \( O_2 \) saturation and content. However, the changes in the latter variables were of lesser magnitude than in Group I. Fetal arterial glucose level was significantly increased from 0.69 ± 0.07 at pre-labor to 1.59 ± 0.21 mmol/L at delivery. There were no significant changes in fetal hemoglobin concentrations throughout labor in either group.

There were similar differences in the pattern of change in the blood gas, acid-base, \( O_2 \) saturation and content values in the ascending aorta (Tables 27 and 28) and umbilical vein (Tables 29 and 30) between the 2 groups. Table 31 gives the mean ratios between ascending aortic (CA) and FA blood gas variables and glucose and lactate concentrations. There were no significant changes in the ratios for \( P_O2 \), \( P_C02 \), lactate and glucose concentrations in either group. In contrast, as labor progressed the CA/FA ratio for \( O_2 \) saturation and content tended to increase in both groups, and in Group II, these ratios were significantly increased in late labor and at delivery. The mean value for fetal lactate and glucose concentrations at each stage of labor were always higher in umbilical venous blood than in descending aortic blood in both groups (Tables 25, 26, 29 and 30).
Table 27. Fetal CA blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascending aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.309 ± 0.019</td>
<td>7.250 ± 0.042</td>
<td>7.135 ± 0.059*</td>
<td>7.008 ± 0.049‡</td>
<td>6.876 ± 0.095‡</td>
</tr>
<tr>
<td>PC02 (mmHg)</td>
<td>49.6 ± 2.4</td>
<td>47.9 ± 1.2</td>
<td>49.7 ± 1.7</td>
<td>51.1 ± 1.8*</td>
<td>52.8 ± 3.5‡</td>
</tr>
<tr>
<td>PO2 (mmHg)</td>
<td>20.8 ± 2.1</td>
<td>17.5 ± 0.7</td>
<td>20.2 ± 1.0</td>
<td>21.1 ± 1.4*</td>
<td>25.3 ± 1.8§</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>-0.9 ± 0.6</td>
<td>-5.0 ± 2.2</td>
<td>-10.9 ± 3.1†</td>
<td>-17.1 ± 2.2‡</td>
<td>-22.6 ± 3.5‡</td>
</tr>
<tr>
<td>HCO3 (mEq/L)</td>
<td>24.4 ± 0.4</td>
<td>21.0 ± 1.6</td>
<td>17.0 ± 2.2†</td>
<td>12.8 ± 1.4‡</td>
<td>9.8 ± 1.6‡</td>
</tr>
<tr>
<td>TCO2 (mmol/L)</td>
<td>25.8 ± 0.4</td>
<td>22.7 ± 1.7</td>
<td>18.3 ± 2.2†</td>
<td>14.2 ± 1.4‡</td>
<td>11.3 ± 1.5‡</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.4 ± 0.2</td>
<td>11.1 ± 0.4</td>
<td>11.2 ± 0.5</td>
<td>10.9 ± 0.5</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td>O2 saturation (%)</td>
<td>38.0 ± 4.0</td>
<td>27.4 ± 2.3*</td>
<td>26.6 ± 3.2*</td>
<td>22.0 ± 1.9*</td>
<td>21.7 ± 3.4*</td>
</tr>
<tr>
<td>O2 content (mmol/L)</td>
<td>2.57 ± 0.25</td>
<td>1.83 ± 0.17*</td>
<td>1.79 ± 0.22*</td>
<td>1.45 ± 0.16*</td>
<td>1.35 ± 0.22†</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.85 ± 0.60</td>
<td>5.11 ± 0.42*</td>
<td>8.23 ± 0.85†</td>
<td>12.89 ± 1.23‡</td>
<td>16.68 ± 2.78§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.45 ± 0.09</td>
<td>0.66 ± 0.10</td>
<td>0.79 ± 0.16*</td>
<td>0.92 ± 0.19†</td>
<td>0.92 ± 0.19†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre labor.
† P <0.05 vs pre and early labor.
‡ P <0.05 vs pre, early and mid labor.
§ P <0.05 vs pre, early, mid and late labor.
n = 5 for pre labor and n = 6 for early, mid and late labor measurements, and n = 4 for delivery measurements.
Except for lactate and glucose where n = 5 for early, mid and late labor and n = 4 for pre labor and delivery measurements.
Table 28. Fetal CA blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.343 ± 0.006</td>
<td>7.332 ± 0.009</td>
<td>7.314 ± 0.013</td>
<td>7.288 ± 0.012†</td>
<td>7.221 ± 0.020§</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>48.9 ± 1.1</td>
<td>53.2 ± 1.4*</td>
<td>53.1 ± 1.4*</td>
<td>53.4 ± 1.4*</td>
<td>55.6 ± 1.5‡</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>21.5 ± 0.8</td>
<td>19.0 ± 0.8*</td>
<td>18.3 ± 1.2*</td>
<td>17.2 ± 1.0†</td>
<td>16.8 ± 1.0‡</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>1.5 ± 0.7</td>
<td>2.2 ± 0.6</td>
<td>1.0 ± 0.6</td>
<td>-0.8 ± 0.6†</td>
<td>-4.5 ± 1.2§</td>
</tr>
<tr>
<td>HCO₃ (mEq/L)</td>
<td>26.2 ± 0.7</td>
<td>27.7 ± 0.6</td>
<td>26.5 ± 0.6</td>
<td>25.1 ± 0.6*</td>
<td>22.5 ± 1.0§</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>27.5 ± 0.7</td>
<td>29.2 ± 0.6</td>
<td>28.0 ± 0.6</td>
<td>26.6 ± 0.6*</td>
<td>24.0 ± 1.0§</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.5 ± 0.4</td>
<td>12.3 ± 0.6</td>
<td>12.2 ± 0.6</td>
<td>12.0 ± 0.6</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>50.5 ± 2.0</td>
<td>36.8 ± 1.4*</td>
<td>32.3 ± 2.7†</td>
<td>25.6 ± 1.8‡</td>
<td>23.6 ± 1.9‡</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>3.47 ± 0.20</td>
<td>2.71 ± 0.15*</td>
<td>2.34 ± 0.18†</td>
<td>1.82 ± 0.12‡</td>
<td>1.69 ± 0.14‡</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.00 ± 0.07</td>
<td>1.31 ± 0.11</td>
<td>1.94 ± 0.22</td>
<td>3.48 ± 0.44‡</td>
<td>5.73 ± 1.11§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.69 ± 0.08</td>
<td>0.88 ± 0.11</td>
<td>0.96 ± 0.13</td>
<td>1.17 ± 0.13*</td>
<td>1.69 ± 0.22§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

*  P < 0.05 vs pre labor.
†  P < 0.05 vs pre and early labor.
‡  P < 0.05 vs pre, early and mid labor.
§  P < 0.05 vs pre, early, mid and late labor.

n = 6 for pre, early, mid, late labor and delivery measurements.
Table 29. UV blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.353 ± 0.024</td>
<td>7.259 ± 0.053</td>
<td>7.147 ± 0.069*</td>
<td>7.006 ± 0.051‡</td>
<td>6.906 ± 0.090‡</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>44.4 ± 1.8</td>
<td>44.6 ± 1.0</td>
<td>46.1 ± 1.3</td>
<td>46.7 ± 1.7</td>
<td>46.4 ± 1.5</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>30.0 ± 2.5</td>
<td>26.6 ± 0.7</td>
<td>30.0 ± 1.6</td>
<td>31.8 ± 1.5**</td>
<td>37.0 ± 3.0§</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>0.0 ± 0.9</td>
<td>-5.5 ± 2.9</td>
<td>-11.0 ± 3.7*</td>
<td>-18.0 ± 2.3‡</td>
<td>-22.1 ± 3.5‡</td>
</tr>
<tr>
<td>HCO₃ (mEq/L)</td>
<td>24.2 ± 0.6</td>
<td>20.1 ± 2.1*</td>
<td>16.5 ± 2.7*</td>
<td>11.7 ± 1.5‡</td>
<td>9.4 ± 1.8‡</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>25.5 ± 0.5</td>
<td>21.3 ± 2.1*</td>
<td>17.7 ± 2.7*</td>
<td>13.0 ± 1.5‡</td>
<td>10.7 ± 1.8‡</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.0 ± 0.2</td>
<td>10.9 ± 0.6</td>
<td>11.0 ± 0.7</td>
<td>10.7 ± 0.6</td>
<td>10.4 ± 0.3</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>60.8 ± 4.7</td>
<td>45.9 ± 3.3*</td>
<td>44.4 ± 3.3*</td>
<td>40.1 ± 1.8*</td>
<td>40.2 ± 1.9*</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>3.97 ± 0.25</td>
<td>3.00 ± 0.26*</td>
<td>2.90 ± 0.24*</td>
<td>2.55 ± 0.13*</td>
<td>2.50 ± 0.10*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.82 ± 0.50</td>
<td>5.34 ± 0.38*</td>
<td>8.56 ± 0.88†</td>
<td>13.35 ± 1.19‡</td>
<td>16.93 ± 2.42§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.59 ± 0.08</td>
<td>0.70 ± 0.12</td>
<td>0.90 ± 0.17*</td>
<td>1.01 ± 0.18†</td>
<td>1.07 ± 0.18†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
*     P < 0.05 vs pre labor.
†    P < 0.05 vs pre and early labor.
‡   P < 0.05 vs pre, early and mid labor.
§  P < 0.05 vs pre, early, mid and late labor.
** P < 0.05 vs early labor.

n = 5 for pre, early, mid and late labor measurements, and n = 4 for delivery measurements.
Table 30. UV blood gas status, oxygen content and acid-base balance before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Umbilical vein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.391 ± 0.003</td>
<td>7.379 ± 0.005</td>
<td>7.366 ± 0.009</td>
<td>7.341 ± 0.011</td>
<td>7.286 ± 0.034§</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>44.3 ± 0.4</td>
<td>45.2 ± 1.2</td>
<td>44.3 ± 1.5</td>
<td>43.8 ± 0.9</td>
<td>42.7 ± 1.5</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>35.8 ± 3.8</td>
<td>31.0 ± 3.0*</td>
<td>28.5 ± 2.9*</td>
<td>29.3 ± 3.8*</td>
<td>32.3 ± 5.2*</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td>2.7 ± 0.3</td>
<td>2.2 ± 0.7</td>
<td>0.8 ± 0.6</td>
<td>-1.0 ± 1.1†</td>
<td>-4.9 ± 1.8§</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>26.5 ± 0.4</td>
<td>26.3 ± 0.8</td>
<td>25.0 ± 0.7</td>
<td>23.4 ± 1.0†</td>
<td>20.1 ± 1.4§</td>
</tr>
<tr>
<td>TCO₂ (mmol/L)</td>
<td>27.8 ± 0.4</td>
<td>27.5 ± 0.9</td>
<td>26.2 ± 0.7</td>
<td>24.6 ± 1.0†</td>
<td>21.3 ± 1.4§</td>
</tr>
<tr>
<td>[Hb] (g %)</td>
<td>11.8 ± 0.7</td>
<td>12.4 ± 1.0</td>
<td>11.9 ± 1.0</td>
<td>11.6 ± 0.9</td>
<td>11.6 ± 0.7</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>79.2 ± 3.8</td>
<td>68.0 ± 3.6*</td>
<td>63.7 ± 4.6*</td>
<td>60.4 ± 5.3*</td>
<td>59.9 ± 7.4*</td>
</tr>
<tr>
<td>O₂ content (mmol/L)</td>
<td>5.59 ± 0.48</td>
<td>5.03 ± 0.37</td>
<td>4.47 ± 0.25*</td>
<td>4.13 ± 0.35†</td>
<td>4.12 ± 0.48†</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.23 ± 0.11</td>
<td>1.55 ± 0.17</td>
<td>2.43 ± 0.16</td>
<td>4.04 ± 0.49‡</td>
<td>6.04 ± 0.98§</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.82 ± 0.12</td>
<td>1.08 ± 0.18*</td>
<td>1.18 ± 0.21*</td>
<td>1.28 ± 0.20*</td>
<td>1.69 ± 0.11§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre labor.
† P <0.05 vs pre and early labor.
‡ P <0.05 vs pre, early and mid labor.
§ P <0.05 vs pre, early, mid and late labor.
n = 4 for pre, early, mid, late labor and delivery measurements.
Table 31. Fetal CA/FA blood gas, oxygen content, lactate and glucose ratio before and during labor and delivery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po2</td>
<td>1.23 ± 0.11</td>
<td>1.18 ± 0.03</td>
<td>1.25 ± 0.07</td>
<td>1.13 ± 0.02</td>
<td>1.15 ± 0.08</td>
</tr>
<tr>
<td>Pco2</td>
<td>0.98 ± 0.02</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>O2 saturation</td>
<td>1.24 ± 0.05</td>
<td>1.34 ± 0.12</td>
<td>1.37 ± 0.06</td>
<td>1.24 ± 0.10</td>
<td>1.11 ± 0.04</td>
</tr>
<tr>
<td>O2 content</td>
<td>1.24 ± 0.06</td>
<td>1.33 ± 0.11</td>
<td>1.36 ± 0.06</td>
<td>1.23 ± 0.10</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.01 ± 0.02</td>
<td>1.00 ± 0.01</td>
<td>1.02 ± 0.01</td>
<td>1.02 ± 0.01</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.04 ± 0.07</td>
<td>0.98 ± 0.03</td>
<td>0.99 ± 0.02</td>
<td>0.99 ± 0.04</td>
<td>0.99 ± 0.02</td>
</tr>
</tbody>
</table>

Group II

| Po2     | 1.10 ± 0.02 | 1.13 ± 0.03 | 1.14 ± 0.02 | 1.15 ± 0.04 | 1.14 ± 0.03 |
| Pco2    | 0.95 ± 0.02 | 0.98 ± 0.01 | 0.98 ± 0.01 | 0.97 ± 0.01 | 0.97 ± 0.01 |
| O2 saturation | 1.23 ± 0.03 | 1.26 ± 0.04 | 1.23 ± 0.03 | 1.35 ± 0.04* | 1.38 ± 0.05* |
| O2 content | 1.19 ± 0.03 | 1.25 ± 0.04 | 1.24 ± 0.02 | 1.33 ± 0.03* | 1.40 ± 0.06* |
| Lactate | 0.98 ± 0.04 | 1.02 ± 0.02 | 1.04 ± 0.02 | 1.01 ± 0.01 | 1.01 ± 0.01 |
| Glucose | 1.00 ± 0.02 | 1.00 ± 0.02 | 1.01 ± 0.02 | 1.03 ± 0.03 | 1.06 ± 0.01 |

Data are expressed as mean ± SEM. CA = ascending aorta blood and FA = descending aorta blood.

* P <0.05 vs pre labor.
† P <0.05 vs pre, early and mid labor.
3.6.4 Fetal Heart Rate, Arterial Pressure and Rate Pressure Product

As noted in section 3.5.2, the overall value for fetal arterial pressure for Group II was significantly increased on the day of labor, compared to the previous day, whereas this was not the case for Group I. Table 32 gives the fetal heart rate and arterial pressure values obtained by averaging the minute values from 15 min immediately before to 15 min after each microsphere injection, to give an overall value for each variable at each stage of labor. Fetal arterial pressure in Group I was minimally changed. It rose from $56 \pm 3$ mmHg at the pre-labor stage to a maximum of $62 \pm 3$ mmHg in early labor, but this change was not statistically significant. Afterwards, fetal blood pressure remained unchanged from the pre-labor value. However, fetal heart rate in Group I significantly increased from $149 \pm 5$ beats/min at pre-labor to $167 \pm 9$ bpm in early labor. It remained higher in mid-and late labor than at pre-labor, but these changes were not statistically significant. At delivery, fetal heart rate dropped to $139 \pm 13$ beats/min, which was a significant fall from the maximum value in early labor. The heart rate-arterial pressure product (a measure of myocardial work) showed a similar change to that in fetal heart rate.

In contrast to the Group I results, fetal heart rate in Group II did not change significantly during labor and at delivery, while there was a significant increase in arterial pressure at each stage of labor. The arterial pressure rose 38% to a maximum of $72 \pm 3$ mmHg at delivery. As a result of this increased blood pressure, the rate pressure product was also significantly increased during labor.
Table 32. Fetal heart rate, arterial pressure and rate pressure product before and during labor and delivery.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>149 ± 5</td>
<td>167 ± 9*</td>
<td>155 ± 7</td>
<td>150 ± 9</td>
<td>139 ± 13†</td>
</tr>
<tr>
<td>Arterial pressure (mmHg)</td>
<td>56 ± 3</td>
<td>62 ± 3</td>
<td>61 ± 3</td>
<td>60 ± 3</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Rate pressure product (beats/min*mmHg)</td>
<td>8202 ± 370</td>
<td>10436 ± 945*</td>
<td>9437 ± 672</td>
<td>9037 ± 820</td>
<td>8320 ± 989†</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>142 ± 9</td>
<td>147 ± 5</td>
<td>151 ± 6</td>
<td>152 ± 8</td>
<td>142 ± 8</td>
</tr>
<tr>
<td>Arterial pressure (mmHg)</td>
<td>57 ± 2</td>
<td>66 ± 2*</td>
<td>68 ± 2*</td>
<td>70 ± 3*</td>
<td>72 ± 3‡</td>
</tr>
<tr>
<td>Rate pressure product (beats/min*mmHg)</td>
<td>8100 ± 512</td>
<td>9759 ± 605*</td>
<td>10187 ± 316*</td>
<td>10500 ± 478*</td>
<td>10155 ± 558*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre labor, † P <0.05 vs early labor, ‡ P <0.05 vs pre and early labor.
n = 6 for pre, early, mid, late labor and delivery measurements.
3.6.5 Fetal Organ and Tissue Blood Flows

