MATERNAL PROGESTERONE AND FETAL CORTISOL RESPONSES TO HYPOXEMIA IN PREGNANT SHEEP

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ABSTRACT

The placenta is an important endocrine organ producing large amounts of steroid and protein hormones which are released into the maternal and fetal circulations. Moreover, it has a very high metabolic rate and consumes a significant proportion of the oxygen and glucose delivered to the uterus and its contents. However, while there have been many studies on the effects of hypoxemia on fetal cardiovascular, metabolic and endocrine functions, there are limited data on the effects of reduced oxygenation on placental endocrine activities.

In the present study, we examined the effects of acute (2-h) moderate (maternal Pao_2 lowered by 27-35%, n=5) and severe (maternal Pao_2 lowered by 41-58%, n=4) hypoxemia on placental progesterone output into the maternal circulation in 7 chronically instrumented pregnant sheep (125 to 136 d). Hypoxemia was achieved by lowering maternal inspired O_2 concentration and the hypoxemia period was preceded and followed by 2 h pre-hypoxia and recovery periods, respectively. Control experiments (n=4), involving 6 h periods of normoxia were also carried out. Samples were taken simultaneously at predetermined time periods from maternal femoral arterial and uterine venous catheters for measurement of progesterone concentration. Blood flow to the uterine horn containing the operated fetus was measured continuously, and utero-placental progesterone output was calculated as the uterine venous - arterial difference in progesterone concentration times uterine blood flow. Blood samples were also collected from the fetal femoral artery and umbilical vein, and in these samples, as well as in the maternal samples, the following variables were measured: Po_2, Pco_2 and pH, hemoglobin concentration, blood O_2 saturation and content, glucose and lactate concentrations and fetal

plasma cortisol level. The following variables were calculated from these data: utero-placental oxygen delivery and consumption and glucose uptake and lactate flux. Maternal and fetal arterial pressure and heart rate were continuously monitored.

In sheep carrying twin fetuses compared to those with a single fetus, the average progesterone concentrations of maternal arterial (6.53±0.19 versus 4.27±0.13 ng/ml) and uterine venous plasma (21.05±0.56 versus 17.82±1.17 ng/ml) were significantly higher and associated with significantly higher values of uterine blood flow (549.5±17.9 versus 339.6±6.2 ml/min) and progesterone output (8,156±426 versus 4,720±243 ng/min).

In the moderate hypoxia experiments, maternal arterial Po_2 was lowered by 27-35% (mean = 30.7±1.8%) or 38.9±4.7 mmHg. This resulted in a fall in fetal arterial oxygen tension by 19.7±2.4% or 4.1±0.5 mm Hg. This was associated with similar decreases in fetal blood O_2 saturation and content, and with a rise in lactate concentration and cortisol level. There were no consistent changes in fetal Pco_2 or pH. Similar changes in the fetal variables were observed with severe hypoxia, when maternal Po_2 fell by 41-58% (mean = 48.5±3.5%) or 59.4±6.6 mmHg, except that in this case the decrease in fetal Po_2 was greater (28.6±2.0% or 4.8±1.0 mmHg) and there was a significant decline in fetal arterial pH and larger increases in fetal lactate and cortisol levels. Likely as a consequence of the acidemia, the fall in O_2 saturation (44.7±5.5%) and content (41.8±9.0 mM) was greater than with moderate hypoxemia. With both degrees of hypoxemia, there was a tendency for the umbilical veno-arterial lactate difference to increase during hypoxemia, suggesting increased utero-placental lactate production. Severe hypoxia was associated with an increase in maternal heart rate, but no change in arterial pressure, whereas neither variable was altered with moderate hypoxia. Severe hypoxia was associated with fetal hypertension and bradycardia, but these changes did not occur with

moderate hypoxia. There were no changes in the maternal and fetal variables during the control, normoxia experiments except for a slight but significant decrease in fetal plasma cortisol concentration.

Uterine blood flow, O_2 delivery and O_2 consumption were not consistently changed during the moderate and severe hypoxia experiments, nor during the control, normoxia protocol. There were also no significant changes in maternal arterial and uterine venous progesterone concentrations. However, with moderate hypoxemia, the progesterone concentration in uterine venous blood increased in 4 of the 5 experiments, and the mean percentage increase was 16.2±7.3%. There was a similar trend for a rise in utero-placental progesterone output, which increased by 18.6±10.5%. However, neither change was statistically significant.

Overall, the results indicate that acute hypoxemia results in significant alterations in fetal cardiovascular, metabolic and endocrine functions, with limited effects on the utero-placental variables measured. Thus the placenta may be more resistant to reduced oxygenation than the fetus. The trend for an increase in utero-placental progesterone production with moderate hypoxia is similar to data in published reports. If such an increase does in fact occur, it may be due to the elevation in placental PGE_2 production that occurs with hypoxia, since PGE_2 has been shown to increase ovine placental progesterone synthesis *in vitro*. The lack of any evidence for a rise in progesterone production with severe hypoxia may reflect an inhibitory effect of severe decreases in maternal and/or fetal oxygenation on placental progesterone production. However further studies are necessary to confirm the results of the current and previous studies and in this regard the effects on placental progesterone output of other methods of inducing fetal hypoxemia, which have a

greater impact on uterine O_2 delivery (e.g. maternal anemia, reduced uterine blood flow), would seem worthy of investigation.

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ABBEVIATIONS

%	percent
20α-diHP	20α-dihydroprogesterone
^{125}I	radioisotope iodine
A	artery
ACTH	adrenocoticotropin
AVP	vasopressin
BNC	binucleate cells
bpm	beat per minute
C	control period
cm	centimeter
CRF	corticotropin-releasing factor
d	day
dl	deciliter
FA	fetal femoral artery
g	gram
GA	gestational age
Glu	glucose
H	intervention period
h	hour
Hb	hemoglobin
HCG	human chorionic gonadotropin
I.U.	international unit
i.v.	intravenous

kg	kilogram
l	liter
Lac	lactate
MA	maternal femoral artery
MCR	metabolic clearance rate
mg	milligram
MH	moderate hypoxia
min	minute
ml	milliliter
mM	millimolar
mRNA	messenger RNA
NADP	nicotinamine adenine dinucleotide phosphate
NDGA	nordihydroguaiaracetic acid
ng	nanogram
P_4	progesterone
$P450_{scc}$	$P450_{side\text{-}chain\ cleavage\ enzyme}$
Pco_2	partial pressure of CO_2 in blood
PG	prostaglandin
PGE_2	prostaglandin E_2
pH	negative log hydrogen ion concentration
Po_2	partial pressure of O_2 in blood
Qut	uterine blood flow
R	recovery period
sec	second
SH	severe hypoxia
UtV	uterine vein

UV	umbilical vein
V	vein
Vo_2	oxygen consumption
µg	microgram
µl	microliter