The mean values of fetal upper and lower body organ and tissue blood flows before and during labor and delivery are presented in Tables 33-36. There were differences in regional blood flow changes between the Group I and Group II animals. As shown in Table 33 and 34, in Group I fetuses, associated with the deterioration in fetal acid-base status, perfusion to the majority of individual fetal organs and tissues was increased during labor, but with a terminal fall at delivery. Blood flow to most regions within the brain was significantly increased over pre-labor values during labor, including the pituitary, spinal cord, cerebellum, medulla and pons. Blood flow to the midbrain and frontal and temporal hemispheres also showed a trend for an increase, but this did not reach statistical significance. Thus, as a percentage change from the pre-labor value, the total brain blood flow rose significantly by 70 ± 48% at mid labor and increased further by 98 ± 57% at late labor, and remained significantly elevated by 91 ± 78% at delivery. Following the onset of labor, the mean total heart blood flow rose by 122 ± 45% in early labor and peaked in late labor with an increase of 221 ± 66% over the pre-labor value. It was still elevated by 184 ± 133% at delivery. A similar change was also observed in blood flow to the adrenal gland (Table 34). It was highest in late labor, representing an increase of 176 ± 37% from pre-labor. Compared to the late labor value, however, adrenal blood flow dropped at delivery, but was still 117 ± 43% above the pre-labor value. There was also a significant increase in blood flow to the diaphragm, stomach, small intestine, large intestine and gall bladder, whereas flow to the spleen was significantly decreased (Table 34). Kidney blood flow tended to increase during labor, but there was a significant decrease at delivery. Similarly, blood flow to brown fat was significantly increased at mid and late labor,
Table 33. Fetal upper body organ/tissue blood flows before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>131 ± 33</td>
<td>151 ± 32</td>
<td>225 ± 50</td>
<td>274 ± 62†</td>
<td>169 ± 28**</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>137 ± 30</td>
<td>192 ± 30</td>
<td>220 ± 46*</td>
<td>319 ± 72‡</td>
<td>372 ± 90‡</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>303 ± 48</td>
<td>374 ± 40</td>
<td>413 ± 36*</td>
<td>459 ± 50*</td>
<td>364 ± 16**</td>
</tr>
<tr>
<td>Medulla</td>
<td>311 ± 64</td>
<td>387 ± 68</td>
<td>392 ± 59</td>
<td>477 ± 98*</td>
<td>438 ± 170</td>
</tr>
<tr>
<td>Pons</td>
<td>334 ± 58</td>
<td>468 ± 61*</td>
<td>473 ± 77*</td>
<td>570 ± 90*</td>
<td>532 ± 34*</td>
</tr>
<tr>
<td>Midbrain</td>
<td>299 ± 58</td>
<td>401 ± 45</td>
<td>402 ± 66</td>
<td>454 ± 62</td>
<td>363 ± 38</td>
</tr>
<tr>
<td>Frontal hemisphere</td>
<td>216 ± 40</td>
<td>264 ± 36</td>
<td>285 ± 41</td>
<td>302 ± 48</td>
<td>257 ± 16</td>
</tr>
<tr>
<td>Temporal hemisphere</td>
<td>210 ± 36</td>
<td>254 ± 36</td>
<td>287 ± 38</td>
<td>302 ± 47</td>
<td>260 ± 10</td>
</tr>
<tr>
<td>Total brain</td>
<td>242 ± 41</td>
<td>309 ± 37</td>
<td>334 ± 42*</td>
<td>388 ± 55*</td>
<td>336 ± 23*</td>
</tr>
<tr>
<td>Total heart</td>
<td>480 ± 113</td>
<td>831 ± 45*</td>
<td>960 ± 73*</td>
<td>1195 ± 126†</td>
<td>935 ± 81*</td>
</tr>
<tr>
<td>Lungs</td>
<td>64 ± 20</td>
<td>50 ± 7</td>
<td>48 ± 10</td>
<td>56 ± 12</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>Nuchal muscle</td>
<td>19 ± 3</td>
<td>17 ± 3</td>
<td>24 ± 6</td>
<td>20 ± 5</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Forelimb muscle</td>
<td>9 ± 2</td>
<td>14 ± 3</td>
<td>14 ± 2</td>
<td>13 ± 3</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>Upper muscle</td>
<td>14 ± 2</td>
<td>16 ± 3</td>
<td>19 ± 4</td>
<td>17 ± 4</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>Upper skin</td>
<td>24 ± 4</td>
<td>14 ± 4*</td>
<td>16 ± 4*</td>
<td>13 ± 5*</td>
<td>6 ± 1‡</td>
</tr>
<tr>
<td>Upper bone</td>
<td>31 ± 10</td>
<td>23 ± 8</td>
<td>22 ± 8</td>
<td>17 ± 7*</td>
<td>4 ± 1§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.
* P <0.05 vs pre labor, † P <0.05 vs pre and early labor, ‡ P <0.05 vs pre, early and mid labor,
§ P <0.05 vs pre, early, mid and late labor, ** P <0.05 vs late labor.
n = 6 for pre, early, mid and late labor measurements, and n = 3 for delivery measurements.
Table 34. Fetal lower body organ/tissue blood flows before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm</td>
<td>26 ± 10</td>
<td>27 ± 3</td>
<td>34 ± 8</td>
<td>46 ± 7†</td>
<td>54 ± 10†</td>
</tr>
<tr>
<td>Stomach</td>
<td>58 ± 8</td>
<td>79 ± 14</td>
<td>79 ± 11</td>
<td>99 ± 18*</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>Small intestine</td>
<td>159 ± 21</td>
<td>209 ± 32</td>
<td>237 ± 30*</td>
<td>282 ± 31*</td>
<td>203 ± 46**</td>
</tr>
<tr>
<td>Large intestine</td>
<td>94 ± 15</td>
<td>143 ± 21</td>
<td>156 ± 19*</td>
<td>190 ± 21*</td>
<td>164 ± 33*</td>
</tr>
<tr>
<td>Liver</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 2</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>42 ± 6</td>
<td>102 ± 17*</td>
<td>91 ± 10*</td>
<td>110 ± 10*</td>
<td>117 ± 42*</td>
</tr>
<tr>
<td>Spleen</td>
<td>360 ± 110</td>
<td>263 ± 55</td>
<td>273 ± 30</td>
<td>94 ± 15‡</td>
<td>69 ± 11‡</td>
</tr>
<tr>
<td>Adrenals</td>
<td>371 ± 63</td>
<td>565 ± 54</td>
<td>638 ± 62*</td>
<td>955 ± 146‡</td>
<td>714 ± 87*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>234 ± 33</td>
<td>298 ± 42</td>
<td>311 ± 45</td>
<td>308 ± 45</td>
<td>128 ± 37§</td>
</tr>
<tr>
<td>Brown fat</td>
<td>70 ± 13</td>
<td>117 ± 23</td>
<td>123 ± 21*</td>
<td>134 ± 22*</td>
<td>56 ± 37‡†</td>
</tr>
<tr>
<td>Lower skin</td>
<td>22 ± 3</td>
<td>26 ± 8</td>
<td>23 ± 4</td>
<td>21 ± 4</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Lower bone</td>
<td>20 ± 3</td>
<td>28 ± 11</td>
<td>22 ± 5</td>
<td>18 ± 5</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Lower muscle</td>
<td>8 ± 2</td>
<td>19 ± 3</td>
<td>20 ± 2</td>
<td>15 ± 4</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>Total skin</td>
<td>22 ± 3</td>
<td>24 ± 8</td>
<td>22 ± 5</td>
<td>20 ± 5</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Total bone</td>
<td>22 ± 4</td>
<td>28 ± 11</td>
<td>22 ± 5</td>
<td>18 ± 5</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Total muscle</td>
<td>12 ± 2</td>
<td>17 ± 3</td>
<td>19 ± 2</td>
<td>16 ± 4</td>
<td>15 ± 5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.
* P <0.05 vs pre labor, † P <0.05 vs pre and early labor, ‡ P <0.05 vs pre, early and mid labor,
§ P <0.05 vs pre, early, mid and late labor, ** P <0.05 vs late labor, †† P <0.05 vs mid and late labor.
n = 6 for pre, early, mid and late labor measurements, and n = 3 for delivery measurements.
Table 35. Fetal upper body organ/tissue blood flows before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>122 ± 14</td>
<td>164 ± 21</td>
<td>168 ± 24</td>
<td>185 ± 20*</td>
<td>285 ± 34§</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>123 ± 10</td>
<td>147 ± 16</td>
<td>161 ± 21</td>
<td>184 ± 15*</td>
<td>272 ± 27§</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>268 ± 22</td>
<td>301 ± 35</td>
<td>313 ± 51</td>
<td>391 ± 73*</td>
<td>453 ± 57‡</td>
</tr>
<tr>
<td>Medulla</td>
<td>320 ± 30</td>
<td>343 ± 38</td>
<td>367 ± 63</td>
<td>409 ± 61</td>
<td>554 ± 65§</td>
</tr>
<tr>
<td>Pons</td>
<td>394 ± 40</td>
<td>395 ± 31</td>
<td>341 ± 61</td>
<td>411 ± 44</td>
<td>493 ± 53‡</td>
</tr>
<tr>
<td>Midbrain</td>
<td>368 ± 37</td>
<td>371 ± 27</td>
<td>332 ± 58</td>
<td>399 ± 48</td>
<td>452 ± 56</td>
</tr>
<tr>
<td>Frontal hemisphere</td>
<td>228 ± 18</td>
<td>240 ± 20</td>
<td>246 ± 47</td>
<td>256 ± 36</td>
<td>302 ± 34</td>
</tr>
<tr>
<td>Temporal hemisphere</td>
<td>217 ± 22</td>
<td>247 ± 25</td>
<td>231 ± 33</td>
<td>246 ± 38</td>
<td>275 ± 28</td>
</tr>
<tr>
<td>Total brain</td>
<td>265 ± 21</td>
<td>278 ± 19</td>
<td>267 ± 41</td>
<td>302 ± 33</td>
<td>373 ± 38‡</td>
</tr>
<tr>
<td>Total heart</td>
<td>304 ± 48</td>
<td>402 ± 34</td>
<td>506 ± 57*</td>
<td>614 ± 67†</td>
<td>710 ± 58‡</td>
</tr>
<tr>
<td>Lungs</td>
<td>83 ± 14</td>
<td>99 ± 20</td>
<td>80 ± 8</td>
<td>80 ± 13</td>
<td>48 ± 4§</td>
</tr>
<tr>
<td>Nuchal muscle</td>
<td>16 ± 5</td>
<td>20 ± 9</td>
<td>21 ± 9</td>
<td>14 ± 4</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Forelimb muscle</td>
<td>17 ± 5</td>
<td>14 ± 4</td>
<td>15 ± 4</td>
<td>13 ± 4</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Upper muscle</td>
<td>16 ± 5</td>
<td>17 ± 7</td>
<td>18 ± 6</td>
<td>14 ± 4</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Upper skin</td>
<td>29 ± 4</td>
<td>21 ± 3*</td>
<td>19 ± 3*</td>
<td>15 ± 2†</td>
<td>10 ± 1§</td>
</tr>
<tr>
<td>Upper bone</td>
<td>22 ± 3</td>
<td>21 ± 2</td>
<td>18 ± 1</td>
<td>17 ± 2*</td>
<td>13 ± 2§</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.
* P < 0.05 vs pre labor, † P < 0.05 vs pre and early labor, ‡ P < 0.05 vs pre, early and mid labor, § P < 0.05 vs pre, early, mid and late labor. n = 6 for pre, early, mid, late labor and delivery measurements.
Table 36. Fetal lower body organ/tissue blood flows before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm</td>
<td>19 ± 4</td>
<td>12 ± 1</td>
<td>14 ± 2</td>
<td>17 ± 1</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Stomach</td>
<td>78 ± 13</td>
<td>76 ± 6</td>
<td>76 ± 7</td>
<td>82 ± 10</td>
<td>73 ± 17</td>
</tr>
<tr>
<td>Small intestine</td>
<td>114 ± 9</td>
<td>109 ± 12</td>
<td>117 ± 11</td>
<td>114 ± 12</td>
<td>113 ± 17</td>
</tr>
<tr>
<td>Large intestine</td>
<td>73 ± 9</td>
<td>67 ± 8</td>
<td>88 ± 13</td>
<td>90 ± 15</td>
<td>92 ± 22</td>
</tr>
<tr>
<td>Liver</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>51 ± 9</td>
<td>62 ± 9</td>
<td>61 ± 10</td>
<td>47 ± 13</td>
<td>74 ± 24</td>
</tr>
<tr>
<td>Spleen</td>
<td>288 ± 45</td>
<td>204 ± 27*</td>
<td>154 ± 24*</td>
<td>127 ± 15†</td>
<td>133 ± 14†</td>
</tr>
<tr>
<td>Adrenals</td>
<td>400 ± 48</td>
<td>506 ± 53</td>
<td>614 ± 144</td>
<td>755 ± 133†</td>
<td>760 ± 116†</td>
</tr>
<tr>
<td>Kidneys</td>
<td>236 ± 22</td>
<td>238 ± 18</td>
<td>291 ± 46</td>
<td>300 ± 52</td>
<td>284 ± 67</td>
</tr>
<tr>
<td>Brown fat</td>
<td>108 ± 40</td>
<td>95 ± 13</td>
<td>114 ± 35</td>
<td>84 ± 16</td>
<td>79 ± 26</td>
</tr>
<tr>
<td>Lower skin</td>
<td>28 ± 3</td>
<td>22 ± 3</td>
<td>21 ± 3</td>
<td>22 ± 5</td>
<td>16 ± 3*</td>
</tr>
<tr>
<td>Lower bone</td>
<td>25 ± 3</td>
<td>20 ± 2</td>
<td>21 ± 3</td>
<td>23 ± 5</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Lower muscle</td>
<td>12 ± 3</td>
<td>10 ± 3</td>
<td>9 ± 2</td>
<td>8 ± 1</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Total skin</td>
<td>28 ± 3</td>
<td>21 ± 3*</td>
<td>20 ± 2*</td>
<td>19 ± 3*</td>
<td>13 ± 2‡</td>
</tr>
<tr>
<td>Total bone</td>
<td>23 ± 3</td>
<td>20 ± 2</td>
<td>19 ± 2</td>
<td>20 ± 4</td>
<td>16 ± 3*</td>
</tr>
<tr>
<td>Total muscle</td>
<td>14 ± 4</td>
<td>13 ± 4</td>
<td>14 ± 3</td>
<td>11 ± 2</td>
<td>13 ± 2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.
* P < 0.05 vs pre labor, † P < 0.05 vs pre and early labor, ‡ P < 0.05 vs pre, early, mid and late labor.
n = 6 for pre, early, mid, late labor and delivery measurements.
but it was decreased significantly at delivery. Although blood flow to the lung, total skin and bone decreased toward the end of labor, the changes did not reach statistical significance. There was no significant alteration of blood flow to the liver (hepatic arterial flow only) and to the skeletal muscles samples (Tables 33 and 34).

In Group II fetuses, there were increases in blood flow to the brain, heart and adrenals, but these were less than in Group I (Tables 35 and 36). The time course of the changes was also later than that in Group I, and there was no terminal fall. Thus overall brain blood flow did not change significantly until delivery. The percentage change from the control in total brain blood flow at delivery was about the same as that at early-labor in Group I (52 ± 30 vs. 50 ± 32%). Total heart blood flow increased by 95 ± 39% at mid-labor and reached its highest at delivery with a rise of 172 ± 52% above the pre-labor estimate. Similarly, adrenal blood flow significantly increased by 60 ± 15% in late labor and by 63 ± 32% at delivery. Blood flow to the diaphragm, stomach, small intestine, large intestine, liver, gall bladder, kidneys, brown fat and muscle was not altered throughout labor and delivery. However, there was a significant fall in blood flow to the spleen. In addition, blood flow to the lungs, skin and bone showed a significant decrease during labor and/or at delivery (Tables 35 and 36).

Blood flow was determined in different parts of the heart including the right ventricle, left ventricle and interventricular septum. During labor and delivery, weight normalized blood flow (ml/min/100g) was highest in the right ventricle in both groups. However, blood flow to each region of the heart was higher in Group I than that in Group II at each stage of labor (Table 37).
## Table 37. Total and regional myocardial blood flow in Group I and II before and during labor and delivery.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>569 ± 156</td>
<td>981 ± 84*</td>
<td>1109 ± 120*</td>
<td>1395 ± 210†</td>
<td>1153 ± 125*</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>386 ± 92</td>
<td>643 ± 52*</td>
<td>816 ± 121*</td>
<td>1012 ± 164†</td>
<td>756 ± 168*</td>
</tr>
<tr>
<td>Ventricles</td>
<td>478 ± 122</td>
<td>812 ± 52*</td>
<td>962 ± 114*</td>
<td>1203 ± 176†</td>
<td>955 ± 141*</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td>542 ± 138</td>
<td>944 ± 71*</td>
<td>1081 ± 109*</td>
<td>1317 ± 136†</td>
<td>1020 ± 20*</td>
</tr>
<tr>
<td>Total heart</td>
<td>480 ± 113</td>
<td>831 ± 45*</td>
<td>960 ± 73*</td>
<td>1195 ± 126†</td>
<td>935 ± 81*</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>410 ± 85</td>
<td>531 ± 53</td>
<td>647 ± 46*</td>
<td>847 ± 110‡</td>
<td>960 ± 86‡</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>309 ± 59</td>
<td>385 ± 37</td>
<td>470 ± 45*</td>
<td>607 ± 90‡</td>
<td>669 ± 34‡</td>
</tr>
<tr>
<td>Ventricles</td>
<td>359 ± 72</td>
<td>458 ± 45</td>
<td>558 ± 43*</td>
<td>727 ± 98‡</td>
<td>815 ± 57‡</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td>288 ± 53</td>
<td>401 ± 53</td>
<td>523 ± 89*</td>
<td>593 ± 86‡</td>
<td>721 ± 101‡</td>
</tr>
<tr>
<td>Total heart</td>
<td>304 ± 48</td>
<td>402 ± 34</td>
<td>506 ± 57*</td>
<td>614 ± 67‡</td>
<td>710 ± 58‡</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Flow = ml/min/100g.
* P <0.05 vs pre labor, † P <0.05 vs pre and early labor, ‡ P <0.05 vs pre, early and mid labor.
For Group I, n = 6 for pre, early, mid and late labor measurements, and n = 3 for delivery measurements.
For Group II, n = 6 for pre, early, mid, late labor and delivery measurements.
Figure 34 and 35 show the relationship of the fetal CA $O_2$ content to brain blood flow and to heart blood flow at pre-labor stage and throughout labor to delivery in both groups. Figure 36 illustrates the relationship between the fetal FA $O_2$ content and the adrenal blood flow at pre-labor and in the course of labor and delivery. In all these 3 organs, blood flow increased in inverse relation to arterial $O_2$ content. There were significant negative correlations between the $O_2$ content and blood flow to the brain ($r = -0.449, P < 0.001$), heart ($r = -0.743, P < 0.001$) and adrenals ($r = -0.711, P < 0.001$).

3.6.6 Fetal Combined Ventricular Output and Its Distribution

Table 38 gives the estimates of fetal combined cardiac output and blood flow to the fetal body and placenta, expressed as both weight-normalized (ml/min/kg of fetal weight) and absolute values (ml/min) for Group I. Table 39 lists the distribution of fetal cardiac output to individual organs and tissues as well as to the fetal body and placenta, expressed as percentage of combined ventricular output (%) for Group I fetuses. The corresponding values for Group II fetuses are given in Table 40 and 41.

In Group I, the observed increase in perfusion to the majority of fetal organs and tissues appears to be the result of an increase in fetal combined ventricular output during the course of labor (Table 38). The increase in absolute fetal cardiac output during labor was significantly different from the pre-labor value, with an increase of $36 \pm 23\%$ in early labor, $40 \pm 17\%$ in mid labor and $50 \pm 22\%$ in late labor. A similar pattern was observed with systemic and
Figure 34. Relationship between fetal CA oxygen content and blood flow to the brain from pre-labor stage throughout labor to delivery. The logarithmic function is shown with an equation: \( y = 395 - 118 \ln(x) \), \( r = -0.449, P < 0.001 \). Group I fetuses, (○); Group II fetuses, (●).

Figure 35. Relationship between fetal CA oxygen content and blood flow to the heart from pre-labor stage throughout labor to delivery. The exponential function is shown with an equation: \( y = 1784e^{0.50x} \), \( r = -0.743, P < 0.001 \). Group I fetuses, (○); Group II fetuses, (●).
umbilical blood flow, although the latter change did not reach statistical significance. However, at delivery, fetal cardiac output and blood flow to the fetal body and placenta returned to values similar to the pre-labor estimates.

In Group I, there was a redistribution of cardiac output with the progress of labor (Table 39). The percentage of combined ventricular output distributed to the brain, heart, adrenals, diaphragm, stomach, small intestine, large intestine and gall bladder was significantly increased. In contrast, the fraction of cardiac output to the skin, bone, spleen was significantly reduced. Toward the end of labor, there was also a decrease in cardiac output distribution to the lung, but this did not reach statistical significance. The proportion of fetal combined cardiac output
Table 38. Fetal combined cardiac output, systemic and umbilical blood flows before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined ventricular output</td>
<td>372 ± 47</td>
<td>453 ± 58</td>
<td>470 ± 42</td>
<td>495 ± 52</td>
<td>350 ± 47</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic blood flow</td>
<td>253 ± 39</td>
<td>326 ± 44</td>
<td>337 ± 33</td>
<td>339 ± 41</td>
<td>246 ± 43</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>119 ± 13</td>
<td>128 ± 16</td>
<td>133 ± 13</td>
<td>157 ± 14</td>
<td>104 ± 18</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. combined cardiac output</td>
<td>956 ± 172</td>
<td>1173 ± 149</td>
<td>1243 ± 151*</td>
<td>1311 ± 173*</td>
<td>955 ± 120†</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. systemic blood flow</td>
<td>654 ± 132</td>
<td>846 ± 119</td>
<td>894 ± 122*</td>
<td>903 ± 137*</td>
<td>675 ± 128†</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. umbilical blood flow</td>
<td>302 ± 48</td>
<td>327 ± 35</td>
<td>348 ± 36</td>
<td>409 ± 40</td>
<td>280 ± 33</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Abs. = absolute values.
* P <0.05 vs pre labor.
† P <0.05 vs early, mid and late labor.
n = 6 for pre, early, mid and late labor measurements, and n = 3 for delivery measurements.
Table 39. The % distribution of fetal combined cardiac output before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic blood flow</td>
<td>66.88 ± 3.14</td>
<td>71.49 ± 1.64</td>
<td>71.47 ± 1.64</td>
<td>67.70 ± 2.11</td>
<td>69.47 ± 5.73</td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>33.12 ± 3.14</td>
<td>28.51 ± 1.64</td>
<td>28.53 ± 1.64</td>
<td>32.30 ± 2.11</td>
<td>30.53 ± 5.73</td>
</tr>
<tr>
<td>Brain</td>
<td>9.77 ± 0.97</td>
<td>11.22 ± 1.72</td>
<td>11.12 ± 1.64</td>
<td>12.03 ± 1.56*</td>
<td>17.39 ± 2.40$</td>
</tr>
<tr>
<td>Heart</td>
<td>6.92 ± 2.31</td>
<td>9.81 ± 2.30*</td>
<td>10.74 ± 2.80*</td>
<td>12.35 ± 3.09†</td>
<td>12.91 ± 4.65†</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.17 ± 1.10</td>
<td>3.44 ± 1.71</td>
<td>2.38 ± 0.74</td>
<td>2.68 ± 0.78</td>
<td>1.14 ± 0.23</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.11 ± 0.02</td>
<td>0.23 ± 0.09§</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.44 ± 0.18</td>
<td>1.55 ± 0.25</td>
<td>1.47 ± 0.19</td>
<td>1.75 ± 0.30*</td>
<td>2.38 ± 0.50‡</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3.76 ± 0.13</td>
<td>4.08 ± 0.40</td>
<td>4.45 ± 0.41</td>
<td>5.08 ± 0.33†</td>
<td>5.38 ± 1.26†</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.72 ± 0.05</td>
<td>0.94 ± 0.11*</td>
<td>0.96 ± 0.08*</td>
<td>1.13 ± 0.09‡</td>
<td>1.37 ± 0.17§</td>
</tr>
<tr>
<td>Liver</td>
<td>0.20 ± 0.07</td>
<td>0.19 ± 0.07</td>
<td>0.25 ± 0.12</td>
<td>0.22 ± 0.08</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>0.02 ± 0.00</td>
<td>0.03 ± 0.00*</td>
<td>0.03 ± 0.00*</td>
<td>0.03 ± 0.00*</td>
<td>0.06 ± 0.02§</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.58 ± 0.43</td>
<td>1.02 ± 0.30</td>
<td>0.97 ± 0.09</td>
<td>0.32 ± 0.05†</td>
<td>0.43 ± 0.07†</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.30 ± 0.05</td>
<td>0.37 ± 0.04</td>
<td>0.38 ± 0.04</td>
<td>0.60 ± 0.16‡</td>
<td>0.67 ± 0.18‡</td>
</tr>
<tr>
<td>Kidneys</td>
<td>5.61 ± 0.49</td>
<td>5.58 ± 0.34</td>
<td>5.61 ± 0.68</td>
<td>5.28 ± 0.57</td>
<td>2.86 ± 0.53§</td>
</tr>
<tr>
<td>Brown fat</td>
<td>1.01 ± 0.16</td>
<td>1.31 ± 0.16</td>
<td>1.36 ± 0.21</td>
<td>1.39 ± 0.21</td>
<td>0.76 ± 0.36</td>
</tr>
<tr>
<td>Skin</td>
<td>9.56 ± 1.93</td>
<td>7.78 ± 1.45</td>
<td>7.18 ± 1.29</td>
<td>5.91 ± 1.10*</td>
<td>4.03 ± 0.85†</td>
</tr>
<tr>
<td>Bones</td>
<td>9.03 ± 1.48</td>
<td>7.94 ± 2.03</td>
<td>6.62 ± 1.26</td>
<td>5.05 ± 1.13*</td>
<td>2.61 ± 0.69‡</td>
</tr>
<tr>
<td>Muscles</td>
<td>14.21 ± 1.93</td>
<td>16.65 ± 1.68</td>
<td>18.21 ± 1.66</td>
<td>14.14 ± 2.98*</td>
<td>17.20 ± 3.90</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P < 0.05 vs pre labor, † P < 0.05 vs pre and early labor, ‡ P < 0.05 vs pre, early and mid labor, § P < 0.05 vs pre, early, mid and late labor.

n = 6 for pre, early, mid and late labor measurements (except for lung and liver where n = 5) and n = 3 for delivery measurements.
Table 40. Fetal combined cardiac output, systemic and umbilical blood flows before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined ventricular output</td>
<td>495 ± 42</td>
<td>454 ± 27</td>
<td>448 ± 30</td>
<td>446 ± 20</td>
<td>423 ± 28</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic blood flow</td>
<td>300 ± 28</td>
<td>284 ± 18</td>
<td>287 ± 19</td>
<td>287 ± 22</td>
<td>289 ± 23</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>194 ± 19</td>
<td>170 ± 16</td>
<td>162 ± 18</td>
<td>159 ± 3</td>
<td>134 ± 8*</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. combined cardiac output</td>
<td>1785 ± 216</td>
<td>1743 ± 172</td>
<td>1755 ± 186</td>
<td>1751 ± 177</td>
<td>1673 ± 188</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. systemic blood flow</td>
<td>1076 ± 123</td>
<td>1091 ± 106</td>
<td>1124 ± 117</td>
<td>1131 ± 140</td>
<td>1146 ± 139</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs. umbilical blood flow</td>
<td>708 ± 106</td>
<td>652 ± 86</td>
<td>631 ± 87</td>
<td>620 ± 51</td>
<td>527 ± 53</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Abs. = absolute values.

*  P <0.05 vs pre labor.

n = 6 for pre, early, mid, late labor and delivery measurements.
Table 41. The % distribution of fetal combined cardiac output before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic blood flow</td>
<td>60.84 ± 2.17</td>
<td>62.75 ± 2.45</td>
<td>64.23 ± 2.60</td>
<td>63.98 ± 1.99</td>
<td>67.97 ± 1.62</td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>39.16 ± 2.17</td>
<td>37.25 ± 2.45</td>
<td>35.77 ± 2.60</td>
<td>36.02 ± 1.99</td>
<td>32.03 ± 1.62</td>
</tr>
<tr>
<td>Brain</td>
<td>9.27 ± 0.94</td>
<td>9.68 ± 0.46</td>
<td>9.13 ± 1.22</td>
<td>10.48 ± 0.87</td>
<td>13.59 ± 1.03$</td>
</tr>
<tr>
<td>Heart</td>
<td>5.86 ± 0.80</td>
<td>8.03 ± 0.92*</td>
<td>9.99 ± 1.21†</td>
<td>11.85 ± 0.55†</td>
<td>14.69 ± 1.01§</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.69 ± 0.86</td>
<td>5.79 ± 1.31</td>
<td>4.83 ± 1.01</td>
<td>4.50 ± 0.73</td>
<td>2.91 ± 0.43‡</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.09 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.70 ± 0.26</td>
<td>1.83 ± 0.29</td>
<td>1.86 ± 0.34</td>
<td>1.86 ± 0.27</td>
<td>1.83 ± 0.40</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3.03 ± 0.73</td>
<td>3.03 ± 0.64</td>
<td>3.21 ± 0.54</td>
<td>3.15 ± 0.55</td>
<td>3.22 ± 0.54</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.69 ± 0.25</td>
<td>0.67 ± 0.23</td>
<td>0.80 ± 0.18</td>
<td>0.79 ± 0.15</td>
<td>0.84 ± 0.19</td>
</tr>
<tr>
<td>Liver</td>
<td>0.27 ± 0.06</td>
<td>0.28 ± 0.08</td>
<td>0.22 ± 0.04</td>
<td>0.17 ± 0.03</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.01*</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.21 ± 0.28</td>
<td>0.79 ± 0.04*</td>
<td>0.63 ± 0.09*</td>
<td>0.50 ± 0.04*</td>
<td>0.57 ± 0.07*</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.16 ± 0.01</td>
<td>0.22 ± 0.03</td>
<td>0.26 ± 0.05*</td>
<td>0.32 ± 0.06†</td>
<td>0.33 ± 0.04†</td>
</tr>
<tr>
<td>Kidneys</td>
<td>4.81 ± 0.70</td>
<td>4.93 ± 0.68</td>
<td>5.79 ± 0.77</td>
<td>5.79 ± 0.48</td>
<td>5.72 ± 0.83</td>
</tr>
<tr>
<td>Brown fat</td>
<td>0.97 ± 0.28</td>
<td>0.95 ± 0.09</td>
<td>1.19 ± 0.42</td>
<td>0.82 ± 0.13</td>
<td>0.85 ± 0.25</td>
</tr>
<tr>
<td>Skin</td>
<td>8.73 ± 0.88</td>
<td>7.22 ± 0.96*</td>
<td>6.76 ± 0.68*</td>
<td>6.35 ± 1.04*</td>
<td>4.87 ± 0.68§</td>
</tr>
<tr>
<td>Bone</td>
<td>7.19 ± 0.59</td>
<td>6.96 ± 0.41</td>
<td>6.47 ± 0.43</td>
<td>6.75 ± 1.13</td>
<td>5.46 ± 0.85</td>
</tr>
<tr>
<td>Muscle</td>
<td>12.06 ± 2.95</td>
<td>12.20 ± 2.78</td>
<td>12.93 ± 2.06</td>
<td>10.47 ± 1.74</td>
<td>12.64 ± 1.77</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

* P < 0.05 vs pre labor, † P < 0.05 vs pre and early labor, ‡ P < 0.05 vs pre, early and mid labor, § P < 0.05 vs pre, early, mid and late labor. n = 6 for pre, early, mid, late labor and delivery measurements.
directed to the liver and muscle was not changed. Although distribution of fetal cardiac output
to the kidney and brown fat was not significantly altered during labor, the proportion was
reduced at delivery. The percentage of the cardiac output distributed to the fetal body and to
the placenta did not change significantly during labor and delivery.

In Group II animals, fetal combined ventricular output and blood flow to the fetal body
appeared to be maintained during labor (Table 40). In contrast, umbilical blood flow tended to
decline progressively during labor, but a significant fall in placental flow occurred only at
delivery, with a 26 ± 9% decline from the pre-labor value. The significant fall in the umbilical
placental blood flow accounted for the slight reduction in combined ventricular output at
delivery, although the latter change was not statistically significant.

The change in fetal cardiac output distribution in the Group II fetuses was also different from
that observed in Group I (Table 41). Associated with the fall in umbilical blood flow, the
proportion of fetal combined cardiac output directed to the placenta tended to decrease,
whereas the proportion to the fetal body tended to increase. The proportion of cardiac output
distributed to the most of lower body organs and tissues was maintained, including the
diaphragm, stomach, small intestine, large intestine, liver, kidneys and brown fat. A similar
result was observed in muscle. In contrast, the proportion of combined ventricular output
distributed to the spleen, skin, bone and lungs was reduced, whereas the percentage distributed
to the brain, heart and adrenal increased. However, the magnitude of this increase was less
than in Group I, since the percentage of cardiac output distributed to these organs remained
higher in Group I than in Group II throughout labor. In contrast, the proportion of cardiac
output distributed to the placenta was lower at each stage of labor in Group I than in Group II.

### 3.6.7 Fetal Oxygen Delivery and Consumption

Fetal oxygen delivery, consumption and extraction before and during labor and at delivery are presented in Tables 42 and 43. In Group I animals (Table 42), as described above, umbilical blood flow was increased during labor, but this was not sufficient to compensate for the significant fall in umbilical venous oxygen content. As a result, fetal oxygen delivery was significantly decreased as labor progressed. Fetal oxygen consumption was maintained until delivery, as a result of a moderate increase in both fetal oxygen extraction and umbilical blood flow. However, fetal oxygen consumption was not maintained at delivery, dropping by 40 ± 9% from the control value. This was accompanied by a significant decrease in the umbilical venous-arterial oxygen content difference (Cvo2-Cao2). In this group of fetuses, there was only a minimal change in oxygen extraction during labor and at delivery. When O2 delivery was reduced by 25 ± 11% in early labor, oxygen extraction increased only from 46.4 ± 5.1 to 52.5 ± 3.6%. When O2 delivery was reduced by 39 ± 17% at delivery, fetal O2 extraction was essentially unchanged (51.1 ± 7.5%).

In the Group II fetuses (Table 43), because of the decrease in both umbilical venous O2 content (Cvo2) and umbilical blood flow, fetal O2 delivery was also significantly reduced. It fell by 24 ± 10% in early labor, 36 ± 12% in late labor and 50 ± 8% at delivery. However, throughout labor fetal O2 delivery in Group II was substantially higher than in Group I. Fetal O2 consumption through the labor process, as with fetal O2 delivery, was higher than in Group I.
Table 42. Fetal oxygen delivery, extraction and consumption before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen delivery (μmol/min/kg)</td>
<td>476 ± 61</td>
<td>334 ± 30*</td>
<td>361 ± 49*</td>
<td>370 ± 29*</td>
<td>272 ± 54*†</td>
</tr>
<tr>
<td>Oxygen Consumption (μmol/min/kg)</td>
<td>213 ± 20</td>
<td>175 ± 18</td>
<td>204 ± 23</td>
<td>202 ± 26</td>
<td>129 ± 16§</td>
</tr>
<tr>
<td>Oxygen extraction (%)</td>
<td>46.4 ± 5.1</td>
<td>52.5 ± 3.6</td>
<td>57.2 ± 3.7</td>
<td>54.6 ± 6.0</td>
<td>51.1 ± 7.5</td>
</tr>
<tr>
<td>Cvo2-Cao2 (mmol/L)</td>
<td>1.81 ± 0.15</td>
<td>1.58 ± 0.18</td>
<td>1.65 ± 0.14</td>
<td>1.37 ± 0.12*</td>
<td>1.27 ± 0.18‡</td>
</tr>
<tr>
<td>Cvo2 (mmol/L)</td>
<td>3.97 ± 0.25</td>
<td>3.00 ± 0.26*</td>
<td>2.90 ± 0.24*</td>
<td>2.55 ± 0.13*</td>
<td>2.50 ± 0.10*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre labor.
† P <0.05 vs late labor.
‡ P <0.05 vs pre, early and mid labor.
§ P <0.05 vs pre, early, mid and late labor.

n = 5 for pre, early, mid and late labor measurements, and n = 4 for delivery measurements. Except for oxygen delivery and consumption where n = 3 for delivery measurements.
Table 43. Fetal oxygen delivery, extraction and consumption before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen delivery (µmol/min/kg)</td>
<td>1128 ± 178</td>
<td>828 ± 150*</td>
<td>704 ± 125*</td>
<td>664 ± 46*</td>
<td>536 ± 86†</td>
</tr>
<tr>
<td>Oxygen Consumption (µmol/min/kg)</td>
<td>513 ± 86</td>
<td>457 ± 81</td>
<td>444 ± 96</td>
<td>455 ± 31</td>
<td>396 ± 70</td>
</tr>
<tr>
<td>Oxygen extraction (%)</td>
<td>45.2 ± 2.4</td>
<td>55.4 ± 1.5*</td>
<td>61.7 ± 2.6†</td>
<td>68.7 ± 1.5‡</td>
<td>73.3 ± 1.6‡</td>
</tr>
<tr>
<td>Cvo2-Cao2 (mmol/L)</td>
<td>2.50 ± 0.17</td>
<td>2.79 ± 0.22</td>
<td>2.76 ± 0.21</td>
<td>2.83 ± 0.22</td>
<td>3.04 ± 0.41</td>
</tr>
<tr>
<td>Cvo2 (mmol/L)</td>
<td>5.59 ± 0.48</td>
<td>5.03 ± 0.37</td>
<td>4.47 ± 0.25*</td>
<td>4.13 ± 0.35†</td>
<td>4.12 ± 0.48†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P < 0.05 vs pre labor.
† P < 0.05 vs pre and early labor.
‡ P < 0.05 vs pre, early and mid labor.
n = 4 for pre, early, mid, late labor and delivery measurements.
The maintenance of consumption was accomplished by a marked increase in fetal oxygen extraction, which rose significantly from 45.2 ± 2.4% at the pre-labor stage to 68.7 ± 1.5% in late labor. There was a slight increase in Cvo2-Cao2 over the pre-labor value throughout labor, although this was not significant. At delivery, fetal O₂ consumption fell, but this was not statistically different.

3.6.8 Umbilical Glucose and Lactate Fluxes

Umbilical glucose and lactate delivery, extraction and uptake, the umbilical venous-descending aorta lactate and glucose concentration difference as well as the lactate/oxygen quotient and glucose/oxygen quotient (lactate/O₂ and glucose/O₂) before and during labor and delivery are presented in Tables 44 and 45. In the Group I animals (Table 44), fetal glucose delivery was progressively increased, becoming significant in late labor. In contrast, the umbilical extraction of glucose was decreased with the progress of labor, but this did not reach statistical significance. As a result, umbilical glucose uptake was maintained during labor and at delivery. There was no significant change in the umbilical venous-descending aorta glucose concentration difference during labor.

A similar trend for an increase in glucose delivery to the fetus during labor was also observed in the Group II animals (Table 45), whereas the umbilical extraction of glucose was essentially unchanged. Umbilical glucose uptake was also maintained during labor. Although the increase in fetal glucose delivery was observed in both groups, the values were much lower in Group I than Group II at each sample time (Tables 44 and 45). With the progress of labor, glucose
Table 44. Umbilical glucose and lactate delivery, extraction, and uptake before and during labor and delivery (Group I).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (μmol/min/kg)</td>
<td>229 ± 80</td>
<td>594 ± 39</td>
<td>1060 ± 146*</td>
<td>1953 ± 237‡</td>
<td>1862 ± 533‡</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>7.40 ± 3.15</td>
<td>4.60 ± 1.85</td>
<td>5.28 ± 1.86</td>
<td>5.56 ± 1.84</td>
<td>2.48 ± 3.12</td>
</tr>
<tr>
<td>Uptake (μmol/min/kg)</td>
<td>12 ± 6</td>
<td>27 ± 11</td>
<td>60 ± 29†</td>
<td>106 ± 35‡</td>
<td>25 ± 44§</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.11 ± 0.05</td>
<td>0.22 ± 0.08</td>
<td>0.46 ± 0.19</td>
<td>0.68 ± 0.17†</td>
<td>0.23 ± 0.40§</td>
</tr>
<tr>
<td>Lactate / O2</td>
<td>0.16 ± 0.07</td>
<td>0.49 ± 0.21</td>
<td>0.91 ± 0.44</td>
<td>1.53 ± 0.36†</td>
<td>0.50 ± 0.79§</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (μmol/min/kg)</td>
<td>71 ± 12</td>
<td>78 ± 13</td>
<td>116 ± 28</td>
<td>154 ± 36†</td>
<td>132 ± 27</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>24.95 ± 4.92</td>
<td>1.51 ± 11.13</td>
<td>13.09 ± 3.48</td>
<td>10.75 ± 1.93</td>
<td>15.54 ± 4.51</td>
</tr>
<tr>
<td>Uptake (μmol/min/kg)</td>
<td>16 ± 3</td>
<td>3 ± 9</td>
<td>13 ± 4</td>
<td>15 ± 4</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.14 ± 0.02</td>
<td>0.03 ± 0.07</td>
<td>0.10 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Glucose / O2</td>
<td>0.46 ± 0.07</td>
<td>0.06 ± 0.32</td>
<td>0.36 ± 0.09</td>
<td>0.44 ± 0.07</td>
<td>0.66 ± 0.14</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

*  P < 0.05 vs pre labor.
†  P < 0.05 vs pre and early labor.
‡  P < 0.05 vs pre, early and mid labor.
§  P < 0.05 vs late labor.

n = 5 for pre, early, mid and late labor measurements, and n = 4 for delivery measurements.
Except for lactate & glucose delivery and uptake where n = 3 for delivery measurements.
Table 45. Umbilical glucose and lactate delivery, extraction, and uptake before and during labor and delivery (Group II).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (µmol/min/kg)</td>
<td>253 ± 45</td>
<td>255 ± 48</td>
<td>390 ± 86</td>
<td>656 ± 96*</td>
<td>754 ± 87*</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>14.79 ± 1.64</td>
<td>13.18 ± 1.36</td>
<td>12.34 ± 2.26</td>
<td>8.16 ± 2.83</td>
<td>10.40 ± 3.05</td>
</tr>
<tr>
<td>Uptake (µmol/min/kg)</td>
<td>36 ± 6</td>
<td>35 ± 9</td>
<td>45 ± 10</td>
<td>53 ± 19</td>
<td>73 ± 23</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.18 ± 0.02</td>
<td>0.21 ± 0.04</td>
<td>0.29 ± 0.05</td>
<td>0.33 ± 0.11</td>
<td>0.59 ± 0.21</td>
</tr>
<tr>
<td>Lactate / O2</td>
<td>0.22 ± 0.02</td>
<td>0.23 ± 0.05</td>
<td>0.32 ± 0.04</td>
<td>0.35 ± 0.13</td>
<td>0.62 ± 0.27</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery (µmol/min/kg)</td>
<td>168 ± 35</td>
<td>185 ± 49</td>
<td>195 ± 55</td>
<td>206 ± 31</td>
<td>216 ± 21</td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>10.94 ± 1.40</td>
<td>10.48 ± 1.12</td>
<td>10.32 ± 1.62</td>
<td>9.26 ± 1.93</td>
<td>10.39 ± 2.45</td>
</tr>
<tr>
<td>Uptake (µmol/min/kg)</td>
<td>19 ± 5</td>
<td>20 ± 7</td>
<td>21 ± 8</td>
<td>20 ± 7</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>UV- FA (mmol/L)</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.03</td>
<td>0.13 ± 0.04</td>
<td>0.13 ± 0.05</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>Glucose / O2</td>
<td>0.21 ± 0.04</td>
<td>0.25 ± 0.05</td>
<td>0.28 ± 0.07</td>
<td>0.25 ± 0.07</td>
<td>0.35 ± 0.09</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* P <0.05 vs pre, early and mid labor.
n = 4 for pre, early, mid, late labor and delivery measurements.
oxygen quotients did not change significantly in either group.

In Group I (Table 44), umbilical lactate uptake was significantly increased in mid and late labor, indicating elevated uteroplacental lactate production. Thus, the rise in blood lactate levels in these fetuses may not have been due solely to increased lactate production in fetal tissues, but could have involved the placenta as well. However, at delivery, fetal lactate uptake from the placenta declined and the umbilical venous-descending aorta lactate concentration difference became significantly less than in late labor, suggesting that there was net fetal lactate production at this time. The increase in umbilical lactate uptake was largely due to the significant increase in umbilical lactate delivery, since umbilical lactate extraction tended to decrease during labor.

In the Group II animals (Table 45), umbilical lactate delivery was also significantly increased during labor, but to a much lesser degree compared to Group I, whereas fetal lactate extraction from the placenta was maintained. Consequently, umbilical lactate uptake showed a trend toward an increase during labor and at delivery, but the values were not significantly different from the pre-labor estimate. In contrast to the situation in Group I, there was no decrease in the UV-FA lactate difference at delivery. With the progress of labor, the lactate/oxygen quotient in Group I was progressively increased and reached a significant difference in late labor, but then fell at delivery. In Group II, although the lactate oxygen quotient was also increased, this did not reach statistical significance.
3.6.9 Fetal Plasma Arginine Vasopressin Concentrations

Fetal plasma arginine vasopressin concentrations before and during labor and delivery are presented in Table 46. The AVP concentrations were especially high in Group I animals throughout labor and delivery. The mean value was 11.7 ± 1.4 pg/ml at pre-labor. With the progress of labor, the level rose rapidly to 67.1 ± 13.8 pg/ml in early labor, 175.8 ± 30.2 pg/ml in mid-labor, 315.5 ± 21.5 pg/ml in late labor and 480.1 ± 102.7 pg/ml at delivery. In contrast, fetal AVP concentrations in Group II were only minimally increased during labor, with a significant rise occurring only at delivery to mean value of 98.3 ± 30.2 pg/ml. In view of the marked increase in the plasma AVP levels in Group I fetuses with severe metabolic acidemia during labor, the relationship between fetal plasma AVP concentrations and fetal FA blood pH was examined. As depicted in Figure 37, the concentrations of AVP were significantly correlated with fetal arterial blood pH (r = -0.825, P < 0.001).

3.6.10 Fetal Breathing Activity

As was discussed in section 3.5.8, fetal breathing activity fell progressively in both groups of fetuses over the last week of pregnancy. In addition the incidence of FBM was consistently lower in Group I and on the day of labor the overall mean values were 1.4 ± 0.6 and 5.9 ± 1.0% in Groups I and II respectively. Table 47 presents fetal breathing activities at the last day of gestation, expressed as percentage of time spent breathing over each 4 hr period. In both groups, the incidence of FBM decreased further as labor approached, but the incidence remained higher in Group II. FBM in both groups were completely abolished over the last 8
Table 46. Fetal plasma arginine vasopressin concentrations before and during labor and delivery.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Labor</th>
<th>Early-Labor</th>
<th>Mid-Labor</th>
<th>Late-Labor</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine vasopressin (pg/ml)</td>
<td>11.7 ± 1.4</td>
<td>67.1 ± 13.8</td>
<td>175.8 ± 30.2*</td>
<td>315.5 ± 21.5†</td>
<td>480.1 ± 102.7‡</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine vasopressin (pg/ml)</td>
<td>4.2 ± 0.8</td>
<td>6.1 ± 1.3</td>
<td>10.1 ± 1.6</td>
<td>21.2 ± 5.1</td>
<td>98.3 ± 30.2‡</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

*  P <0.05 vs pre labor.
†  P <0.05 vs pre, early and mid labor.
‡  P <0.05 vs pre, early, mid and late labor.

For Group I, n = 4 for pre, early, mid and late labor measurements, and n = 3 for delivery measurements.
For Group II, n = 6 for pre, early, mid, late labor and delivery measurements.
Figure 37. Relationship between fetal plasma AVP concentrations and fetal arterial blood (FA) pH value from pre-labor stage throughout labor to delivery. Fetal plasma AVP concentrations are plotted on the logarithmical scale against fetal FA pH. The regression equation is $y = 27.55 - 3.62x$, $r = -0.825$, $P < 0.001$; where $y$ is log plasma AVP concentrations. Group I fetuses, (○); Group II fetuses, (●).

hours of labor. We also examined the time interval between the onset of labor (as assessed from the pattern of uterine activity) and the final cessation of FBM. In Group I, the interval ranged from -28 min (i.e. FBM ceased before labor onset) to 399 min, with a mean of $200.2 \pm 81.2$ min ($3.3 \pm 1.4$ h). In Group II, the interval ranged from -124 to 398 min, with a mean of $94.8 \pm 103.4$ min ($1.6 \pm 1.7$ h). The mean values in the 2 groups were not significantly different. Thus during the study, the cessation of FBM in each animal was a useful sign that labor had really commenced.
### Table 47. Fetal breathing movements on the day of labor.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time Period</th>
<th>n=5</th>
<th>n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-24 to -20 hr</td>
<td>-20 to -16 hr</td>
<td>-16 to -12 hr</td>
</tr>
<tr>
<td>% Time breathing</td>
<td>4.3 ± 2.1</td>
<td>2.9 ± 1.7</td>
<td>0.7 ± 0.5*</td>
</tr>
<tr>
<td>% Time breathing</td>
<td>18.3 ± 3.2</td>
<td>10.0 ± 1.9*</td>
<td>5.0 ± 1.2†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Data are calculated on the last day of gestation (i.e., on the day of delivery) from the time of delivery of the fetus retrospectively for 24 hours and expressed as each 4 hour period before delivery, with the time of delivery being hour 0. The overall incidence of fetal breathing movements are expressed as percent of time spent breathing over each 4 hr period.

* P <0.05 vs. -24 to -20 hr.
† P <0.05 vs. -24 to -20 hr and -20 to -16 hr.
‡ P <0.05 vs. -24 to -20 hr, -20 to -16 hr and -16 to -12 hr.
4. DISCUSSION

4.1 Method Development

4.1.1 Use of Fluorescent Microspheres to Measure Regional Blood Flow in the Fetal Sheep

Measurement of regional blood flow is important in assessing fetal cardiovascular function. The radioactive microsphere technique is an established method, but is becoming increasingly problematic due to radiation exposure and regulatory restriction. Thus a non-radioactive method is desirable. The current study appears to be the first in which fluorescent-labeled microspheres were employed to measure fetal blood flow in all tissues and organs and also the first in which a 96-well plate fluorescent reader was successfully employed for the fluorescence determination. However, there is a recent report of cerebral blood flow measurement in fetal lambs using colored microspheres (McCrabb and Harding 1995), and a recent abstract on the use of fluorescent-labeled microspheres and a conventional fluorimeter to measure fetal adrenal blood flow (Buchwalder et al. 1996).

Four methods have been employed in adult studies to recover fluorescent microspheres from tissues and reference samples following tissue digestion: vacuum filtration (Glenny et al. 1993), density gradient centrifugation using sucrose (Austin et al. 1993), a sedimentation method (Van Oosterhout et al. 1995) and a method involving addition of Cellosolve to the KOH containing the dissolved tissue, followed by phase separation of the organic and aqueous layers (Abel et al.
1993; Li et al. 1996). However, this latter method has only been applied to myocardial tissue to date, and there was an under-representation of fluorescence in the blood reference samples, perhaps due to incomplete extraction of the dye into the organic phase (Li et al. 1996). Van Oosterhout et al. (1995) have calculated that both the time and cost/sample are lowest with the sedimentation method, and as estimation of regional and placental blood flows in fetal lambs generates many samples per animal, we chose this method. However, several modifications were made to the sedimentation method described by Van Oosterhout et al. (1995), largely because of the larger amounts of tissue involved in our study. The main modifications were a larger size (50 ml vs. 10) and different material (polypropylene vs. glass) for the sample digestion tubes and additional deionized water washes of the digested samples. For most tissues, this procedure was effective in yielding a clear supernatant for fluorescence determination. However for skin, and brown adipose tissue, an additional step involving a 2 h incubation at 60°C was required. In addition, bone required direct extraction of the fluorescent dye following crushing of the tissue. The overall estimated recovery of spiked microspheres from all tissues averaged 94.3 ± 2.5% and the % loss of 5.7 ± 1.0% is significantly different from 0, indicating a small loss of microspheres during the tissue processing procedures. Using a similar approach to estimate microsphere recovery, Van Oosterhout et al. (1995) found a 4% loss, but this was not statistically significant. The apparent lower recovery with our method may be due to the increased number of washing steps. However, as there was no apparent relationship between the number of microspheres added to the tissue and blood samples and microsphere recovery, the loss should not affect the flow calculations and hence was ignored.
Automated microsphere quantification is one of major issues with the fluorescent microsphere technique (Prinzen and Glenny 1994). The most frequently performed method for determination of fluorescence signals in the tissues is the cuvette method in which the fluorescence of single samples is measured one at a time. However, using this method to measure fluorescence of numerous tissue samples is labor-intensive, time consuming and difficult to automate. A previous attempt to automate spectrometry using a surface-reading 96 microwell plate reader has been reported (Glenny et al. 1993), but this suffered from increased fluorescent quenching with higher microsphere numbers (> 2000 microspheres/ml), resulting in nonlinearity in the concentration-fluorescence intensity relationship. An improved multi-well plate reader with a robotic system to fill the multi-well plates is however being investigated (An et al. 1996). Recently, a fully automated system that reads samples via an automated flow cell unit has been developed for measurement of regional lung perfusion. Analysis time of this system is ~3 min/sample, which is much longer than with the microwell plate reader in the current study, although the former method can analyze up to 280 samples unattended (Schimmel et al. 1996).

In the present work we describe use of a CytoFluor™ 2350 96-well plate reader, coupled to translucent, polypropylene 96-well plates which are resistant to the solvent used. Measurement can be completed within 40 min for all tissues from one animal. Excellent linearity was observed in the microsphere number-fluorescent intensity relationship, up to at least 20,000 microspheres/ml. This range is considerably wider than that obtained with a 96-well plate fluorescence surface reader (Glenny et al. 1993), probably because quenching effects are more significant with surface reading instruments (Guilbault 1990). The wide linear range is a
particular advantage with organs which receive a high blood flow and hence large microsphere numbers, since serial dilution of the samples can be avoided. The performance characteristics of our method are in accordance with the validated cuvette methods (Glenny et al. 1993; Van Oosterhout et al. 1995). The precision is $3.4 \pm 1.1\%$ for within-run CV and $4.5 \pm 1.1\%$ for between-run CV. The potential for well to well crosstalk is small, since the overall CV for the blood flows estimated from the triplicate wells for each tissue was $2.8 \pm 1.5\%$.

A potential disadvantage of our method involves the use of fixed wavelength excitation and emission filters with a 1/2 bandpass of 20-40 nm, compared to the monochromators used in conventional fluorimeters, which can vary excitation and emission wavelengths continuously and with a narrow bandwidth (e.g. 4-6 nm). This could lead to increased problems of spillover of one fluorescent label into the emission window of another label. In addition, there is the potential for one fluorophore to quench the signal of another by absorbing the emitted photons. Evidence for this phenomenon was obtained for the crimson and red fluorescent dyes; in the presence of the former dye, the emitted fluorescence of the red dye was reduced by $-3\%$ at the peak emission wavelength (Prinzen and Glenny 1994). We did observe significant fluorescence overlap for 3 of the fluorophore pairs: orange into yellow green and red, and red into crimson. However, in no case was there spillover of one label into more than an immediately adjacent label or spillover of more than one label into a single emission band. Hence the matrix correction method that involves solving simultaneous linear equations (Heymann et al. 1977; Schosser et al. 1979) and which is employed to correct for the multiple spectral overlap of radioactive microspheres was not required with our method, at least with the color combination used. Previous estimates of spillover with both radioactive and fluorescent
microspheres have utilized a single concentration of each label (Heymann et al. 1977; Schosser et al. 1979; Glenny et al. 1993; Van Oosterhout et al. 1995). However, because it seemed possible that quenching of one fluorophore by another could occur in our measurement system, and because the magnitude of both spillover and quenching are in part determined by the concentration of the interfering dye, we examined the effect of differing concentrations of the fluorophore pairs for which these 2 problems could occur. In terms of spillover, this occurred with orange into yellow green, orange into red and red into crimson. However, the magnitude of the fluorescence overlap signal was relatively small (4-9%) and only slightly larger than the values reported for the same labels in conventional fluorimeters (Glenny et al. 1993). Moreover, the degree of spillover, expressed as a percentage of the fluorescent intensity of the interfering fluorophore in its own emission band, was constant over a wide range of concentrations of both interfering and affected dye. If quenching occurred with these 3 fluorophore pairs it was not obvious, and with crimson effects on red, there also was no evidence of quenching of the red emission signal with increasing concentrations of crimson, at least up to the maximum crimson/red ratio of 8 examined. This result differs from that of Prinzen and Glenny (1994), who found a slight quenching of the red signal, even with a crimson/red concentration ratio of ~1. This difference may be due to the fact that Prinzen and Glenny examined the entire emission spectrum.

With the exception of the blue emission band, there was no endogenous color in the extracted tissue and samples. However, when reading the blue fluorophore (i.e. ~420-500 nm), endogenous color was present in both the tissues and blood samples, and was particularly large in the latter samples. This phenomenon has also been observed in other studies that employed
sedimentation and phase separation methods to recover the fluorophores (Abel et al. 1993; Van Oosterhout et al. 1995; Li et al. 1996), but not with the filtration method (Glenny et al. 1993). Although the blue endogenous color has been suggested to be due to the Cellosolve acetate and Tween 80 (Van Oosterhout et al. 1995), we tested Cellosolve acetate and the final wash supernatant from tissue and blood samples and did not find any color in the blue emission band. We did find that bubbling nitrogen through the final wash of the blood samples completely eliminated the interfering blue color, although the mechanism of this effect is not clear. We did not try nitrogen bubbling with the tissue samples as the intensity of the interference was much less and because to do this for all tissue samples would not be practical. Given these problems with the blue microsphere encountered by us and others, an option would be to omit it and use one of the other available fluorescent labels (e.g. blue green).

As noted in the section 1.11.2, flow estimates obtained using fluorescent microspheres have been compared with measurements obtained with coadministered radioactive microspheres in several adult studies, and the correlation was excellent (Abel et al. 1993; Austin et al. 1993; Glenny et al. 1993; Chien et al. 1995; Van Oosterhout et al. 1995). Thus we did not feel it necessary to coadminister the two microsphere types in fetal lambs. However, we did compare fluorescent microsphere flow estimates obtained in 3 fetal lambs with those obtained using radioactive microspheres in 4 other lambs at the same gestational age. For 88% of the tissues/organs examined, the fluorescent microsphere estimate was within 1 SD of the radioactive measurement, and for all but 1 of the remaining tissues was within 2 SD. The exception was forelimb skin, where the difference was greater (3 SD). In other studies in which fetal skin blood flow has been measured, the mean values vary considerably from 19-32
mL·min$^{-1}$·100g$^{-1}$ (Knight et al, 1996), suggesting that flow to this tissue may fluctuate normally in the fetus. Thus overall, there was good agreement between the 2 methods, particularly given the fact that they were obtained in different animals. The measurements are also similar to the many published estimates of blood flows in the sheep fetus (e.g. Cohn et al. 1974; Court et al. 1984; Richardson et al. 1989b; Rurak et al. 1990b). The fact that bone blood flow in the two groups were similar indicates that the fluorophore recovery method that we developed for bone (i.e. direct extraction of the dyes by Cellosolve from crushed bone) results in reliable flow estimates. This appears to be the first time that fluorescent microspheres have been used to obtain quantitative flow estimates in bone in either fetal or adult studies.

4.1.2 Measurement of Fetal Plasma Arginine Vasopressin Concentrations

In the present study, fetal plasma AVP measurement by radioimmunoassay was validated by running a parallel assay to examine the immunologic reactivity between the antibody and the ligands. The validation test yielded parallel concentration-response curves between the AVP standard and fetal sheep plasma extracts, which indicates radioimmunological identity of the extraction of fetal lamb plasma with the AVP standard. Once validated, the method was applied successfully to the measurement of AVP in fetal sheep plasma, with the lowest detection limit of 1.5 pg/ml and between assay precision of 7.02%. The validated method appears to be more precise than the others using similar radioimmunoassay methods for measurement of fetal sheep plasma AVP. In those measurements, the interassay coefficient of variation ranged from 9.9 to 14.6% (Wlodek et al. 1988, Akagi and Challis, 1990). Recovery of AVP from the extraction step in the
present study was 74.10 ± 3.93%, which is also higher than the reported recovery value of 57-59% (Wlodek et al. 1988; Akagi and Challis, 1990).

4.2 Fetal and Placental Growth

As noted in the RESULTS section and discussed more fully later, the initial division of the study animals into Groups I and II was accomplished on the basis of the FA pH change during labor. However, it was subsequently found that the Group I fetuses were spontaneously and asymmetrically growth restricted. Thus this study provides data about a naturally occurring form of fetal growth restriction, in which both fetal and placental weight were significantly reduced compared to the Group II fetuses. In terms of the separation of the 2 groups, it is not as distinct with fetal weight as with FA pH at delivery, since the smallest Group II fetus (2.927 kg) weighed slightly less than the heaviest animal in Group I (3.071 kg). However, there was a clear separation in the placental weights of the 2 groups (see section 3.4). The decreased fetal weight in Group I was accompanied by the reduction in relative fetal organ weights, including the liver, spleen, stomach, kidney and lung, with the exception of the relative weight of the brain, adrenal and heart (Table 11). Thus, there is an alteration in the pattern of fetal growth in Group I, featuring asymmetrical growth restriction with a larger brain-to-liver weight ratio. The relative sparing of brain, heart, and adrenal growth thus may have distinct survival value in these fetuses. The increase in fetal adrenal weight in the Group I may be due to adrenal hypertrophy or hyperplasia resulting from chronic stress, which will be further discussed in the following section. Another characteristic of the Group I animals is the marked
decrease in the placental weight, which is reduced to a greater degree than fetal weight, thus resulting in a larger fetal weight-to-placental weight ratio (i.e., smaller placental weight-to-fetal weight ratio) in these fetuses than in normally grown ones (Table 11). In addition, fetal and placental weights were positively related (Figure 8). The decrease in placental weight-to-fetal weight ratio also had a close positive correlation with placental weight (Figure 9).

The extent and pattern of fetal growth restriction in Group I is similar to that observed in a number of fetal growth restriction models in sheep, in which fetal morphometric changes have been evaluated. These include maternal undernutrition (Wallace, 1948; Mellor, 1983; Godfrey and Barker, 1995), heat stress (Alexander and Williams, 1971; Brown et al. 1977; Bell et al. 1987), restriction of implantation area (Robinson et al. 1979; Harding et al. 1985; Owens et al. 1986), embolization of the utero-placental circulation (Creasy et al. 1972; Clapp et al. 1982b; Charlton and Johengen, 1987), mechanical occlusion of the maternal iliac artery (Lang et al. 1997) and long term embolization of the umbilical circulation (Gagnon et al. 1997). Also, our results on fetal growth pattern are comparable to that reported on spontaneously hypoxemic fetal sheep with growth restriction (Goetzman et al. 1984). Thus, with both experimentally induced and naturally occurring fetal growth restriction, the common feature is asymmetric growth restriction, in which the brain is relatively spared along with the heart and adrenals, and the liver and spleen suffer a great reduction in size. Accordingly, brain weight increases and that of liver decreases as a proportion of body weight, with a resultant greater brain-to-liver weight ratio. These
results suggest that both experimental and spontaneous fetal growth restriction may involve the same mechanisms.

The etiology of the fetal growth restriction in the present study is unclear. It could have arisen from a variety of causes, since fetal growth and development are the outcome of many factors. Studies of domestic animals have shown that fetal growth is influenced by intrinsic factors such as the size, age and parity of the mother (Walton and Hammond, 1938; Hunter, 1956; Alexander, 1964a). The size of the fetus is externally affected by the environmental conditions (Alexander and Williams, 1971; Brown et al. 1977; Thompson et al. 1982; Symonds et al. 1986; Bell et al. 1987; Symonds et al. 1992). Prolonged environmental heating of pregnant sheep, particularly during mid-gestation, results in substantially reduced placental size and fetal growth, a decreased placental weight-to-fetal weight ratio and a curvilinear association between fetal and placental weights (Alexander and Williams, 1971; Bell et al. 1987). Placental and fetal growth restriction also occurs as a result of maternal undernutrition from early in pregnancy (Wallace, 1948; Everitt, 1964; Mellor, 1983). Variations in placental weight could account for up to 91% of that in fetal weight when nutritional restriction occurs throughout gestation and up to 72% even when nutrition is adequate (Mellor, 1983). After severe underfeeding of sheep in early pregnancy (About 0.6 gestation), placental weight is significantly reduced as early as 90 days (Everitt, 1964) and is associated with a decreased placental/fetal weight ratio, suggesting a primary effect on placental growth. However, as discussed in the section 1.1.5.1, the effect of food restriction on fetal weight is more severe during the later part of pregnancy than during early development. This is probably because during early
pregnancy, the ewe can draw on her own body reserves to meet fetal requirements, whereas during late gestation, an external supply of good quality food, such as a balanced ration which can provide the proper proportions and amounts of all the required nutrients, is needed to nourish the fetus (Mellor and Matheson, 1979; Mellor, 1987).

Any factor which reduces uterine blood flow may restrict placental and fetal growth. Experimental interference with the circulation supplying the gravid uterus in late gestation, such as embolization of the utero-placental circulation after 0.6 gestation in sheep, results in fetal growth restriction (Creasy et al. 1972). Placental weight and the placental weight-to-fetal weight ratio are also decreased. In addition, a reduction in utero-placental blood flow could be one of the mechanisms by which fetal growth rate is reduced in heat-stressed sheep (Alexander et al. 1987; Bell et al. 1987). Abnormalities of the placenta are often associated with reduced umbilical blood flow, which may also be associated with fetal growth restriction. Following embolization of the umbilical circulation at 0.74-0.88 of gestation, there was a reduced umbilical blood flow associated with a decrease in fetal and placental weights. However, the placental weight-to-fetal weight ratio was unaltered (Dr. Gagnon, personal communication), suggesting that compensatory fetal growth in response to the reduced placental mass may not occur at this relatively late stage. In some instances, limited oxygen availability may also be a contributory factor. A deficient supply of oxygen, which is a major fetal substrate, is associated with reduced placental and fetal growth (Jacobs et al. 1988a, Boyle et al. 1996). When fetal O₂ delivery (but not fetal Po₂) is reduced by fetal hemorrhage, there is also decreased fetal growth, but the effect on placental growth is not known (Kwan et al. 1995a).
It is not possible at this point to fully elucidate the mechanisms of the growth restriction in the Group I fetuses. For instance, we did not measure uteroplacental blood flow in our study. But we have examined several factors, which may affect fetal growth and development. To assess the possible effect of maternal size on fetal birth weight, we have assessed the ratio of fetal birth weight to maternal weight in the 2 groups. The fetal weight-to-maternal weight ratio was significantly lower in Group I than in Group II (see section 3.4), suggesting that maternal size was not a major determinant of fetal growth in the current study. In addition, both groups of ewe were of comparable maternal age at conception, maternal body weight, birth weight of mother, parity and breed (Table 7 and 9). Both group of animals were also housed in our laboratory under identical conditions and given identical food before and after surgery.

As noted in Table 7, the animals in Group I and II were studied in different breeding seasons. With 4 of the 6 ewes in Group I, it was the 1992-93 season, with the others being used in 1991-92 and 1994-95. In contrast in Group II, 3 of the 6 ewes were bred in the 1994-95 season, 2 in 1995-96, while 1 was bred in 1993-94. To determine whether these differences in the breeding years of the 2 groups could have contributed to the differences in fetal weight, the weight and gestational age data for all operated fetuses studied in the laboratory in each breeding season was examined. The mean results are presented in Table 48 from 1991-92 to 1994-95, which gives the mean weight, the gestational age at which the weight was obtained and the number of fetuses/pregnancy. Fetal weight in 1992-93, the season in which 4 of 6 Group I ewes were studied is significantly less than the mean value for 1994-95, when 3 of 6 Group II animals were
Table 48. Average values for fetal weight and number of fetuses at the time of delivery or death for all fetuses studied between 1991 and 1995.

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Fetal weight</th>
<th>Gestational age</th>
<th>Number of fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991-92</td>
<td>25</td>
<td>3.075 ± 0.147</td>
<td>133.6 ± 1.3</td>
<td>1.8 ± 0.1\textsuperscript{a}</td>
</tr>
<tr>
<td>1992-93</td>
<td>39</td>
<td>2.783 ± 0.103\textsuperscript{a}</td>
<td>132.7 ± 0.9</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>1993-94</td>
<td>39</td>
<td>3.117 ± 0.115\textsuperscript{b}</td>
<td>131.5 ± 0.8</td>
<td>1.5 ± 0.1\textsuperscript{b}</td>
</tr>
<tr>
<td>1994-95</td>
<td>40</td>
<td>3.372 ± 0.106\textsuperscript{b}</td>
<td>134.2 ± 0.9</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. N, the number of animals studied in that season. Values with different letter superscripts are significantly different.

used, while there are no significant differences in gestational age or fetal number between these 2 seasons. Fetal weight also tended to be low in 1991-92, but there was no significant difference from any of the other seasons. Data for 1995-96 are not given in Table 48 as only 8 animals in total were studied. However, average weight (3.273 ± 0.428 kg) was similar to that in 1994-95, while gestational age (136.8 ± 2.2 d) and number of fetuses/pregnancy (2.4 ± 0.4) were higher than in any of the other years. Figure 38 plots fetal weight against gestational age and against the time between surgery and delivery (or death) for the 1992-93 and 1994-95 seasons, as these contain the majority of the Group I and II animals, respectively. Weight was significantly related to both X variables in both seasons, but there were no obvious differences between the 2 seasons. However, the data in Table 48 and Figure 38 are not segregated in terms of litter size, and for all years except 1991-92 and 1995-96, there was a significant negative relationship between fetal number and weight of the operated fetus. The lack of the relationship between fetal weight and litter size in 1991-92 and 1995-96, as well as in the labor study
Figure 38. Plots of fetal weight against gestational age and days between delivery and surgery for all animals studied in 1992-93 (left panels) and 1994-95 (right panels).
animals (Figure 7) is likely due to the smaller number of animals in those groups compared to the other breeding seasons. The negative impact of fetal number on fetal growth is consistent with the lower birth weight often seen in multiple pregnancies in sheep (Alexander, 1964a; Stegeman, 1974; Barlow et al, 1987). Multiple regression analysis was performed for the 1992-93 and 1994-95 seasons, with the equation: $FW = A*GA + B*#F + C$, where $FW$ is fetal weight, $GA$ is gestational age and $#F$ is the number of fetuses in each animal. For 1992-93 and 1994-95, the regression coefficient $A$ averaged $0.0666 \pm 0.0134 \ (P < 0.001)$ and $0.0560 \pm 0.0153 \ (P < 0.001)$, respectively, while $B$ averaged $-0.4851 \pm 0.1183 \ (P < 0.001)$ and $-0.2881 \pm 0.1207 \ (P < 0.05)$. The intercept value, $C$, was $-5.195$ and $-3.681$ for 1992-93 and 1994-95, respectively, and the overall $r$ values were $0.6975 \ (P < 0.001)$ and $0.5826 \ (P < 0.001)$. In Figure 39, the regression equations for the 2 seasons were used to predict the relationship between $GA$ and $FW$ for singleton and twin pregnancies. In both cases, the curve for 1992-93 is below that for 1994-95, and this is largely due to the lower intercept value in 1992-93; the greater discrepancy for twins is due to the larger effect of fetal number in the earlier season. One interpretation of the data in Figure 39 is that some factor in early gestation, i.e. before the animals were studied in the laboratory, reduced fetal growth potential in 1992-93 and that twins (or triplets) in that season had a greater negative impact on growth than in 1994-95. Given that 4 of the 6 Group I ewes were pregnant in 1992-93 and all of these were carrying twins or triplets, this could partly explain the growth restriction in that group. Moreover, all of the Group I twins were studied in 1992-93, and on the basis of the data in Figure 39 they would be expected to be smaller than twins in 1994-95 (i.e. Group II), which was in fact the case.
Figure 39. Relationship between fetal weight and gestational age (115 -145 days). The linear curves are derived from the multiple regression model: Fetal weight = A\times\text{gestational age} + B\times\text{number of fetuses} + C, which examines the simultaneous effect on fetal weight of gestational age and the number of fetuses in each pregnancy of individual animals. Upper panel shows the derived lines for singletons in 1994-95 (solid line) and 1992-93 season (dashed line). Lower panel shows the derived lines for twins in 1994-95 (solid line) and 1992-93 season (dashed line).
However, the precise mechanisms involved are unclear since the sheep used in the laboratory all come from the same flock and are subjected to the same housing and feeding regimens. Minor differences in food intake or composition (e.g. different levels of trace nutrients) in different years could be involved, but this cannot be determined from the information available in the sheep unit. Certainly, restricted maternal nutrient intake has a greater impact on twin compared to singleton pregnancies (Mellor and Murray, 1981).

The data in Figures 38 and 39 allow an assessment of two other differences between Group I and II animals that could potentially have contributed to the lower fetal weight in the former group. One is the mean gestational age at delivery, which is 2 days shorter in Group I than in Group II. However, as shown by the figures, such a relatively small difference in gestational age would contribute little to the fetal birth weight difference between the two groups. The daily growth rate in fetal lambs rate in late gestation is 2-3% (Koong et al. 1975; Mellor and Murray, 1981; Fowden et al. 1986; Owens et al. 1986; Kwan et al. 1995a) with a terminal decrease, which usually starts between 120 and 130 days of gestation (Mellor and Murray, 1981). From the data in Figure 39, average growth rate was 2.5 ± 0.5%/d for 1992-93 and 1.7 ± 0.5%/d for 1994-95, respectively. These are not significantly different and are similar to the estimate (1.6 ± 0.6%/d) obtained in an earlier study in the laboratory (Kwan et al, 1995a).

Another potential confounding factor in the difference in weights between the 2 groups relates to the time of surgery during gestation and the effect of surgical instrumentation on fetal growth. The gestational age at surgery was earlier in Group I (121-125 days
gestation) than in Group II (127-133 days gestation). Chronic surgical instrumentation has been reported to produce significant alterations in fetal growth. In a study of pregnant sheep carrying twins, Mellor and Murray (1981) found that placental weight, crown-to-rump length and fetal weight were all significantly lower in the twin exposed to surgery than in the contralateral control fetus (Mellor and Murray, 1981). The authors concluded that the surgical intervention acted to decrease placental weight. However, surgery in their study was performed between 86-92 d gestation (~ 0.61 of term), i.e., at a time when placental weight is likely still increasing. A negative impact of chronic instrumentation on fetal growth has also been demonstrated in 2 commonly used fetal sheep preparations (Clark et al. 1990), one instrumented with fetal catheters and electromagnetic flow probes on the uterine arteries, and the other with the addition of a UV catheter and UA flow probe. In both instrumented groups, fetal birth weight was significantly decreased by 11-15%, as compared to a non-surgically instrumented control group. In both groups, the brain/liver weight ratio was less than in the controls, while the brain/thymus weight ratio was increased. In addition, placental weights were not decreased in either instrumented group. Thus, it appears that the change in growth pattern in the instrumented fetal sheep is not consistent with asymmetric growth restriction. The results on placental growth (Clark et al. 1990) differ from those of Mellor and Murray (1981), suggesting that at different stages of gestation, the effect of surgery on placental growth can vary. Overall, the effect of surgical instrumentation on fetal growth pattern is different from the findings of a number of studies on experimental manipulation of fetal growth in the sheep, and also different from the observations in the present study, in which the growth-restricted fetuses have a larger brain/liver weight ratio and a smaller placenta. Moreover, the plot in Figure
38 of fetal weight against the surgery-delivery interval is not consistent with an effect of surgical intervention on fetal growth, since there is a linear relationship between the 2 variables, which is similar to the relationship between fetal weight and gestational age.

There is one additional factor to be discussed in relation to confounding effects in the study. This is the fact that experiments from other studies were carried out in some of the sheep in the antepartum period and that a greater number of Group I than Group II sheep were involved (Table 6). The rationale for conducting these experiments was to maximize the information obtained from the animals, and previous research in the laboratory indicated that the types of experiments did not interfere with the normal vaginal delivery of healthy, well-grown lambs. Thus it seemed that the experiments would not confound the labor study as initially planned. However, if it had been realized at the outset of the study that it would ultimately come to include comparison of normally grown and growth restricted fetal lambs, then the other experiments would likely not have been carried out, so as to remove this as a potential confounding element. However, for several reasons we do not believe that they affected the outcome of the study, particularly in terms of the fetal growth differences in the 2 groups. Firstly, the experiments involved maternal or fetal administration of drugs, or a 60 minute period of modest hypoxemia, with sampling of blood from the operated fetus and replacement of this with maternal blood. Obviously all of the Group I sheep subjected to these experiments were carrying twins or triplets, and the impact on the unoperated fetuses would be less, as they would not be subject to blood sampling (or fetal drug administration). Yet the weight of the unoperated twins was also reduced in Group I, compared to Group II. Secondly, the time in gestation at which the
experiments were carried out was unlikely to have allowed for an effect on placental growth, since the placenta appears to be more susceptible to disturbance or restriction during the first half of gestation, when it undergoes most of its growth by rapid hyperplasia (Wallace, 1948; Alexander, 1964a; Stegeman, 1974; Rosenfeld et al. 1974; Kelly et al. 1987; Ehrhardt and Bell, 1995). In contrast, over the last 50 to 60 days of pregnancy, the weight of the placenta remains relatively constant (Mellor and Murray, 1981). And the most dramatic growth difference between the groups was that placental weight in Group I was reduced by 48% as compared to that in Group II. It seems highly unlikely that such a dramatic difference between the 2 groups could be due to the other experiments that were predominately carried out in Group I. Thirdly, the antepartum data (Figure 13-17) indicate differences between Group I and II at least up to 10 days prior to labor. As this is before most of the experiments were conducted, it indicates that the Group I fetuses were different from Group II before experimentation started.

As noted above, a striking difference between the 2 groups was a markedly lower average placental weight in Group I; moreover there was no overlap between the individual placental weights in the 2 groups. Thus, although placental weight (/fetus) in normally fed ewes carrying twins has been reported to be less (Alexander, 1964a) or the same (Mellor and Murray, 1981) as in singleton pregnancies, this cannot explain the difference between Group I and II. In terms of the mechanism of the reduced weight, there was a non-significant difference in average cotyledon number (38 ± 4 vs. 53 ± 10, Group I vs. Group II) and a significant difference in the average weight of individual cotyledons (4.11 ± 0.16 vs. 5.62 ± 0.20 g). As illustrated in Figure 12, the difference in placental weight between
the 2 groups was associated with differences in the frequency distribution of individual cotyledon weight. The distribution in Group II is similar to that previously described for normally grown fetal lambs in late gestation. (Robinson et al. 1979). Group I differed from this pattern in having a shift in the distribution to lower cotyledon weights and the absence of larger weight cotyledons (i.e. > 12 g). When placental weight is reduced by carunclectomy, there is a similar shift to lower weight cotyledons (Robinson et al, 1979). However, there are also small numbers of large cotyledons (up to 60 g), and this tends to compensate for the reduced cotyledon number in terms of overall placental weight. Thus, the marked reduction in the placental weight in Group I is due to a larger proportion of small size cotyledons, and an apparent failure to produce larger ones. However, the reason for this is not clear. Moreover, elucidation of the mechanism will require an understanding of the factors that affect growth of individual placentomes.

Cotyledon number (59 ± 22) and weight (260.9 ± 11.0 g) in the Group II singletons are at the low end of the ranges (59-91 and 274-538 g, respectively) of published values for sheep in late gestation (Wallace, 1948; Alexander and Williams, 1971; Stegeman, 1974; Robinson et al. 1979; Worthington et al. 1981; Kelly et al. 1987; Bell et al. 1989; Clark et al. 1990; McCrabb et al. 1991; Phillips et al. 1996). The wide ranges could in part be due to breed differences, since the placental weight in Group I singletons is similar to that found in the same breed of sheep (274 g) by Worthington et al (1981). In the 3 singleton pregnancies in the present study, there was a wide variation in cotyledon numbers (33, 42 & 102). However, the low cotyledon numbers in two of the fetuses were compensated by a greater average cotyledon weight (6.62 and 8.01 vs. 2.36 g) so that total placental
weight was very similar (277.90 and 264.39 vs. 240.29 g). The reasons for the lower cotyledon number in these 2 animals are not clear, but may be partly due to the age or parity difference (Table 7). The number of cotyledons tends to be lower in older ewes than in young ones (Alexander, 1964a). The two animals associated with lower cotyledon numbers were older than the remaining one (4.7 and 6.7 vs. 1.5 year; parity 3 and 5 vs. 1). In contrast to the situation with the Group II singletons, both cotyledon number (48 ± 2) and weight (338.3 ± 54.9 g) in the Group II twins were similar to published values (42-55 and 277-398 g), whereas in the Group I twins, placental weight (145.3 ± 13.8 g) but not cotyledon number were well below the published ranges (Stegeman, 1974; Robinson et al. 1979; Bell, 1989). There are limited published data on cotyledon numbers and weights in triplet pregnancies in sheep (Stegeman, 1974), but as with the Group I twins, cotyledon number in the Group I triplets (34 ± 4) was similar to the value (34 ± 3) reported by Stegeman (1974), while placental weight was less (167.0 ± 24 vs. 199 ± 16 g). Figure 40 plots the average placental and fetal weights for singleton, twins and triplets in the current study and the published results for sheep at a comparable gestational age. The Group II animals, particularly the twins are within the range of most published studies, whereas the Group I fetuses are clearly at the lower end. The overall correlation coefficient is 0.865 (P < 0.001). Thus, differences in placental weight would account for 75% (r² × 100) of the variance in fetal weight in the combined data, indicating the predominant influence of the placenta on fetal growth.

Thus, the asymmetric pattern of fetal growth restriction in the Group I animals was likely due to an underdeveloped placenta. Evidence for a relationship between placental and
Figure 40. Relationship between placental and fetal weights at 138-147 d gestation. The data points are from the current study (▼ Group I twins, ▲ Group I triplets, ● Group II singletons, ■ Group II twins) and from various published studies of singletons (○), twins (□) and triplets (△) (1, Wallace, 1948; 2, Phillips et al. 1996; 3, Clark et al. 1990; 4, McCrabb et al. 1991; 5&10, Robinson et al. 1979; 6, Bell et al. 1989; 7, Alexander and Williams, 1971; 8, Kelly et al. 1987; 9,11&13, Stegeman, 1974; 12, Worthington et al. 1981).
fetal growth is also provided by our observations of a significant correlation between the two variables. These results are consistent with that of other models of experimentally reduced placental growth and its consequence for fetal growth (Wallace, 1948; Alexander, 1964b; Alexander and Williams, 1971; Creasy et al. 1972; Robinson et al. 1979; Clapp et al. 1982b; Mellor, 1983; Harding et al. 1985; Owens et al. 1986; Bell et al. 1987; Charlton and Johengen, 1987). In these studies, placental growth was restricted or placental mass reduced in a variety of ways either before or during pregnancy in the sheep. These data indicate that the placenta does influence or limit fetal growth when its own growth is restricted. The decreased placental weight-to-fetal weight ratio in the present study is also observed in experimental animals with fetal growth restriction such as maternal undernutrition (Wallace, 1948; Mellor, 1983), maternal heat exposure (Alexander and Williams, 1971; Bell et al. 1987), interference with the utero-placental blood flow (Creasy et al. 1972). It is not known why such fetuses outgrow their placentas. The change in placental weight-to-fetal weight ratio may merely represent an acceleration of the normal relative patterns of fetal and placental growth and development. In addition, fetal compensation may also contribute to a decreased placental weight-to-fetal weight ratio (Mellor, 1983).

As pointed out by Mellor (1988), inadequate placental development is usual in a small proportion of ewes in most flocks and its most obvious consequence is fetal growth restriction. Consistent with this view, we found that in Group I animals, both fetal and placental growth are restricted, but the weight of the placenta in Group I falls to a greater extent than fetal body weight. Therefore, in the present study, it is placental weight that is
a major determinant of fetal weight. In other words, naturally occurring growth restriction of our fetuses most likely results from restriction of placental growth. Fetal growth restriction may represent a fetal adaptation to the decreased placental growth with a limited ability to supply oxygen and nutrients.

4.3 Fetal Condition in the Antepartum Period

4.3.1 Fetal Metabolic, Hemodynamic and Endocrine Status

As noted previously, several experimental models have been developed in the sheep and the fetal morphometric data from such studies indicate that these experimental perturbations all produce asymmetric fetal growth restriction. Moreover, similar changes in fetal hemodynamic and metabolic functions have also been found in these various experimental models of fetal growth restriction. These include a significant reduction in fetal placental blood flow (Creasy et al. 1972; Morriss et al. 1980; Block et al. 1984; Owens et al. 1986; Bell et al. 1987), selective redistribution of blood flow to the fetal vital organs such as the brain, heart and adrenal glands (Creasy et al. 1972; Block et al. 1984), chronic fetal hypoxemia (Alexander and Williams, 1971; Harding et al. 1985; Bell et al. 1987; Owens et al. 1987a; Owens et al. 1987b; Thureen et al. 1992; Gagnon et al. 1994; Boyle et al. 1996; Daniel et al. 1996; Phillips et al. 1996; Gagnon et al. 1997), hypoglycemia (Alexander and Williams, 1971; Harding et al. 1985; Bell et al. 1987; Owens et al. 1987b; Thureen et al. 1992; Daniel et al. 1996) and hyperlactic acidemia (Owens et al. 1987b; Jacobs et al. 1988b). An exception to this general pattern is that
when low birth weight is due to maternal undernutrition, chronic prenatal hypoxemia is absent, but hypoglycemia remains (Mellor, 1983; Mellor and Cockburn, 1986). Furthermore, several studies have demonstrated that in fetuses with experimentally induced growth restriction, there is reduced fetal oxygen delivery (Owens et al. 1987a; Boyle et al. 1996) and oxygen consumption (Clapp et al. 1981; Owens et al. 1987a), reduced umbilical glucose delivery (Owens et al. 1987b) and glucose uptake (Clapp et al. 1981; Chandler et al. 1985; Owens et al. 1987b; Leury et al. 1990a), increased umbilical glucose extraction (Owens et al. 1987b), and decreased umbilical lactate uptake (Clapp et al. 1981).

An interesting finding in the present study was that not only was the fetal growth restriction of the Group I animals similar to that observed in a number of fetal sheep growth restriction models, but also that the antepartum metabolic and hemodynamic status in the group had many aspects in common with those of experimental fetal growth restriction. During the last 10 days of pregnancy, Group I fetuses were hypoxemic, hypoglycemic and hyperlacticemic as compared to Group II (Table 12). Fetal oxygen saturation and content in Group II fetuses decreased only slightly over the last 10 days of gestation, whereas the decrease in Group I fetuses was significantly steeper (Table 16). At least 2-3 d prior to labor, these changes were associated with higher vasopressin concentrations and a higher fractional distribution of fetal cardiac output to the adrenals (Table 19), but with lower values of cardiac output, umbilical placental blood flow (Table 18), fetal O₂ delivery and consumption (Table 20), umbilical glucose delivery and fetal lactate uptake from the placenta (Table 21). The reduction in glucose delivery to the fetus
was a result of both a decreased umbilical blood flow and a lower UV glucose concentration. However, Group I fetuses were able to compensate for the reduced glucose delivery by increasing umbilical extraction of glucose to maintain fetal glucose uptake (Figure 16). Umbilical lactate uptake at the pre-labor stage was lower in Group I, due to a reduced placental blood flow and a relatively smaller UV-FA lactate concentration difference as compared to Group II (Table 21). The latter suggests that in response to chronic fetal hypoxemia, there is a slight but significant increase in circulating lactate of fetal origin in Group I. In addition, assuming that umbilical blood flow over the antepartum period was similar to that measured at the pre-labor stage, the UV-FA substrate concentration differences may reflect differences in substrate uptake. Thus, it appears that a low fetal O₂ uptake in Group I animals was maintained throughout the last 10 days of gestation (Figure 13), while umbilical glucose and lactate uptake in Group I would be gradually decreased with advancing gestation (Figure 15 and 17). Taken together, our data suggest that fetal oxygenation and substrate availability in Group I fetuses became gradually compromised prior to the onset of labor. The elevated AVP concentrations in Group I before labor are consistent with chronic fetal compromise (Oosterbaan et al, 1985; Wintour et al, 1985).

In many ways our observations on fetal condition in Group I during the antepartum period resemble those observed in the growth restricted human fetus, including hypoxemia, hypoglycemia and hyperlacticemia (Soothill et al. 1987; Economides and Nicolaides, 1989; Nicolaides et al. 1989; Soothill et al. 1992). The human growth restricted fetus also responds to chronic hypoxemia with a blood flow redistribution to the
vital organs at the expense of peripheral organs, as demonstrated by Doppler studies (Soothill et al. 1986a; Wladimiroff et al. 1986; Di Renzo et al. 1992). Furthermore, studies have shown abnormal flow velocity waveform patterns in the human growth-restricted fetuses using Doppler recordings of the umbilical artery (Erskine and Ritchie, 1985; Fleischer et al. 1985; Karsdorp et al. 1994; Okagaki et al. 1994; Jackson et al. 1995; Yoshimura et al. 1997). These changes may reflect inadequate growth of the placenta and are most likely to be an indication of increased impedance to blood flow, thus implying reduced umbilical blood flow. Our experimental data in the present study support these clinical observations. Thus, although the initiating causes for fetal growth restriction may be quite different and not easily identifiable, the consequences for both the human and sheep asymmetrically growth restricted fetuses appear similar in terms of the final functional consequences.

Glucose and lactate are quantitatively important nutrients of the ovine fetus for fetal growth and metabolism (Battaglia and Meschia, 1986c). It is generally accepted that in fetuses of well-fed ewes near term and under undisturbed in utero conditions, umbilical glucose uptake normally accounts for approximately 35-50% of fetal oxidative metabolic requirements and umbilical lactate uptake for about 20-25% (Tsoulos et al. 1971; Hay et al. 1981; Sparks et al. 1982; Faber and Thornburg, 1983; Hay et al. 1983). Assuming that all of the antepartum fetal blood samples in the present study were taken when the ewes were in the fed-state, the glucose/oxygen quotient in Group II was 0.21 and the lactate/oxygen quotient was 0.22 (Table 21). The former value is low most probably because glucose uptake is low relative to oxygen uptake in this group. Together, glucose
and lactate could account for 43% of the substrates for fetal oxidative metabolism. Thus, it appears that the Group II fetuses may have utilized large portions of substrates other than glucose and lactate at this stage. In contrast, the glucose/oxygen quotient in Group I animals was 0.46 and lactate/oxygen quotient was 0.16 (Table 21). The low value of lactate/oxygen quotient is due to lower lactate uptake relative to oxygen uptake. But the results of glucose/oxygen quotient in this group of animals are consistent with those reported by others regarding the glucose oxidation fraction in late gestation (Tsoulos et al. 1971; Hay et al. 1981; Hay et al. 1983). Moreover, maternal undernutrition is usually associated with a decrease in both umbilical glucose/oxygen quotient and lactate/oxygen quotient (Hay et al. 1983; Chandler et al. 1985; Leury et al. 1990a). Clearly, this was not the case for animals in this study.

In association with the modest antepartum fetal hypoxemia, Group I significantly increased oxygen extraction over the last 10 days of gestation (Figure 14). While this compensatory response helps to maintain fetal oxygen consumption, the margin of safety, defined as a difference between substrate delivery and consumption (Owens et al. 1987a; Owens et al. 1987b), is reduced (Figure 28). As a consequence, these growth restricted fetuses became vulnerable to a further reduction in oxygen supply, particularly during labor. Similarly, the increased glucose extraction with the decreased glucose fetal concentrations could have actually improved maternal to fetal glucose transfer because of an increased concentration gradient for glucose between the maternal and fetal circulations (Hay and Meznarich, 1989; Molina et al. 1991). But again the increased extraction of
glucose by the growth restricted fetuses indicates that there is also a reduction in the margin of safety for glucose in these animals (Figure 29).

When placental growth is restricted, a limitation of placental transfer of oxygen due to reduced placental O\textsubscript{2} diffusion capacity could occur. In addition, a reduction in fetal and/or maternal placental blood flows would also affect not only placental transfer of highly diffusible substances such as oxygen, but also rates of delivery of other substrates to the gravid uterus and fetus (Battaglia and Meschia, 1986b; Rurak, 1995). In the present study, we did not measure uterine blood flow, so it is not known if a reduction of uterine blood flow occurred in Group I, however given the markedly reduced placental weight and umbilical blood flow in this group it seems possible. Certainly, it would be interesting in a future study to measure the uterine blood flow and substrate metabolism and further explore the cause of the reduced placental and fetal growth in naturally occurring growth restriction in sheep. Nevertheless, the lower oxygenation observed in Group I fetuses appears to be related to the smaller placental weight. A significant correlation between umbilical venous oxygen saturation and placental weight was observed (Figure 18), suggesting that the deceased placenta weight was associated with a reduced ability to supply oxygen to the fetus. The uptake of glucose by the placenta and its transfer to the fetus are mediated by transporters (Battaglia and Meschia, 1986c). Placental to fetal glucose transport capacity increases with gestation (Molina et al. 1991), probably due to an increase in placental surface area and thus the number of glucose transporters in placental tissue (Hay and Wilkening, 1994). When placental growth is restricted, placental surface area may also be reduced along with placental mass (Marconi
et al. 1990). As a result, placental transfer of glucose could be altered. In the present study, there is a significant correlation between placental weight and umbilical glucose delivery (Figure 29). This is associated with a decrease in UV glucose concentrations in Group I as compared to Group II (Table 14). Thus, our data suggest that as in experimentally induced fetal growth restriction, a deficit in the supply of oxygen and glucose may have limited fetal growth in Group I, probably directly through their own effects and indirectly through hormonal influences. It has been shown that the metabolic adaptations of the growth restricted fetuses are accompanied by endocrine changes to compensate for reduced fetal oxygen and nutrient supply. These hormone-induced mechanisms, such as reduced insulin (Robinson et al. 1980; Harding et al. 1985), thyroxine, triiodothyronine (Mellor and Pearson, 1977; Robinson et al. 1980; Harding et al. 1985; Jones, 1985), and the IGF-I and II (Owens et al. 1994; Kind et al. 1995) concentrations, could in turn modulate intrauterine fetal growth.

Fetal oxygen consumption rate (V02) has been measured in many fetal sheep studies during late gestation (Itskovitz et al. 1983; Wilkening and Meschia, 1983; Edelstone et al. 1985; Milley, 1988; Edelstone et al. 1989; Rurak et al. 1990a; Van der Weyde et al. 1992; Kwan et al. 1995b). Its normal range has been defined as 260-360 μmol/min/kg fetal body weight (Battaglia and Meschia, 1986c). In the present study, fetal V02 as measured at pre-labor stage averaged 213 ± 20 and 513 ± 86 μmol/min/kg in Group I and II respectively. Thus, in Group I, fetal V02 is below the lower limit of defined normal range, whereas in Group II, it is well above the upper limit. The decrease in fetal V02 in Group I, thus lowering fetal metabolic requirements, reflects a major adaptive mechanism designed
to balance demand to supply in these growth restricted fetuses. However, the reasons for the high mean Vo$_2$ value in Group II animals are not clear. Some of the factors which may influence fetal Vo$_2$ include fetal breathing movements (Rurak and Gruber, 1983a; Rurak and Gruber, 1983b), body movements (Natale et al. 1981; Harding et al. 1983) and cardiac work (Fisher, 1984b). These activities may require a significant fraction of the total fetal Vo$_2$. In the present study, there is no difference between the two groups in terms of fetal heart rate and pressure product at pre-labor measurement (8202 ± 370 vs. 8100 ± 512), so it can be assumed that there would be a similar myocardial oxygen consumption in these two groups at this stage. On the other hand, the percentage of time spent on fetal breathing movements at -3 days prior to labor was 21.6 ± 3.9 and 33.5 ± 4.2%; while at -2 days, it averaged 18.6 ± 1.4 and 25.8 ± 3.3% in Group I and II respectively. This comparison indicates that during these two days, in which our pre-labor measurements were made, fetal breathing movements were higher in Group II than in Group I. This may partly contribute to the increased fetal Vo$_2$. However, fetal Vo$_2$ in Group II is still high relative to published data even in early labor (457 ± 81 µmol/min/kg), that is, at the time when fetal breathing movements had ceased. Thus, some other mechanisms may be involved, which cannot be identified at this point. There appears to be only one other study that has measured umbilical blood flow, and O$_2$ delivery and consumption in fetal lambs as late in gestation as in the present study. In 7 fetal lambs at 137.7 ± 0.8d, umbilical flow, Do$_2$ and Vo$_2$ were 191 ± 10 ml/min/kg, 922 ± 46 µmol/min/kg and 312 ± 41 µmol/min/kg, respectively (Rurak et al, 1987a). The umbilical blood flow value is very similar to that in Group II (194 ± 19 ml/min/kg) and to other studies in late gestation (Cohn et al. 1974; Block et al. 1984; Rurak et al. 1990a; Jensen...
and Lang, 1992), whereas Do2 and Vo2 are lower than in Group II (see Table 43). The differences are largely due to the higher values for umbilical venous O2 content (5.59 ± 0.48 vs. 4.83 ± 0.28 mmol/L) and especially the umbilical veno-arterial O2 difference (2.50 ± 0.17 vs. 1.66 ± 0.18 mmol/L) and O2 extraction (45.2 ± 2.4 vs. 33.3 ± 3.7%) in Group II. Given the relatively small numbers of animals in Group II and in the study of Rurak et al (1987a), further measurements of these variables in the fetal lamb in the immediate prepartum period are warranted. It may be that some of the fetal endocrine changes (e.g. in thyroid hormones; Fraser and Liggins, 1988; Fraser and Liggins, 1989; Fowden and Silver, 1995) at this time may act to increase fetal metabolic rate. On the other hand, the umbilical blood flow in Group I fetuses at the pre-labor stage (119 ± 13 ml/min/kg) is much lower than in Group II and than the published values. Thus, the decreased placental flow may partly explain the reduced fetal Vo2 in Group I. Furthermore, after correcting for placental weight, the umbilical blood flow is also lower in Group I than in Group II (201.35 ± 24.07 vs. 253.40 ± 21.00 ml/min/100 g).

Although placental function appears markedly reduced in Group I animals, umbilical blood flow (Figure 21 and 22), fetal cardiac output (Figure 23 and 24), umbilical glucose delivery (Figure 29), as well as fetal oxygen delivery (Figure 25) and oxygen consumption (Figure 26) are closely matched to the placental weight. Moreover, the magnitude of fetal growth restriction is directly related to the decrease in placental size (Figure 8). These parallel adjustments are consistent with the concept of a programmed reduction in fetal growth, perhaps via the endocrine and/or growth factor mechanisms mentioned above, so as to maintain the balance between placental nutrient supply and fetal metabolic demands.
In addition to a decrease or cessation of fetal growth, other adaptive mechanisms may also be brought into play, including a decrease in FBM and perhaps body movements, and a redistribution of cardiac output by diversion of blood flow to vital organs as discussed previously. These metabolic, behavioural and cardiovascular adjustments would appear to have a distinct survival value for these fetuses, as the absolute utilization of oxygen and metabolic substrates can be decreased and supply of oxygen and glucose to the major organ system maintained.

Finally, regardless of how fetal growth is restricted, either by experimental manipulation or through naturally occurring form from unknown causes, as in the present study, a remarkably consistent picture of the fetal compensatory mechanisms emerges. This consistency suggests that there may be common underlying regulatory mechanisms, which need to be further elucidated. Moreover, similar to the findings of a number of studies on experimental manipulation of placental growth and its effect on fetal growth, the restricted placental growth in Group I appears to have also limited fetal growth, with a resultant smaller placental/fetal weight ratio. The restricted placental growth is closely associated with evidence of compromised placental function. Because of the different time course of placental and fetal growth during gestation (section 1.1.4.2), an early limitation of placental growth can have consequences for fetal growth later in gestation. Thus, it is possible that it was early placental growth impairment which led to the fetal growth restriction in Group I, but verification of this requires further work.
4.3.2 Fetal Heart Rate and Blood Pressure

As noted in the section 1.8.1, previous studies have demonstrated that with increasing gestational age in the sheep fetus, basal fetal heart rate falls (Boddy et al. 1974; Walker et al. 1978; Dawes et al. 1980; Dawes, 1985; Blanco et al. 1988a; Kitanaka et al. 1989; Wakatsuki et al. 1992a; Gagnon et al. 1994) and arterial blood pressure rises (Boddy et al. 1974; Dawes, 1985; Kitanaka et al. 1989; Gagnon et al. 1994). The rate of the fall of mean fetal heart rate is 0.67 beats/min per day and the rise of mean arterial blood pressure is 0.46 mmHg per day between 100 days and 145 days gestation (term 147 days) (Boddy et al. 1974). However, in the previous studies, the monitoring of fetal arterial pressure and heart rate ended several days prior to labor onset. Also, that data in most studies were obtained only for relatively short times (e.g., several hours per day). Thus it appears that no comparable data exist for the daily changes over 24 h in fetal heart rate and arterial blood pressure up to and during parturition. In the present study, fetal heart rate and arterial blood pressure were continuously monitored every day throughout last 8-9 days of gestation to delivery. Thus the information gained from 24 h recordings can be utilized not only to assess the cardiovascular signals for imminent onset of labor but also to compare the sequence of changes occurring in fetal heart rate and arterial blood pressure between our two groups of animals over the antepartum period.

Fetal arterial blood pressure increased progressively as parturition approached in both groups, although the daily rate of increase in Group I was significantly less than in Group II. A similar reduction rate of fetal arterial pressure increase has been observed in ewes subjected to
reduced food intake in early pregnancy compared to normally fed ewes (Crowe et al. 1997). There is not yet a precise explanation for this phenomenon, though it may be due to the tendency for a reduced combined ventricular output in Group I compared to Group II (Table 18), and if peripheral resistance was equal in the 2 groups, this could result in a lesser rise in arterial pressure in Group I. The observed increase in fetal arterial blood pressure with advancing gestation is consistent with previous observations (Boddy et al. 1974; Dawes, 1985; Kitanaka et al. 1989). However, it appears that the rate of mean arterial blood pressure rise in both groups is larger in the last 7-8 days of gestation than that previously reported over the last third gestation, with an increase of 1.8 mm Hg per day in Group II and 0.9 mm Hg per day in Group I. Since the increase in fetal arterial blood pressure over the last 1-2 days prior to labor is not dramatic, fetal arterial blood pressure appears not to be a reliable indicator of impending parturition in sheep.

In keeping with previous observations (Boddy et al. 1974; Walker et al. 1978; Dawes et al. 1980; Dawes, 1985; Blanco et al. 1988a; Kitanaka et al. 1989; Wakatsuki et al. 1992a; Gagnon et al. 1994), our data from the present study also demonstrate a decrease in fetal heart rate between 7 and 2 days prior to labor in Group II animals, but also with a faster rate of decline (-4.4 bpm/d) over the last several days of gestation than has been reported earlier in gestation (-0.67 bpm/d, Boddy et al, 1974). One noteworthy finding of the present study is that following the gradual fall in fetal heart rate from day -7 to day -2, thereafter there is a significant increase in rate on day -1. This was especially obvious in Group II (Figure 20), but a similar trend was also apparent in Group I.
The pattern of fetal heart rate in relation to the timing of spontaneous labor which we observed in the present study has not been previously described. This 'rebound' increase in fetal heart rate coincided with the significant decrease in the incidence of fetal breathing movements on day -1 in both groups (Table 22). Thus, a marked decrease of incidence in fetal breathing movements in combination with an increase in fetal heart rate from day -2 to day -1 before parturition appear to be highly suggestive of the imminent onset of spontaneous labor.

In the late gestation, autonomic control of the heart is well established in the fetal lamb (Walker, 1984; Walker, 1994). \( \beta \) adrenergic stimulation increases fetal heart rate, whereas the parasympathetic nervous system via muscarinic receptor activation depresses fetal heart rate. Throughout the last third gestation, the parasympathetic nervous system exerts a greater influence on the heart rate than does the sympathetic nervous system and is therefore mainly responsible for the gradual decrease in fetal heart rate over this period (Walker, 1984; Battaglia and Meschia, 1986d; Walker, 1994). However, the 'rebound' pattern of fetal heart rate observed in the present study suggests that at term just before parturition, there appears to be a switch from the parasympathetic to the sympathetic influence on the fetal heart rate. This switch may reflect the time course of fetal heart rate change in relation to the timing of parturition rather than to gestational age. Clearly, the accelerating basal heart rate from day -2 to day -1 prior to labor is not reflex in nature since there is a concurrent increase instead of decrease in fetal arterial blood pressure at the same time period. We postulate that this shift of autonomic control of the heart may help the fetus in preparing for the impending transition from fetal to post-natal life, because the sympathoadrenal system has
an important role in maintaining fetal cardiovascular and metabolic homeostasis in the perinatal period. The latter subject has been discussed in detail in section 1.9. Whether or not this changeover with an increased sympathetic stimulation is due to an increase in sympathetic nervous efferent activity or the direct action of circulating catecholamines remains to be elucidated. It is also possible that the some of the fetal endocrine changes immediately prior to parturition could be involved, for example the rapid increase in fetal circulating cortisol concentration (section 1.2).

4.4 Fetal Breathing Movements before and during Labor

Fetal breathing activity has been suggested to be a useful indicator of fetal well-being (Boddy and Dawes, 1975; Boddy, 1979). As noted in the section 1.10, the incidence of fetal breathing movements decreases during hypoxaemia in the sheep fetus (Bissonnette et al. 1989; Moore et al. 1989; Koos et al. 1992; Giussani et al. 1993; Koos et al. 1994b). More severe hypoxaemia produces a cessation of fetal breathing movements, accompanied by fetal gasping activity (Moore et al. 1989). In the human fetus, there is also a reduction in the incidence of FBM with hypoxaemia and acidemia (Bekedam and Visser, 1985; Ribbert et al. 1993a). Some studies have shown that a diminution or absence of breathing movements may predict fetal compromise in utero (Boddy and Dawes, 1975; Manning, 1977; Manning, 1990; Manning et al. 1993). Furthermore, FBM are significantly decreased in sheep fetuses with IUGR induced by restriction of placental growth using surgical excision of endometrial caruncles prior to pregnancy (Worthington et al. 1981; Maloney et al. 1982), and the decrease correlates with the reduction in oxygen tension
A low incidence in FBM is also observed in growth restricted human fetuses (Trudinger et al. 1979; Sival et al. 1992; Ribbert et al. 1993b). Conversely, an increase in FBM occurs during maternal hyperoxygenation in human IUGR fetuses (Ruedrich et al. 1989; Gagnon et al. 1990; Bekedam et al. 1991). These results suggest that there is a causal relationship between a reduction in the incidence of fetal breathing movements and the impairment of fetal oxygenation.

In the present study, the antepartum analysis on the incidence of fetal breathing movements indicates that the percentage of time spent breathing over 24 hr period during 4-6 days before delivery is present for about 24 to 29% in Group I and 37 to 40% in Group II respectively. Gasping efforts (i.e. large inspiratory efforts), which may represent a fetal analog to regurgitative activity in adult sheep (Harding, 1984), occurred intermittently for 4% of the time in Group I and 2% in Group II during these days. FBM were consistently lower in Group I than in Group II over each of these days, whereas the incidences of fetal "gasing" was consistently higher (Table 22). These observations on FBM in Group I are thus in accordance with those previously reported from the other studies either on human fetus (Trudinger et al. 1979; Sival et al. 1992; Ribbert et al. 1993b) or sheep fetus (Worthington et al. 1981; Maloney et al. 1982).

In Group I, the reduced incidence of FBM is associated with fetal chronic hypoglycemia and hypoxemia as compared to Group II during the prenatal period. As noted in the section 1.10, fetal blood glucose is involved in the modulation of fetal breathing activity. Thus, the reduced fetal breathing movements could be related to impaired glucose supply,
acting as a protective mechanism for the fetus when energy substrate is lacking. Spontaneous hypoglycemia with glucose concentration < 10mg/dl (0.56 mmol/L) is associated with reduced FBM in sheep (Boddy and Dawes, 1975; Lewis and Boylan, 1979). The average blood glucose concentration throughout last 10 days of gestation in Group I fetuses is 0.49 mmol/L, only 67% of Group II. Thus, it appears that this factor may contribute to the diminution in fetal breathing in Group I.

Similarly, the mean fetal arterial Po<sub>2</sub>, O<sub>2</sub> saturation and O<sub>2</sub> content are all significantly lower in Group I than in Group II over the antepartum period, although this was of a modest nature. Thus, chronic hypoxemia in Group I may also have contributed to the reduced breathing activity. Our findings thus support the view that in the growth restricted fetuses a causal relationship exists between reduction of fetal breathing movements and fetal chronic hypoxemia due to placental insufficiency. The reduction of fetal breathing activity may be considered as one of the compensatory mechanisms to the decreased oxygen supply, since FBM can increase fetal O<sub>2</sub> demand (Rurak and Gruber, 1983a). Thus, such an inhibitory action on fetal breathing movements may assume particular importance during periods of chronic oxygen deprivation. In the present study, fetal plasma adenosine concentrations were not measured. However, in human growth restricted fetuses, the change in plasma adenosine concentration correlates inversely and significantly with the incidence of fetal breathing movements (Yoneyama et al. 1994). Thus, it might be speculated that adenosine formation may also be involved in the observed reduction in the incidence of fetal breathing movements in Group I fetuses.
Fetal breathing movements are subject to many influences, such as time of day and maternal plasma glucose levels (Lewis and Boylan, 1979). These influences are difficult to control. Besides, fetal breathing movements depend on fetal behavioral states as discussed in section 1.10. Therefore, a longer time period of observation on FBM is necessary to assess the health of fetuses. The results in the present study indicate that measurement of the amount of time spent breathing over a 24 hr observation period could provide additional information about antepartum fetal well-being in utero particularly when fetal growth is restricted.

Our results indicate that both groups of fetuses had a similar significant decrease in the incidence of fetal breathing movements over the last 2-3 days before delivery (Table 22). The maximum average daily incidence of FBM over the last 7 days of gestation was 29 ± 3% in Group I and 40 ± 3% in Group II, and this fell to 16 ± 2% and 18 ± 1%, respectively on the day prior to delivery. There was a further dramatic decrease in FBM with the onset of labor. But FBM continued in both groups during the early stage of labor with a higher incidence in the normally grown fetuses (Table 47). At the mid labor stage, no episodes of FBM were present in either group. These results are similar to published observations in chronically instrumented fetal lambs, which have reported a decrease in FBM 2-3 d before the onset of labor, and a virtual absence during labor (Dawes, 1973; Berger et al. 1986). In the human fetus, there is also a significant decrease in FBM during the last 3 days before spontaneous parturition at term (Carmichael et al. 1984). The incidence is further decreased with the onset of labor and almost completely abolished during the active labor stage (Richardson et al. 1979; Boylan and Lewis, 1980). Thus, our
results in the present study are in agreement with the previous work and confirm that a decrease in the incidence of fetal breathing movements at the end of gestation can be used as a predictor of impending spontaneous labor (Richardson et al. 1979; Boylan et al. 1985). There are also reports of the use of FBM monitoring to distinguish "true" from "false" preterm labor, with "true" labor being associated with an absence of FBM (Jaschewatzky et al. 1986, Besinger et al. 1987). However, because of the relatively short time between the FBM monitoring and delivery in the "true" group, monitoring of FBM would seem most useful as a confirmatory method, rather than as a predictor of preterm labor (Lockwood, 1995). This latter view is supported by the present results, since in Group I and II, the cessation of FBM followed the onset of labor on average by ~200 and 95 min, respectively. Moreover, there was a wide variability in the time interval between the 2 events in the individual animals.

The potential mechanisms for the decrease in FBM in late gestation and with the onset of labor were discussed in the section 1.10. The complete explanation for the phenomenon is lacking as yet. Decreased fetal oxygenation, either during the course of labor as in the current study or intermittently during the uterine contractures (Nathanielsz et al. 1980a; Harding et al. 1981), and/or increased circulating levels of adenosine (Bissonnetette et al. 1990; Koos and Matsuda, 1990; Bissonnetette et al. 1991; Koos and Doany, 1991; Koos et al. 1994b) or PGE\(_2\) (Kitterman et al. 1979; Kitterman et al. 1983; Fowden et al. 1989; Walker, 1990; Thorburn, 1992; Savich et al. 1995; Hollingworth et al. 1996) have all been implied in the diminution and/or cessation of FBM. In addition, a recent study has demonstrated that when placental extract is administered to fetal sheep, fetal breathing is
decreased in 79% of the experiments and completely abolished in 71% of animals, thus, indicating the presence of a factor produced by the placenta which inhibits spontaneous fetal breathing (Alvaro et al. 1996). In terms of the antepartum decrease in FBM, studies in the human fetus have demonstrated that umbilical venous and arterial \( P_{O_2} \) fall with gestational age (Soothill et al. 1986b; Nicolaides et al. 1989). A similar situation may exist in the fetal sheep (Bell et al. 1986). However, it is not known if this fall in \( P_{O_2} \) with approaching parturition could trigger adenosine secretion especially during the last 2-3 days before delivery and account for the gradual decrease in breathing activity prior to labor. Moreover, although fetal arterial \( P_{O_2} \) did decrease in Group I over the last 10 days of gestation, this was not the case in Group II, so that this mechanism does not seem likely in the latter group, at least prior to the onset of labor. Nevertheless, it may be reasonably assumed that release of adenosine would likely occur in response to the hypoxemia that occurs during labor, which could explain the cessation of FBM at that time. However, testing this hypothesis via experiments involving fetal adenosine receptor blockade during labor have not yet been done.

Many hormonal changes occur during the days before the onset of parturition (see INTRODUCTION), and an earlier study in fetal sheep suggested that the decrease in fetal breathing movements may be related to the fall in placental progesterone output before labor (Parkes et al. 1988). However, a recent study does not support this view, since reducing maternal progesterone synthesis leads to an increase in FBM in pregnant sheep (Nicol et al. 1997). To date no other circulating hormone has been shown to have a consistent significant effect on fetal breathing movements except PGE\(_2\). However, some
studies argue against circulating PGE$_2$ being the sole mediator, since there is the absence of a consistent correlation between changes in plasma PGE$_2$ and changes in breathing activity (Adamson et al. 1991). Others have shown that the inhibition of breathing within 48 of labor, although correlated with changes in plasma concentrations of PGE$_2$, is not abolished by prostaglandin synthesis inhibitor administration (Wallen et al. 1988). If the decrease in breathing movements closely before and during labor is not due to an increase in plasma PGE$_2$ concentrations (Wallen et al. 1988), there must be the other as yet unidentified respiratory inhibitors produced at that time.

4.5 Identification of the Onset of Parturition

In terms of predicting when parturition would likely occur, we primarily used FBM (see section 4.4) and FHR changes (see section 4.3.2), as well as maternal physical signs such as enlargement and swelling of the vulva in the ewe. The combination of these predictors yield valuable information for us regarding the approximate time (i.e. within ~24-48 h) of labor onset in individual animals. Previous studies suggest that the sheep fetus underwent significant changes in its fluid and electrolyte balance during the last few days before delivery (Mellor and Slater, 1971; Mellor and Slater, 1972a; Mellor and Slater, 1972b; Mellor et al. 1975). They include a rise in fetal urine osmolality 1-2 days prior to delivery, accompanied by an increase in fetal urine sodium and especially in fetal urine potassium concentrations and a decrease in fetal urine pH. There are also similar patterns of osmolalities and electrolyte changes in amniotic fluid. Furthermore, plasma protein concentrations in the sheep fetus appear to increase progressively towards term
(Nathanielsz et al. 1980b; Bland et al. 1982). Thus, in the present study, we also measured amniotic and fetal plasma osmolality, sodium, potassium and chloride concentrations as well as fetal plasma protein concentrations in 3 animals of Group II (data not presented). However, the results obtained from these animals were not particularly convincing in terms of the use of these variables as predictors of impending parturition.

On the other hand, as described in the EXPERIMENTAL section, the onset of labor in the present study was identified mainly by the switch from the pre-labor type uterine contractures to the early-labor uterine contraction pattern. Continuous monitoring of intraamniotic pressure allows for observation and interpretation of spontaneous changes in uterine activity. When labor appeared to be imminent, as judged by gestational age, diminution in FBM, daily changes in fetal heart rate with gestation and maternal physical signs, we closely monitored the intrauterine pressure recorded on the polygraph chart and assessed the change of uterine contraction pattern. By this continuous monitoring and "real time" analysis of the transition pattern of uterine activity, together with assessment of cessation of FBM and maternal behavioral changes such as pawing the ground and frequent urination, the onset of labor was reliably detected in all the animals.

4.6 Changes in Uterine Activity during Labor

In the present study, the frequency, intensity and duration of each uterine contraction in spontaneous labor were measured by a visual assessment of intraamniotic pressure
The total work performed by the uterus during labor was quantified by our modified Alexandria unit, which incorporates the sum of the intensity of the uterine contractions, the sum of their duration and the contraction frequency per one hour period at each stage of labor. Use of these measures demonstrated that with the progress of labor, a steady and significant increase in uterine activity occurred in both groups of animals. These changes in uterine activity during labor are consistent with the findings from other studies (Harding et al. 1982; Verhoeff et al. 1985; Lye and Freitag, 1990). In these studies, myometrial electromyograms (EMG) were recorded in sheep during labor and delivery. During labor, the mean duration of each EMG burst is decreased and the frequency of the bursts increased; whereas an increase in intrauterine pressure is associated with each EMG burst. More importantly, the present study clearly shows that uterine activities are not different in Group I from those seen in Group II animals in relation to the successive stages of labor. As discussed in section 1.5, during labor uterine contractions intermittently impede uteroplacental blood flow to a degree proportional to the intensity, duration and frequency of the contractions (Assali et al. 1958; Assali et al. 1961; Greiss, 1965; Caton et al. 1980; Harbert, 1992). Thus, the similar changes in uterine activity during labor in the 2 groups indicate that this cannot account for the different pattern of intrapartum cardiovascular and metabolic changes in Groups I and II.
4.7 Fetal Acid-Base and Oxygen Characteristics during Labor

Labor in the normally grown fetuses (Group II) was characterized by a mild impairment of fetal respiratory gas exchange across the placenta with a gradual fall in fetal \( O_2 \) saturation and content, presumably secondary to uterine contractions. This was associated with a progressive decline in fetal pH and moderate degree of fetal acidemia at delivery, largely due to a progressive increase in fetal lactate concentrations during late labor. This appears to be a normal, physiologic response of the normally grown fetus to labor and delivery. The mean values of fetal blood gas and acid base changes in the present study are similar to the findings of previous studies in the fetal sheep during labor and at delivery (Comline and Silver, 1972; Stark et al. 1979; Stark et al. 1981; Rurak et al. 1987a). Also, the blood gas and acid-base values at delivery are comparable with the cord blood values obtained from human healthy fetuses following term vaginal delivery (Low et al. 1974; Eskes et al. 1983; Yeomans et al. 1985; Ingermarsson et al. 1986; Rurak et al. 1987b, Thorp et al. 1989; Riley and Johnson, 1993). Thus, the normally grown fetus appears to be able to cope well with labor and adapt appropriately to meet the challenge. It is of interest that in Group II, the CA/FA ratio in \( O_2 \) saturation and \( O_2 \) content was significantly increased in late labor and at delivery (Table 31). This suggests that the preferential delivery of oxygenated blood may occur during labor, perhaps as a mechanism to maintain normal cerebral oxygenation.

In the growth restricted fetuses (Group I), there is a rapid deterioration in fetal metabolic blood gas and acid-base status during labor. These animals developed severe fetal hypoxia
and profound metabolic acidemia throughout labor, associated with a higher mean lactate level, lower pH, and more pronounced base deficit than those in Group II (Table 25). This suggests that severe metabolic acidosis and lactic acidemia occurs more frequently in the growth restricted fetus. However, there appear to be no other data on blood gas status, oxygen content and acid-base balance in growth restricted fetal lambs during labor, nor comparable results in other animal species. There are also limited studies on the growth restricted human fetus during labor. Lin et al (1980) compared cord blood lactate, pH, and blood gas values in 37 IUGR and 108 AGA infants at the time of delivery and found that the IUGR fetus develops acidosis and lactic acidemia of greater severity than the AGA fetus. Low et al (1981) demonstrated that the probability of fetal metabolic acidosis during labor increased two fold between the normally grown and the growth-restricted fetus and threefold in the case of severe growth restriction (Low et al. 1981). Also, when fetal pH was measured in scalp blood samples at the beginning of the labor and in umbilical artery blood immediately after birth, fetal acidemia appeared to develop more rapidly in the growth restricted fetuses, as opposed to the normally grown fetuses, and cord arterial pH at delivery was slightly, but significantly lower in the former group (7.23 ± 0.08 vs. 7.27 ± 0.08, Nieto et al. 1994) despite the fact that fetal Po2 levels in labor remain within the “normal” range (Modanlou et al. 1974). Thus, biochemical observations in our growth restricted fetuses in the present study appear to be broadly in agreement with those made in the human fetus. Also, in this study, we observed that the severe acidemia in growth restricted fetuses produced a higher fetal arterial Po2 in late labor and at delivery. This is probably because fetal severe acidemia decreases Hb-O2 affinity (shifts
Hb-O₂ dissociation curve to the right) with a resultant increased umbilical venous Po₂, and thus fetal arterial Po₂ (Table 25).

In Group I animals at delivery, fetal FA pH ranged from 6.671 to 7.112 with an average of 6.876, representing severe fetal acidosis. Studies in the human fetus have demonstrated that acidosis and injury have a dose-response relationship (Goodwin et al. 1992; Low, 1993); the more severe the acidemia below a pH of 7.00 at delivery, the more likely the neonate is to have major organ injury and complications such as hypoxic encephalopathy, neonatal cardiac, renal and pulmonary morbidity. However, most of the infants observed in these studies appeared healthy before the acute intrapartum acidemia. The growth restricted fetus with chronic stress before labor may not tolerate such extreme degrees of acidosis.

The severe acidemia in Group I fetuses could have resulted from diminished utero-placental perfusion or from umbilical cord compression. A comparison of pH values in UV and UA can be used to determine whether fetal acidemia is due primarily to reduced umbilical placental perfusion or reduced utero-placental perfusion (Gordon and Johnson, 1985). Our blood acid-base studies showed that the pH values in both UV and FA blood are decreased and that there are small differences between FA and UV pH values in Group I animals throughout labor and delivery (Table 25 and 29). This is suggestive of reduced utero-placental perfusion as a major cause for fetal severe acidosis in these growth restricted fetuses, but measurements of uteroplacental blood flow would be required to substantiate this.
At the beginning of this study, we selected pH 7.15 as a cutoff value to classify our fetuses. This was based upon the results of several large human studies which have redefined the range of umbilical cord arterial pH values associated with fetal hypoxic distress as being 7.10-7.15, i.e. > than 2 standard deviations below the mean pH value in normal term deliveries (Thorp et al. 1989; Ramin et al. 1989; Winkler et al. 1991; Vintizileos et al. 1992). This range of pH is also far below what is accepted as normal for the fetal lamb in utero, which in our lab ranges from ~7.31-7.40 (van der Weyde et al. 1992; Kwan et al. 1995b). However, blood pH is altered by a change in either the carbon dioxide pressure or the bicarbonate concentration. Increasing the carbon dioxide pressure causes the pH to fall, resulting in respiratory acidosis. Decreasing the bicarbonate concentration, as occurs with the accumulation of lactic acid, also causes the pH to fall and results in metabolic acidosis. Thus, base excess or buffer base are often used as a measure of acid-base status to distinguish between metabolic and respiratory acidosis. The present study suggests that the moderate fetal acidosis in Group II at delivery is a mixed acidosis rather than purely metabolic or respiratory in nature, because the fetal P<sub>co2</sub> is increased and HCO<sub>3</sub> is decreased to a similar degree when expressed as a % change from the control level (12% vs.15%). In Group I fetuses, the fetal P<sub>co2</sub> is also increased, but HCO<sub>3</sub> is decreased to a larger degree (9% vs. 60%) with a very high buffer-base deficit (-22.3 mEq/L). Thus, in the growth restricted fetuses, metabolic acidosis predominates during labor. Low and colleagues (Low et al. 1975a; Low et al. 1975b; Low et al. 1975c; Low et al. 1981; Low et al. 1988; Low et al. 1995; Low, 1996) have used a buffer base of < 34 mmol/L as a criterion for intrapartum fetal asphyxia in the human and this is equivalent to a base deficit of > 12 mEq/L. Use of this latter cutoff
value would have resulted in the same division of the animals into the 2 groups as the base
deficit in all Group I fetuses (12.3-29.8 mEq/L) was below the cutoff while the values in
Group II (0.2-8.2 mEq/L) were all above it.

The possibility has been raised that the increase in catecholamine concentration that
normally occurs with birth and its attendant hypoxemia may mediate the hormonal and
metabolic alterations, since catecholamines decrease plasma insulin level and glucose
clearance and increase plasma glucagon level and hepatic glucose production in the fetal
sheep (Sperling et al. 1980; Bristow et al. 1983; Jones et al. 1983). Infusion of
catecholamines into fetal sheep increases both fetal glucose and lactate concentrations
(Jones and Ritchie, 1978; Palmer et al. 1984b; Apatu and Barnes, 1991). Thus, the rise in
the fetal glucose and lactate concentrations observed during labor may be partially due to
the rise in catecholamine levels. However, the severe lactic acidemia in Group I animals is
unlikely to have been due solely to elevated catecholamine levels. Rather it could have
occurred as a result of a shift to anaerobic glycolysis (Buchalter et al. 1989), indicating
that there is inadequate perfusion of certain tissues with progressive fetal hypoxia during
labor. Lactic acid can accumulate rapidly because, being non-volatile, it can be excreted
only through the fetal kidneys or placenta, neither route being particularly efficient (Rurak,
1994). In the present study, the fetal blood lactate concentrations were dramatically
increased in Group I from a mean value of 1.71 mM/L at pre-labor to that as high as 16.71
mM/L at delivery. Such substantial anaerobic metabolism did not occur in Group II
fetuses during the intrapartum period. Thus, our observation of the marked rise in fetal
blood lactate and fall in fetal pH levels during labor indicate that Group I fetuses were
seriously compromised so that placental and fetal compensatory mechanisms appear to be insufficient to sustain the growth restricted fetuses adequately through labor. This finding may be of clinical significance, since respiratory acidosis is not associated with neurologic damage, but metabolic acidosis is often associated with long term neurologic deficit and mental retardation (Low et al. 1994; Low, 1996).

The elevated lactate concentrations observed in Group I at delivery (16.71 ± 2.78 mmol/L) are much higher than those reported cord blood of human fetuses with intrapartum asphyxia. Suidan and Young (1984) in a study of 468 newborn infants used 3.70 mmol/L as the dividing point between normal and pathologic lactate levels. In that study, 12 depressed fetuses (1-minute Apgar score < 7) had the lactate concentrations of 5.70 to 7.69 mmol/L. The highest values were found in 5 fetuses with lactate concentrations being ≥ 7.70 mmol/L. Lin et al. (1980) demonstrated that in fetal cord blood from 15 infants with intrauterine growth restriction and FHR decelerations, the lactate levels were 5.47 ± 3.31 mmol/L. Eguiluz et al. (1983) reported that lactate concentrations averaged 5.89 ± 0.59 mmol/L in a group of neonates with Apgar score < 6 and pH < 7.20. Lactate levels in cord arterial blood were much higher in this group than in maternal blood, thus indicating that increased production of lactate is primarily of fetal origin. This is in contrast with our observations in the current sheep study in Group I, in which a relatively large portion of the lactate rise may have resulted from increased placental lactate production. Also, the mean lactate values in the UA at birth have been reported in the normal human fetuses, with a mean range of 1.87 to 3.70 mmol/L (Lin et al. 1980; Smith et al. 1983; Nordstrom et al. 1994; Westgren et al. 1995). Thus, Group II
fetuses in the present study also had a higher mean value at delivery (5.65 ± 1.11 mmol/L), being similar to those reported in the distressed human fetuses as noted above. These comparisons suggest that there may be differences of fetal and placental lactate metabolism between the human and sheep during labor and delivery, which will be further discussed in the section 4.9.2.

4.8 Fetal Cardiovascular Function during Labor

4.8.1 Changes in Heart Rate and Blood Pressure

The pattern of fetal heart rate and arterial pressure response to labor is different in Group I and Group II (Table 32). In Group I, fetal heart rate initially increased, but thereafter, it fell progressively and decreased to its lowest value at delivery, while in Group II, mean fetal heart rate was relatively constant during labor and at delivery. In fetal sheep at late gestation, the fetal heart rate and arterial pressure respond to acute hypoxemia with a hypertension and reflex autonomic bradycardia (Yaffe et al. 1987; Block et al. 1990a; Giussani et al. 1993). Several events contribute to the bradycardia, which include chemoreceptor stimulation of the vagus nerve, baroreceptor response to fetal hypertension, or direct myocardial depression if the hypoxia is severe enough (Itskovitz et al. 1982; Bocking, 1993; Giussani et al. 1993). The fetal hypertension seen with acute hypoxemia is the result of peripheral vasoconstriction (Reuss et al. 1982). However, if hypoxia prolonged for several hours, fetal heart rate is elevated, reaching a peak at 6 h (Rurak et al. 1990b). Also, more prolonged hypoxaemia in the absence of acidemia
secondary to a restriction in uterine blood flow leads to an initial bradycardia followed by a tachycardia lasting for up to 24 h (Bocking et al. 1988b). These results suggest that adrenal stimulation under prolonged hypoxaemia produces an elevation of catecholamines that may overwhelm the vagal drive. From these observations, it appears that the moderate increase in fetal heart rate at early-labor in Group I may be indicative of the initial sympathetic response to acute hypoxemia in these chronically hypoxemic fetuses; but afterward, with the progress of labor, the fetal heart rate was gradually decreased. This change is accompanied by a progressive fetal acidemia. Thus, later in labor, fetal heart rate was likely affected by the fetal metabolic status, perhaps a compromise in myocardial function.

Earlier clinical studies suggested that associated with uteroplacental insufficiency during labor, there were characteristic changes in fetal heart rate pattern, including late decelerations, loss of beat-to-beat variability, and baseline tachycardia (Martin, 1978). The late type of periodic deceleration suggests that the fetus is responding to a hypoxic stress (Martin, 1978). Maintenance of beat-to-beat variability indicates that although the fetus is stressed, metabolic acidosis and consequent depression of brain stem function have not yet occurred (Krebs et al. 1979; Kariniemi and Ammala, 1981; Fleischer et al. 1982; Gilstrap et al. 1984). Some reports suggest that the fetus entering labor with no previous chronic deficiencies tolerates intermittent stress well, with prompt recovery of baseline heart rate after each contraction. The chronically stressed infant, whose baseline heart rate has adapted to chronic hypoxemia, demonstrates deeper and longer lasting decelerations when faced with further reductions in oxygen delivery during labor contractions (Martin,
In the present study, data on fetal heart rate patterns during labor were not obtained largely because the recording method used did not allow analysis of beat-to-beat variability. Moreover visual inspection of the polygraph heart rate traces obtained during labor to identify gross patterns (e.g. decelerations) would take considerable time and this was not available within the time frame of the thesis. However, these data will be examined in the future. In addition, abnormal fetal heart rate patterns are not always associated with an adverse fetal outcome during labor and they have a rather low predictive value in identifying the fetus with significant oxygen deprivation (see section 1.7). Thus, it appears that a more detailed analysis of fetal heart rate variation during labor has remained elusive. This is partly because visual measurement is highly subject to observer error and bias (Donker et al. 1993; Strong and Jarles, 1993; Cibils, 1996). As a means to overcome the inherent error associated with visual analysis of fetal heart variation, and to increase the yield of information concerning fetal condition contained within the varying heart rate signal, Dawes and associates have developed a computerized method for objective analysis of the fetal heart rate pattern including the baseline heart rate, heart rate variability, the number and duration of fetal heart rate accelerations and decelerations (Dawes et al. 1990; Dawes et al. 1991a). However, the application of computerized analysis of fetal heart rate record in labor is still in the investigative phase (Dawes et al. 1991b; Pello et al. 1991).

Perhaps one of the most striking findings in the present study was the difference in arterial blood pressure response to labor between Group I and II. The response to labor in Group II was characterized by progressive development of hypertension during labor and at
delivery, while Group I fetuses showed no significant increase in arterial blood pressure. The results on the fetal hypertensive response to labor in Group II are in agreement with those previously reported during sheep labor (Comline and Silver, 1972; Rurak et al. 1987a; Chapman et al. 1994). However, the lack of hypertensive response observed in Group I during labor and at delivery was surprising, in that we observed that in response to labor, plasma vasopressin levels were very much higher in Group I than in Group II fetuses (Table 46). Moreover, it has been reported that there is a substantial increase in the plasma catecholamine concentration in fetal sheep during labor (Eliot et al. 1981; Brown et al. 1983; Richet et al. 1985; Habib et al. 1991; Oyama et al. 1992) and the severe hypoxia and metabolic acidemia developed in Group I during labor would further stimulate catecholamine secretion in these fetuses. Thus, the considerable increase in fetal plasma vasopressin and catecholamine concentrations during the course of labor and at delivery would be expected to produce a marked pressor response with increased peripheral vascular resistance, since the hypertension is thought to be mediated by α-adrenergic (Reuss et al. 1982) and vasopressin mechanisms (Rurak and Gruber, 1984; Tomita et al. 1985). However, arterial blood pressure did not change significantly in this group. Therefore, it appears that the peripheral vasoconstrictive effect of vasopressin and perhaps catecholamines was not as efficient in Group I as in Group II fetuses. The mechanism for the absence of the hypertensive response in Group I is unknown, but it may be related to the underdevelopment of blood vessels or immaturity of sympathetic innervation of vascular smooth muscle in these undergrown fetuses. It is also possible that the fetal severe metabolic acidemia in Group I that developed with the progress of labor could directly impair vascular responsiveness. For instance, following prolonged
hypoxaemia in fetal sheep, there is an initial pressor response at about 4h. When hypoxaemia is continued, fetal blood pressure returns to the control level at 6 h, but tends to decline at 8 h in the presence of marked acidemia (Rurak et al. 1990b). In addition, hypoxic lactic acidosis can cause vasodilation and hypotension in the dog (Landry and Oliver, 1992). The responses are thought to be a result of activation of an ATP-regulated K⁺ channel, which has recently been found to be an important modulator of vascular smooth muscle tone.

4.8.2 Changes in Cardiac Output and Patterns of Blood Flow Distribution

The fetal cardiac output and blood flow to individual organs and tissues during labor and at delivery were different between Group I and Group II (Table 33-41). In Group I, there appeared to be an increase in combined cardiac output, associated with an increased blood flow to most fetal organs, including placenta, brain, heart, adrenals, diaphragm, stomach, small intestine, large intestine and gall bladder. Blood flow to the lungs, skin, bone was moderately reduced, whereas the flow to the spleen was markedly reduced. However, at delivery, flow to most organ fell. In contrast, fetal combined cardiac output in Group II animals was maintained during the course of labor, whereas placental blood flow was progressively decreased. There was only a moderate increase in blood flow to the brain, myocardium and adrenals. Although blood flow to the spleen, lungs, skin and bone are reduced, flow to the other organs and tissues was maintained.
In response to labor, both groups demonstrated a redistribution of fetal cardiac output towards the vital organs (brain, heart and adrenals) at the expense of the non-vital and peripheral organs, but this was of a different degree. The distribution of cardiac output to the brain, myocardium, adrenals increased markedly in Group I, but less so in Group II. On the other hand, the percentage of combined ventricular output perfusing the placenta was lower in Group I than Group II. The pattern of cardiac output distribution to other individual fetal organs was also altered in a different manner in these 2 groups. In Group I, the percentage of combined ventricular output distributed to the diaphragm, stomach, small intestine and large intestine was increased, whereas in Group II, the proportion of cardiac output distributed to these organs was maintained.

There is little information regarding fetal cardiac output and patterns of blood flow distribution in response to hypoxic stress during labor. However, the cardiovascular responses to acute or prolonged hypoxemia and acidemia have been studied extensively in fetal lambs at late gestation using chronically instrumented pregnant sheep, who have been made hypoxemic by reducing maternal arterial oxygen content, decreasing uterine blood, or umbilical cord compression. With fetal hypoxemia caused by maternal hypoxemia, the fetal combined ventricular output does not fall as long as blood pH is maintained (Cohn et al. 1974). However when hypoxemia is accompanied by acidemia, cardiac output falls by ~20%. Umbilical blood flow is maintained, while blood flow to the fetal body is reduced by 40% (Cohn et al. 1974; Parer, 1980). The circulating blood is redistributed to the brain, heart, and adrenals at the expense of peripheral organs, including lungs, kidneys, gastrointestinal tract, and carcass (Cohn et al. 1974; Peeters et al. 1979). Unlike the effect
of maternal hypoxemia, a reduction of umbilical blood flow does not decrease umbilical venous oxygen content (Itskovitz et al. 1983; Itskovitz et al. 1987). A compression of the umbilical cord is accompanied by a fall in fetal cardiac output and a redistribution of blood flow to the fetal organs. But the pattern of fetal cardiac output distribution is different from that observed during maternal hypoxemia. Blood flow to the brain, heart and adrenals increases. Perfusion to the peripheral organs, including those to the kidneys, gastrointestinal tract, and spleen does not change, and that to the carcass increases. Only blood flow to the lungs falls (Itskovitz et al. 1983; Itskovitz et al. 1987). The cardiovascular effects of reduced uterine blood flow depend largely on the severity and type of the reduction. For instance, with a 50% reduction in fetal O₂ delivery achieved via graded reduction in uterine blood flow, blood flow and the fraction of the cardiac output distributed to the brain, heart, and adrenal gland increased and that to the lungs, carcass, skin, and scalp decreased, while umbilical blood flow was maintained (Jensen et al. 1991). Repeated brief arrest of uterine blood flow also causes a circulatory redistribution to the brain and adrenals at the expense of many peripheral organs, particularly of the skin, but blood flow to the lungs increases, while myocardial blood flow does not continue to increase beyond 4 min following repeated interruption of uterine blood flow (Jensen et al. 1985; Jensen et al. 1987). When hypoxia is prolonged by reducing maternal arterial oxygen content, the patterns of redistribution of blood flow to the vital organs are also maintained, but blood flow to the gut and skeletal muscle is sustained, whereas blood flow to brown adipose tissue increases and to the spleen and kidneys decreases (Rurak et al. 1990b). Moreover, umbilical blood flow initially increases but falls when severe acidemia develops (Rurak et al. 1990a). Thus, fetal responses to maternal hypoxemia and reduced
uterine blood flow were similar in many aspects, with a decrease in peripheral blood flow to the most fetal organs and tissues. On the other hand, decreased umbilical blood flow results in less peripheral vasoconstriction. The difference in response appears to be accounted for by the difference in the decrease of carotid arterial oxygen saturation. With umbilical cord compression, the decrease in carotid arterial oxygen saturation was considerably less than during maternal hypoxemia and reduced uterine blood flow, and therefore, chemoreceptor stimulation was less dramatic (Cohn et al. 1974; Edelstone et al. 1980; Reuss et al. 1982; Itskovitz et al. 1987; Jensen et al. 1991). Further exploration showed that the difference in response of carotid arterial oxygen saturation is related to streaming patterns of umbilical venous blood passing through the ductus venosus and foramen ovale, which is greatest during umbilical cord compression and least during hypoxic hypoxaemia (Edelstone et al. 1980; Reuss et al. 1982; Itskovitz et al. 1987; Jensen et al. 1991). Thus, maintenance in oxygen delivery to the brain and myocardium is not only due to the relative increase in their blood flow, but also to the preferential streaming pattern of the oxygenated blood. The response of the growth restricted fetuses to acute maternal hypoxemia has also been examined in fetal sheep (Block et al. 1984). Fetal growth restriction was induced following chronic embolization of the placental bed by microspheres. During imposed acute hypoxemia there was preferential perfusion of vital organs, the adrenal glands, brain, and heart in control and embolized fetuses. This preferential perfusion to the vital organs during hypoxemia was significantly more pronounced in embolized animals. As a consequence, the blood flow to other organs, such as the spleen, kidneys and lungs decreased.
By comparison with the studies described above with the present labor study, it is clear that in response to fetal hypoxia, a common feature is a circulatory centralization of blood flow distribution in favor of the brain, heart, and adrenals. This holds true even for growth restricted fetuses. Thus, preservation of oxygen delivery to these vital organs is an important adaptive mechanism during labor stress. In fact, the growth restricted fetuses had a more pronounced increase in blood flow to the brain, heart and adrenals than the normally grown fetuses during labor, expressed as either % of distribution of fetal combined cardiac output or ml/min/100 g flow value. This is consistent with the results of a study on the cardiovascular responses to acute hypoxemia in the fetal lamb with growth restriction secondary to embolization of the uteroplacental vascular bed (Block et al. 1984).

Although it is not completely clear which factors mediate metabolic regulation of blood flow in the fetal brain, heart, and adrenals, several studies have demonstrated that the blood flow to these organs increases in inverse relation to arterial oxygen content (Jones et al. 1978; Peeters et al. 1979; Sheldon et al. 1979; Ashwal et al. 1984). This relationship was also demonstrated in the present study (Figure 34-36). With the progressive decrease in fetal arterial O₂ content in both groups during labor, there is a corresponding increase in blood flow to the brain, heart and adrenals. The magnitude of this circulatory adjustment appears to be larger in Group I than in Group II, in that Group I fetuses had a larger decrease in fetal O₂ content, but a larger increase in blood flow and also % distribution of cardiac output to these 3 organs, as compared to Group II fetuses. Thus, arterial O₂ content may be a key variable in determining the magnitude of the blood flow to these
organs during labor. However, it is noted that the basal level of oxygen content before labor in Group I fetuses is already lower than in Group II. In addition, at the time of delivery in Group I, the mean values of blood flow to the brain, heart and adrenals did not continue to increase, rather there is a terminal fall, associated with a trend for decreased cardiac output. One likely mechanism for these changes is the fall in fetal heart rate in Group I at delivery. In late gestation, cardiac output falls in proportion to the fall in heart rate. However, in Group I, the % fall in heart rate at delivery from the late labor value (~7%) is less than the fall in cardiac output (~29%). Thus, other mechanisms could be involved (perhaps impaired myocardial function due to the acidemia). Nevertheless, in Group I an important compensatory mechanism during labor appears to be an increase in cardiac output. The absolute cardiac output during labor was significantly increased by 36% in early labor, 40% in mid labor and 50% in late labor. But at delivery, it returned to a level similar to the control. The changes in blood flow to the brain, heart, adrenals, umbilical placenta, diaphragm, stomach, small intestine, large intestine and gall bladder in Group I animals closely follow the change in fetal cardiac output. This, together with the more pronounced % distribution of cardiac output to the brain, heart and adrenal in Group I than in Group II, suggest that for the growth restricted fetuses both an increase in fetal cardiac output and a redistribution of blood flow are important to compensate for a progressive reduction in fetal oxygen delivery during labor. However, it appears that these mechanisms are available only for a limited time. With severe metabolic acidosis at the time of delivery, these fetuses appear to be no longer able to maintain preferential perfusion, so that decompensation occurs. The causes of this late decompensation warrant further study.
As in Group I, it appears that for the normally grown fetuses, a redistribution of regional blood flow is one important compensatory response to labor, which preserves oxygen delivery to the vital organs. However, as noted above, the magnitude of the flow changes were less than in Group I. Moreover other compensatory mechanisms (e.g., an increase in oxygen extraction) also occur (see section 4.9.1). In addition, although the present study did not directly assess the preferential streaming patterns of oxygenated blood, indirect evidence obtained from the changes in the CA/FA ratio in O₂ saturation and O₂ content during labor in Group II suggest there may be an increase in the preferential distribution of O₂ to the upper body. These responses, particularly in terms of the blood flow changes, are in some respects similar to those seen during acute hypoxemia in a number of fetal sheep models as described previously (Cohn et al. 1974; Peeters et al. 1979; Reuss et al. 1982; Itskovitz et al. 1983; Itskovitz et al. 1987; Jensen et al. 1987; Jensen et al. 1991). However, differences exist in the changes of blood flow to some organs and tissues. For instance, blood flow to the gastrointestinal tract and skeletal muscle are maintained throughout labor and delivery in this group. This is comparable to the results of a sustained hypoxaemia study (Rurak et al. 1990b), but unlike with sustained hypoxemia, blood flow to the kidneys and brown adipose tissue are also maintained during labor. These differences may be due to a different duration and extent of hypoxia and degrees of reduction in oxygen delivery through these vascular beds. More importantly, labor itself is a much more complicated, dynamic process compared to isolated hypoxia induced by various experimental manipulations, since several different events can occur simultaneously during labor (e.g., reduction in uterine blood flow and umbilical blood
flow, neurohormonal responses, mechanical stimulation, progressive disruption of the fetal-maternal interface, and maternal stress).

4.9 Fetal Metabolic Status during Labor

4.9.1. Changes in Fetal Oxygen Delivery, Consumption and Extraction

Fetal O₂ consumption is the product of umbilical blood flow and the umbilical veno-arterial difference in oxygen content (Cvo₂ - Cab₂), whereas fetal O₂ delivery is the product of the rate of umbilical blood flow and the oxygen content in umbilical venous blood (Cvo₂) and fetal O₂ extraction is the ratio between fetal O₂ consumption and delivery (Vo₂/Do₂), which can also be expressed as (Cvo₂ - Cab₂)/Cvo₂. Thus, fetal O₂ extraction can be determined without requiring measurement of umbilical blood flow. The mean values of fetal O₂ extraction obtained from two studies in our laboratory measured at 125-134 days of gestation are 30-32% (Van der Weyde et al. 1992; Kwan et al. 1995b). The present study has a mean value of 45% and 46% in Group I and II, respectively, thus considerably higher than those previously measured. However, the coefficient of variation (CV) in fetal O₂ extraction is 26% in one study (Kwan et al. 1995b) and 10% in the other (Van der Weyde et al. 1992). Similarly, CV values for fetal O₂ extraction from the other laboratories are reported to be 14% (Itskovitz et al. 1983) and 24% (Jensen et al. 1991) at the control measurement. In the present study, the CV in fetal O₂ extraction for Group I is 24% and for Group II is 11%. This consistency in variations of measurements indicated that the values obtained from the present study are not a result of experimental error. The
data in the present study are obtained from pre-labor measurement 2-3 days before delivery. Thus, the difference observed may partly be due to changes in $O_2$ extraction with gestational age when animals approach labor.

The most important stresses placed on the fetus are those related to reduced delivery of oxygen. In the rationale of this thesis, it is stated that labor and delivery are times when the fetus is most at risk from reductions in $O_2$ delivery. The present study demonstrates that during labor and at delivery, fetal $O_2$ delivery was indeed reduced in both groups. In Group I, the fall in $O_2$ delivery was largely due to the decrease in the umbilical venous $O_2$ content, since umbilical placental blood flow was slightly increased during the course of labor until delivery. In Group II, the decrease in both umbilical placental blood flow and umbilical venous $O_2$ content contributed to the fall in fetal $O_2$ delivery. However, the magnitude of decrease in the umbilical venous $O_2$ content was quite different in these two groups, with a larger fall from the control value in Group I than in Group II (24 vs. 10% at early labor, 27 vs. 20% at mid-labor, 36 vs. 26% at late labor and 37 vs. 26% at delivery).

In Group II, fetal $O_2$ consumption was maintained as a result of marked increase in $O_2$ extraction, indicating that these fetuses had sufficient oxygen reserve to compensate for the reduced $O_2$ supply. In contrast, in Group I there was an increased fetal cardiac output and thereby blood flow to most of fetal organs to compensate the fall in fetal $O_2$ delivery. It appeared that this increase in cardiac output was still insufficient to maintain normal systemic $O_2$ delivery. Consequently, blood flow was redistributed to the most vital
organs. Another compensatory response in Group I fetuses is that overall fetal O_2 consumption declined to the values as low as about 40% of Group II. This low level of fetal O_2 consumption was maintained during labor until delivery. Such degree of fetal O_2 consumption indicates that oxygen demands were reduced in these smaller fetuses. However, accompanied with this decrease in fetal O_2 consumption was severe metabolic acidemia during labor and at delivery, suggesting that Group I had minimal oxygen reserves and were much less able to tolerate labor.

In Group I, the change in fetal O_2 extraction was minimal during labor and at delivery. The inability for Group I fetuses to extract more oxygen, as compared to Group II, may be explained as follows. The equation of fetal O_2 extraction described above can be arranged as: fetal Cao_2 = Cvo_2 \times (1 - \frac{Vo_2}{Do_2}) (Edelstone, 1984). By rearranging his equation, \frac{Vo_2}{Do_2} (fetal O_2 extraction) = 1 - \frac{Cao_2}{Cvo_2}. The latter equation indicates that there is an inverse relationship between fetal O_2 extraction and fetal arterial O_2 content. Thus, it is the fall in arterial O_2 content (Cao_2) that will eventually limit the extent to which fetal O_2 extraction can increase to compensate for the reduced fetal O_2 delivery, since there is a lower limit below which oxygen diffusion into tissue can not occur (Rurak, 1994). Group I animals developed severe metabolic acidosis and hyperlacticemia. As discussed previously, fetal lactic acidosis is a result of anaerobic metabolism, which indicates that hypoxia existed due to exhausted oxygen reserves in some fetal tissues and/or in the placenta. Hence, tissue hypoxia itself may have played a major role in limiting the increase in O_2 extraction in these fetuses.
The present study appears to be the first in which fetal O$_2$ delivery and consumption during labor have been measured. However, a number of studies have been performed in the sheep fetus at late gestation to assess the effects of reduced O$_2$ supply on the fetus. Among these, experiments involving reductions in umbilical blood flow and in uterine blood flow appear to be most relevant to the labor situation. For instance, cord compression during labor would reduce umbilical blood flow; and prolonged severe uterine contractions would restrict uterine blood flow by increasing uterine vascular resistance and hence O$_2$ delivery to the fetus. When fetal O$_2$ delivery is acutely reduced by reducing uterine blood flow, umbilical venous O$_2$ content falls, but umbilical blood flow is maintained (Wilkening and Meschia, 1983; Jensen et al. 1991). Because total O$_2$ delivery to the fetus is the product of umbilical blood flow and umbilical venous O$_2$ content, O$_2$ delivery is reduced in proportion to the fall in umbilical venous O$_2$ content (Wilkening and Meschia, 1983; Jensen et al. 1991). When umbilical blood flow is reduced by cord compression, umbilical venous O$_2$ content does not change significantly, so O$_2$ delivery is decreased in proportion to the fall in umbilical blood flow (Itskovitz et al. 1983; Edelstone et al. 1985).

Reducing O$_2$ delivery to the fetus by about 50%, by either uterine blood flow restriction or decreasing umbilical venous return, has little effect on fetal O$_2$ consumption because O$_2$ extraction increases (Itskovitz et al. 1983; Wilkening and Meschia, 1983). Also, in these studies, there is no evidence of fetal acidosis with the reduction of fetal O$_2$ delivery to 50%. However, the increased extraction is not able to compensate for reductions in O$_2$ delivery of more than 50%, and O$_2$ consumption falls precipitously with a further decrease
in O\(_2\) delivery (Itskovitz et al. 1983; Wilkening and Meschia, 1983). Normally the fetal lamb extracts about 30% of delivered oxygen. With progressive reductions in O\(_2\) delivery, the fetal lamb has a remarkable ability to extract oxygen, despite the low P\(_{\text{O}}\(_2\) of the fetal blood; with a 75% reduction in O\(_2\) delivery, O\(_2\) extraction is increased to an average of 68%, but in some fetuses extraction reaches 75-80% (Itskovitz et al. 1983).

In the present study, results on the general relationship between D\(_{\text{O}}\(_2\) and V\(_{\text{O}}\(_2\) during labor are similar to those described as with these studies, that is, fetal O\(_2\) delivery can be reduced to a certain degree without fetal O\(_2\) consumption being significantly changed, although there is a difference of D\(_{\text{O}}\(_2\) and V\(_{\text{O}}\(_2\) between Group I and II fetuses as discussed above. Thus, in Group I, when fetal O\(_2\) delivery is reduced by \(~25\)% during labor, O\(_2\) consumption still appears to be maintained. Fetal O\(_2\) consumption falls significantly only at the time of delivery when fetal O\(_2\) delivery is reduced by 39%. In Group II fetuses, when fetal O\(_2\) delivery is reduced by 50% at delivery, there is no significant change in fetal O\(_2\) consumption, though the mean value is lower than the control.

However, important difference exists in the growth restricted fetuses, when compared with the other studies regarding effects of reductions in umbilical blood flow and in uterine blood flow on fetal O\(_2\) consumption (Itskovitz et al. 1983; Wilkening and Meschia, 1983). When the fetal metabolic status is examined in more detail, a different physiological picture is seen. The 25% decrease in fetal oxygen delivery in Group I during labor is already accompanied by fetal acidosis and lactic acidemia and an increase in base deficit, which indicate the presence of anaerobic metabolism resulting from hypoxia at least in
some hypoxic fetal tissues such as the fetal hindlimbs (Boyle et al. 1992) and/or in the placenta, since there was a marked increase in lactate production by the placenta in Group I (see section 4.9.2). Nevertheless, it appears that fetal O$_2$ consumption may be relatively insensitive to reflect changes in fetal O$_2$ delivery during labor in these growth restricted fetuses. It is also possible that there was a balance between an increase in O$_2$ consumption in some vascular beds and a decrease in other vascular beds, so that total O$_2$ consumption was not altered initially. Similar results have also been observed in a previous study on O$_2$ consumption during sustained hypoxemia with progressive acidemia in the normally grown fetuses (Rurak et al. 1990a); even when fetal acid-base balance was severely compromised, fetal O$_2$ consumption was well maintained. Nevertheless, the concept of the oxygen reserve, which is defined as the difference between the normal fetal O$_2$ delivery and the lowest O$_2$ delivery associated with aerobic metabolism (Edelstone, 1984), still holds true during labor. Group II fetuses appear to be able to maintain oxidative metabolism with a 40-50% reduction in fetal O$_2$ delivery during labor. In contrast, Group I fetuses can not tolerate even a 25% decrease in fetal O$_2$ delivery during labor without developing fetal hypoxia and anaerobic metabolism. Based upon these observations, it is concluded that the oxygen reserve available to these growth restricted fetuses must be reduced.

### 4.9.2 Changes in Umbilical Glucose and Lactate Delivery, Uptake and Extraction

Fetal glucose is derived from the maternal circulation and lactate is mainly produced within the placenta and transferred to the fetus (Battaglia and Meschia, 1986c). Both are
major nutrients which the fetus receives. Thus, the net uptake of glucose and lactate into the umbilical circulation from the placenta is important for understanding fetal metabolism. However, similar to the situation with fetal O₂ delivery and consumption during natural parturition, there have been no previous studies which have measured umbilical glucose and lactate delivery, uptake and extraction during this process in any species. Nevertheless, data on the relationship between uterine blood flow and umbilical uptake of glucose and lactate in the fetal sheep in late gestation are available (Simmons et al. 1979; Wilkening et al. 1985; Hooper et al. 1995). There is also a recent report on changes in umbilical glucose uptake during induced labor (Barbera et al. 1997). Thus, some comparison between the results found in the present study and those reported from these studies might be pertinent.

Several studies have demonstrated that the relationship of placental glucose transfer to uterine blood flow is curvilinear (Simmons et al. 1979; Wilkening et al. 1985). In a series of experiments in which uterine blood flow was decreased while maternal arterial glucose concentration was kept constant, a decrease in uterine blood flow by ~50% had no effect on umbilical glucose uptake and no effect on umbilical blood flow (Wilkening et al. 1985). Because there was no change in the fetal arterial glucose concentration as uterine blood flow was decreased to the 50% level, it is clear that uterine blood flow had a negligible effect on placental glucose transfer under these experimental conditions. When uterine blood flow was reduced below 50% of normal levels, there is an increase in fetal arterial glucose concentration which is coincident with a decrease in umbilical glucose uptake. An increase of fetal glucose concentration opposes fetal glucose uptake via the placenta
because the rate of glucose transfer from placenta to umbilical circulation depends upon the glucose concentration gradient between maternal and fetal blood (Hay and Meznarich, 1989; Molina et al. 1991). The decrease in umbilical glucose uptake induced by the reduction of uterine blood flow below the 50% level was associated with fetal hypoxia (Wilkening et al. 1985), of a degree that causes fetal hyperglycemia (Jones, 1977). The hypoxic hyperglycemia in fetal sheep is secondary to an initial decrease in fetal glucose utilization, followed by increased fetal glucose production (Jones et al. 1983). These changes in fetal glucose metabolism are mediated by catecholamines (Robinson et al. 1977; Jones et al. 1983) via an α-adrenergic mechanism (Jones and Ritchie, 1978; Jones et al. 1983). Thus, although a large decrease in uterine blood flow may have some direct effect on decreasing placental glucose transfer, the most important effect is indirect, via the alternation of fetal glucose metabolism that occurs in severe hypoxia. In a recent study, a 50% reduction in uterine blood flow caused a significant reduction in fetal arterial oxygen saturation by 56% (Hooper et al. 1995). Fetal O₂ consumption and umbilical glucose uptake were not altered by reduced uterine blood flow, whereas umbilical lactate uptake was greatly reduced after both short term (4h) and long term (24h) reductions in uterine blood flow with high negative values of the umbilical venoarterial lactate concentration difference, which indicates placental lactate uptake from the fetal circulation. The authors concluded that during reduced uterine blood flow, the placenta is a major site of lactate clearance from the fetal circulation (Hooper et al. 1995). In addition, during fetal hypoxia, increases in fetal blood lactate levels are associated with declines in both umbilical lactate uptake and the umbilical venoarterial lactate concentration difference; while an increase in fetal blood glucose concentrations is
associated with decreases in both umbilical glucose uptake and the umbilical venoarterial glucose concentration difference (Milley, 1988). During parturition induced at 131 days gestation with a fetal infusion of dexamethasone, fetal oxygenation remained normal. Fetal arterial plasma glucose concentrations increased, but umbilical glucose uptake decreased (Barbera et al. 1997). It is important to note that all glucose and lactate uptakes as described above refer to net umbilical uptake and provide a minimum estimate of fetal utilization of these substrates.

The observations in the present study are in several respects very different from those reported in the studies discussed above. During labor, with the likely reduction in uterine blood flow due to uterine contractions, umbilical glucose uptake was maintained in both groups, not decreased as reported in some studies (Wilkening et al. 1985; Barbera et al. 1997). The maintained umbilical glucose uptake occurs even when fetal glucose concentrations are progressively increased during labor. Accordingly, there must be a concomitant increase in UV blood glucose levels to maintain the umbilical glucose uptake, since umbilical blood flow did not change significantly during labor in Group I, and gradually decreased in Group II. The rise in fetal blood glucose concentration is associated with an unchanged (Group I) or slightly increased (Group II) umbilical venoarterial glucose concentration difference. Thus, the extent to which glucose levels rose in both fetal arterial and umbilical venous blood was more or less the same. These data suggest a rise either in uteroplacental glucose production or in maternal glucose concentrations would raise umbilical venous glucose levels. Considerable evidence has demonstrated that under nonstressed conditions, fetal glucose is derived virtually entirely
from the mother via placental transfer (Battaglia and Meschia, 1986c). In fact, we did measure maternal arterial and uterine venous blood glucose concentration in two animals (data not shown). The uterine venous blood glucose levels were consistently much higher than in umbilical venous blood. There were parallel changes in maternal arterial and both uterine and umbilical venous blood glucose concentrations during labor. These findings are consistent with those observed at a previous labor study in sheep (Comline and Silver, 1972), in which a pronounced maternal hyperglycemia was found during parturition. Thus, it is clear that increased glucose supply from the maternal circulation leads to enhanced umbilical venous blood glucose levels. Our data indicate that the exogenous glucose is still available to support fetal metabolism during labor, even in the presence of fetal hypoxemia. But this does not exclude the possibility that individual organs within the fetus may locally produce glucose. In addition, during labor, there was an increase in umbilical glucose delivery in both groups, but the delivery was much lower in Group I than Group II at each measurement point. Umbilical glucose extraction in both groups was not significantly changed in the course of labor.

Umbilical lactate delivery was also increased with the progress of labor, but this was much higher in Group I than Group II. Umbilical lactate uptake rapidly increased in Group I, and in late labor there was almost a 9 fold increase compared to the pre-labor value. At delivery, the umbilical veno-arterial lactate difference became narrowed, perhaps due to net fetal production of lactate. In Group II, lactate uptake from the placenta was also increased, but only two fold at delivery. Thus, there is also a difference in the results on umbilical lactate uptake, as compared to the studies discussed above (Milley, 1988;
Hooper et al. (1995). Our data demonstrated persistently higher lactate concentrations in the umbilical vein compared to the descending aorta in the fetal lamb throughout labor. We also observed that a larger quantity of lactate was delivered into the fetal circulation than into the maternal circulation despite a higher lactate concentration in the fetus (Observation from 2 animals with a uterine vein catheter, data not shown). One study also observed that plasma lactate concentrations in the ewe during labor were much lower than in the fetus (Comline and Silver, 1972). Thus, the lactate delivered to the fetus via the umbilical circulation is produced by the placenta, and the increase in fetal lactate levels during labor may be due in part at least to the rise in placental lactate production. The rise in fetal lactate concentrations may therefore have resulted from increases in both fetal endogenous and placental lactate production.

Perhaps, one of the strongest arguments for uteroplacental production of lactate during labor is the mean positive umbilical venoarterial lactate concentration differences observed in the present study. The positive sign and the magnitude of the umbilical venoarterial lactate concentration differences are closely dependent upon placental lactate production and lactate transfer from the placenta to the fetus. Changes in the umbilical venoarterial lactate difference can first result from an increase of fetal lactate production, mainly due to anaerobic metabolism with fetal hypoxia. A decrease in the umbilical venoarterial lactate concentration differences should thus be observed: the higher the fetal lactate production, the smaller the umbilical venoarterial lactate concentration difference. A fall in placental lactate transfer capacity may also occur during uterine contractions. Such a decrease will result in an increase in umbilical venous lactate concentrations, involving a progressive
increase of the umbilical venoarterial lactate concentration difference. Our results show that mean umbilical venoarterial lactate concentration differences are positive and increased with the progress of labor. These results suggest that a decrease of placental lactate transfer capacity may partly explain the observed umbilical venoarterial lactate concentration differences. Also, studies have shown that a substantial proportion of the glucose taken up by the placenta is extracted from the fetus (Sparks et al. 1983; Hay et al. 1984; Gu et al. 1987). A major proportion of the glucose extracted is metabolized by the placenta to lactate, which helps retain carbohydrate in the fetal compartment and restricts its loss back to the maternal circulation (Sparks et al. 1982; Gu et al. 1987; Jones, 1991). Finally, it seems reasonable to infer that the substantial rise in uteroplacental lactate production may also be due to very low placental oxygenation itself, such as in the case of Group I animals. If local oxygenation in some areas of the placenta is dependent primarily on fetal arterial blood, these areas would be perfused by fetal arterial blood with low oxygen content. In response to lowered local oxygen availability, there could be local anaerobic metabolism resulting from local hypoxia with an increase in local production of lactate. The latter would then be distributed into the fetal circulation. Thus, our data suggest that at least during labor, the placenta is a place of lactate production rather than a site of lactate clearance from the fetal circulation.

Sheep studies have demonstrated that lactate is produced by the placenta under physiological and unstressed conditions (Burd et al. 1975; Sparks et al. 1982) and that the fetus is a net lactate consumer. The finding in human third trimester pregnancies of higher blood lactate concentration in the UV than in the UA suggested that lactate is also
produced by the placenta and consumed as an energy substrate by the human fetus under physiological conditions (Soothill et al. 1986b). Furthermore, in some human fetuses, placental contribution to the increased fetal lactate levels could also occur at the time of cesarean section. For instance, UV lactate concentration was higher than UA in 6 of 21 AGA and 12 of 34 IUGR fetuses (Marconi et al. 1990), and this was only found in association with low UA arterial lactate concentrations. However, the situation at the time of vaginal delivery is quite different between the human and sheep. Several clinical studies reported that there were negative umbilical venoarterial (UV-UA) lactate concentration difference and high fetomaternal gradient in normal human fetuses (Eguiluz et al. 1983; Schneider et al. 1984; Marconi et al. 1990) and also in growth-restricted human fetuses (Lin et al. 1980). In addition, it was reported that in the IUGR human fetuses at the time of cesarean section, the higher umbilical arterial lactate concentration, the more negative the umbilical venoarterial lactate concentration difference (Marconi et al. 1990). These results indicate that lactate levels in human fetal blood mainly represent lactate production by the fetus. Lactate is clearly an excretory product of fetal metabolism in tissue hypoxia during human labor. In contrast to the data obtained in human fetus, the present study found UV lactate concentration to be constantly higher than FA concentrations throughout labor in Group II and also throughout most time of labor until delivery in Group I fetuses. These findings, together with the more pronounced lactate levels observed in sheep fetuses at delivery as compared to human fetuses (see section 4.7), suggest that there might be a fundamental difference in placental and fetal lactate metabolism between these two species during labor and delivery. Whether this difference is due to placental clearance difference of lactate or placental lactate metabolism is not
clear at this time. We hypothesize that there might be an efficient clearing mechanism for lactate within the human placenta in response to labor. In contrast, uteroplacental lactate production may be an important process in the sheep, as in the case of Group II. It is possible that lactate produced by the placenta represents a mechanism for trapping carbohydrate within the fetal circulation so that it can be used as an alternative substrate to glucose during labor. However, the most likely explanation for the marked increase of lactate concentrations in Group I fetuses during labor and delivery is that lactate may come from a combination of two sources (i.e., fetal origin and placental origin). The rise in the lactate concentrations could be caused by reduced oxygen supply to some fetal tissues and to the placenta, with a resultant inhibition of the Krebs cycle and increased conversion of pyruvic acid to lactic acid as an anaerobic source of energy for these growth restricted fetuses. Moreover, observations in normal pregnant sheep in late gestation have suggested that the degree of oxygenation of blood returning to the placenta from the fetus is a major determinant of lactate output by the placenta (Sparks et al. 1983). Similar findings were obtained with fetal growth restriction achieved via carunclectomy; uteroplacental production of lactate per kg of placenta was significantly higher in carunclectomized small fetuses compared to controls and placental lactate production was further increased as oxygen content in fetal FA blood decreased (Owens et al. 1987b). Also, fetal lactate consumption per kg of fetus increased as the concentration of UV blood lactate levels was increased, suggesting that the small fetuses utilize lactate to a greater extent than the normally grown fetuses in the antepartum period. Thus, it may be assumed that such changes would also occur during labor, at least in the growth-restricted sheep
fetuses with smaller placenta, thus contributing partly to the dramatic rise in lactate concentrations seen in Group I.

Both glucose and lactate have been shown to have fairly high oxidation rates (Hay et al. 1983). If their concentrations in fetal blood are increased, their contribution to oxidation would also increase, sparing the use of other fuels for oxidation. The fraction of oxidative metabolism (glucose/O2) that could be supported by glucose did not change significantly with labor in both group of fetuses, but the proportion of oxidative metabolism supported by lactate (lactate/O2) is gradually increased during labor. The increase in lactate/O2 reached statistical significance in Group I fetuses during labor. However, this increase does not mean that the fraction of oxidative metabolism accounted for by lactate is actually increased. It merely reflects the increased umbilical venoarterial lactate concentration difference and decreased umbilical venoarterial O2 concentration difference in Group I fetuses. Thus, using the oxygen quotient to estimate substrate requirements for energy may not be appropriate for these fetuses with severe hypoxia and metabolic acidosis under the stressful conditions of labor.

The reason for the discrepancy of fetal glucose and lactate metabolism between the present labor study and the studies including acute reductions in uterine blood flow and fetal hypoxia may be due to the more complex changes which occur during labor. The precise mechanisms that alter fetal and placental glucose and lactate metabolism during labor remain unclear. Certainly, the fetal neurohormonal environment is dramatically changed. For instance, plasma concentrations of catecholamines are markedly increased in
fetal sheep during labor (Eliot et al. 1981; Brown et al. 1983; Richet et al. 1985; Habib et al. 1991; Oyama et al. 1992). Thus, further studies are needed to explore how the fetal endocrine changes during labor alter substrate metabolism in the fetus and placenta.

4.10 Fetal Arginine Vasopressin Concentrations during Labor

An earlier study using a bioassay has shown that in the chronically instrumented fetal sheep, there is little change in circulating fetal plasma concentrations of AVP in the period from 20 days before delivery until about 3 days before delivery (Alexander et al. 1974). Another study using a radioimmunoassay method has found that increased AVP concentrations occurred only after the onset of spontaneous uterine contractions (Stark et al. 1979). In the present study, pre-labor fetal blood samples for measurement of plasma AVP concentrations was collected at 2-3 days before delivery. Since in the present study we did not follow the time course of the preparturient change in fetal plasma AVP concentrations, it is not known if the AVP concentration change observed in the earlier study would also occur in our sheep fetuses. However, the pre-labor AVP concentrations in the normally grown fetuses (Group II) averaged 4.2 ± 0.8 pg/ml. The value is in the range of normal resting plasma AVP concentrations of 2-5 pg/ml at late gestation in sheep fetus reported by others (Stark et al. 1979; Kelly et al. 1983; Stark et al. 1985). Thus, it is most likely that fetal AVP concentrations would start to rise after the onset of labor.

During labor and at delivery, fetal AVP concentrations rose markedly in Group I, whereas it did not increase significantly until delivery in Group II. In spite of the great variation of
AVP between animals, the concentration of fetal AVP in Group I was much higher than that in Group II at each stage of measurement. A rise in fetal plasma AVP levels has also been observed previously during either spontaneous (Stark et al. 1979; Cummings et al. 1995) or ACTH-induced (Stark et al. 1981) parturition in sheep. A wide range (7.5-8,000 pg/ml) has been reported (Stark et al. 1979). A variety of contributing stimuli could be responsible for the elevated plasma AVP concentrations during labor, such as the hypoxic stress (Rurak, 1978; Daniel et al. 1983; Stark et al. 1984), hypoxia combined with acidosis (Rurak, 1978; Daniel et al. 1983), surge in plasma catecholamines (Ervin et al. 1989; Ervin et al. 1991), hypoxia-induced rise in fetal adenosine levels (Koos et al. 1994a) and cranial compression (Hadeed et al. 1979). The present study supports the view that the elevated AVP levels would occur in response to hypoxia stress with fetal acidosis during labor, since a significant correlation was observed between fetal plasma AVP concentrations and fetal arterial blood pH (Figure 37). This finding agrees with a previous study on the relation between hypoxia and AVP levels during labor (Stark et al. 1981).

The functions of the increased concentrations of AVP during labor are not clear. It has been suggested that AVP, like catecholamines, plays an important role in modulating the significant changes in fetal cardiac output redistribution necessary for successful cardiovascular transition from the intrauterine to extrauterine environment (Iwamoto et al. 1979; Rurak and Gruber, 1984). Also, AVP may be important for the maintenance of perinatal arterial blood pressure, due to its effect on peripheral vascular tone with an increase in peripheral resistance (Rurak and Gruber, 1984; Tomita et al. 1985). Thus, although the physiologic significance of the increase in AVP secretion observed in
response to labor and delivery remain to be elucidated, AVP secretion appears to be an essential part of the fetal adaptive response responsible for maintaining fetal homeostasis.

It is also possible that the remarkably high AVP concentrations in Group I may have had some adverse effects. In fetal sheep, acute hypoxic stress with a marked increase in fetal plasma AVP concentrations is associated with massive expulsion of meconium into the amniotic fluid (DeVane et al. 1982). AVP infusion at high concentrations in the fetal sheep also produces meconium passage (Wiriyathian et al. 1983). Based upon these observations, increased secretion of AVP has been proposed to play a role in meconium passage and development of necrotizing enterocolitis, because AVP is known to produce marked vasoconstriction of the mesenteric circulation and bowel evacuation (DeVane et al. 1982, Wiriyathian et al. 1983). In Group I fetuses at delivery, the presence of meconium in the amniotic fluid was observed. The fetal plasma AVP concentrations started to rise markedly from early-labor and continued to increase exponentially to delivery in each animal. Thus, it is possible in this group of fetuses that there may be a link of meconium staining to not only high AVP concentrations but also the duration of the elevated levels.
5. SUMMARY AND CONCLUSIONS

1. A method has been developed for measuring regional blood flow by means of fluorescent microspheres in all fetal organs and tissues, including bone, and in the placenta. The method appears to be a viable alternative to the use of radioactive microspheres, thereby eliminating the regulatory, environmental and health concerns associated with the latter. Moreover, the use of an automated fluorescent microplate reader greatly decreases analysis time compared to use of a conventional fluorimeter, and this is most valuable when assessing blood flow in large numbers of samples, as in the case of most studies in fetal lambs.

2. Thirty three chronically instrumented pregnant sheep preparations were set up to study fetal cardiovascular and metabolic functions during labor and delivery. Of these, 12 preparations were successful.

3. With these 12 animals, 2 groups (n = 6 in each) were distinguishable in terms of the fetal responses to labor. In Group I, there was the progressive development of severe fetal metabolic acidemia during labor, whereas in Group II, there was only a minimal and late onset deterioration in acid-base balance. The groups were separated on the basis of femoral arterial pH being greater (Group II) or less (Group I) than 7.15 at delivery.

4. Subsequent analysis of the animal data indicated asymmetric growth restriction in Group I, with fetal (2.627 ± 0.164 vs. 3.921 ± 0.301 kg) and placental (156.2 ± 13.2 vs. 299.6 ± 30.5 g) weights and the placental/fetal weight ratio (5.9 ± 0.3 vs. 7.7 ± 0.6%)
being significantly lower than in Group II, whereas the brain/liver weight ratio (0.59 ± 0.07 vs. 0.44 ± 0.03) was significantly higher. Placental weight in Group I was lower primarily because of fewer numbers of larger weight cotyledons compared to Group II.

5. In the antepartum period, the growth restricted fetuses (Group I) exhibited modest hypoxemia, hypoglycemia and hyperlactic acidemia. They also had lower values of cardiac output (372 ± 47 vs. 495 ± 42 ml/min/kg), umbilical blood flow (119 ± 13 vs. 194 ± 19 ml/min/kg), fetal oxygen delivery (476 ± 61 vs. 1128 ± 178 μmol/min/kg) and consumption (213 ± 20 vs. 513 ± 86 μmol/min/kg), umbilical glucose delivery (71 ± 12 vs. 168 ± 35 μmol/min/kg) and umbilical lactate uptake (12 ± 6 vs. 36 ± 6 μmol/min/kg), but higher arginine vasopressin concentrations (11.7 ± 1.4 vs. 4.2 ± 0.8 pg/ml) and higher fractional distribution of fetal cardiac output to the adrenals (0.30 ± 0.05 vs. 0.16 ± 0.01%), as compared with the normally grown fetuses (Group II). There was the maintenance of a balance between fetal oxygen consumption and the reduced fetal oxygen delivery in the growth restricted fetuses, perhaps due to a programmed reduction in fetal growth. However it appears that fetal oxygenation and substrate availability in these fetuses became compromised before the onset of labor.

6. The restricted placental growth appeared to be related to insufficient placental function, since fetal hypoxemia and hypoglycemia was associated with the reduced placental weight. Thus, it may be the placental growth impairment which initiates the fetal growth restriction in these fetuses. A deficit in the supply of oxygen and glucose may act directly through their own effects and indirectly through hormonal influences such as reduced
insulin, thyroxine, triiodothyronine and IGF-I and II concentrations to restrict fetal growth.

7. In both groups, the incidence of fetal breathing movements decreased over the last 3 days before delivery. In addition, there was a significantly lower incidence of fetal breathing movements in the growth restricted fetuses (19.7 ± 2.0 vs. 28.0 ± 1.8%). This reduction of fetal breathing activity may be one of the compensatory mechanisms to the reduced O₂ delivery and hypoglycemia in the growth restricted fetuses.

8. In Group II from day 7 to day 2 prior to the day of labor, there was a progressive decrease in the daily average fetal heart rate (4.4 ± 0.6 bpm/d), but with a significant increase (8.0 ± 2.5 bpm) on the day prior to labor onset. In Group I (growth restricted) fetal heart rate did not change consistently between days 7 and 2 prior to labor, but there was a tendency for the rate to rise on the day prior to labor. Thus, in the growth restricted fetuses, there appears to be a heightened sympathetic stimulation or an inadequate parasympathetic influence on the fetal heart rate in late gestation, perhaps due to a delay in the normal maturational changes of the autonomic control of fetal heart rate.

9. In both groups, the decrease in fetal breathing over the last few days of gestation and the increase in heart rate on the day prior to labor were useful indicators of the impending onset of parturition.
10. In both groups, fetal arterial pressure increased progressively over the last week of gestation. However, the rate of increase in Group I (0.9 ± 0.1 mm Hg/d) was significantly less than that in Group II (1.8 ± 0.3 mm Hg/d). As a consequence arterial pressure on the day prior to labor onset was significantly higher in the normally grown fetuses (67.2 ± 2.5 vs. 58.4 ± 3.0 mm Hg).

11. With the progress of labor, a steady and significant increase in uterine activity occurred in both groups of animals. The changes in uterine activity in relation to the successive stages of labor were similar in the 2 groups, as was the total length of labor (17.9 ± 1.3 h, Group I, vs. 15.1 ± 1.5 h, Group II).

12. The growth restricted fetuses developed severe intrapartum hypoxemia and lactic acidemia, with the fall in blood $O_2$ content being largely due to the acidemia likely via a Bohr shift in hemoglobin $O_2$ affinity. In contrast, fetal blood gas and acid-base status were largely maintained in the normally grown fetuses until delivery. Thus at delivery arterial pH (6.876 ± 0.095 vs. 7.215 ± 0.019) was much lower and [lactate] (16.71 ± 2.78 vs. 5.65 ± 1.11 mmol/L) much higher in Group I.

13. In the normally grown fetuses there was the progressive development of hypertension during labor and at delivery, while in the growth restricted fetuses no such change was apparent, in spite of a marked increase in plasma vasopressin and likely catecholamine levels during labor in this group. The reason for the lack of pressure increase is unclear, but it may be due to developmental and maturational differences, or the effect of the
marked metabolic acidemia on vascular smooth muscle function. There was fetal
tachycardia in Group I initially during labor, with a return to prelabor values later, whereas
in Group II heart rate was not altered.

14. In the growth restricted fetuses there was increased blood flow to most organs and
tissues during labor, associated with an apparent increase in combined ventricular output,
and an increased distribution of cardiac output to the heart, brain and adrenals. However,
there was a fall in blood flow to most organs at delivery. In contrast, in the normally
grown fetuses there was only a moderate increase in blood flow to the heart, brain and
adrenals and maintenance of perfusion to most other organs, and this persisted through
labor and delivery. However umbilical blood flow fell progressively in this group.

15. In both groups, fetal O₂ delivery fell during labor. In the normally grown fetuses, the
fall in O₂ delivery was accompanied by a marked increase in O₂ extraction, so that O₂
consumption was maintained. In contrast, there was only a minimal change in O₂
extraction in the growth restricted fetuses, with the result that O₂ consumption fell
significantly at delivery (from 213 ± 20 to 129 ± 16 µmol/min/kg). The terminal fall in
metabolic rate, coupled with the decreased perfusion to most fetal organs and tissues at
delivery suggests that the growth restricted fetuses were severely compromised by the end
of labor.

16. In the growth restricted fetuses, the increase in lactate concentration during labor was
associated with a progressive rise in fetal lactate uptake from the placenta (12 ± 6 to 106 ±
35 \( \mu \text{mol/min/kg} \) in late labor). Thus it appears that the placenta was contributing to fetal lactic acidemia, perhaps as a consequence of inadequate placental oxygenation. In contrast in Group II, there was no significant change in umbilical lactate uptake during labor. In both groups, fetal blood glucose concentrations increased during labor, but at delivery arterial [glucose] was lower in Group I (0.92 ± 0.19 vs. 1.59 ± 0.21 mmol/L). Umbilical glucose uptake was not changed significantly in either group.

17. During labor and at delivery, fetal arginine vasopressin concentrations rose markedly in the growth restricted fetuses, whereas it did not increase significantly until delivery in the normally grown fetuses. There was a significant correlation between fetal plasma vasopressin concentrations and fetal arterial blood pH.

18. Overall, the responses of the growth restricted fetuses to labor and delivery were distinctively different from those in the normally grown fetuses, and they appeared to be especially vulnerable. Thus our results suggest that fetal and placental growth are important modifiers of the fetal responses to labor. Moreover, since in Group I increased placental lactate production during labor contributes to the marked fetal lactic acidemia that is largely due to the decrease in fetal oxygenation, it appears that the reduced placental growth in this group is involved in both the antepartum and intrapartum fetal compromise.
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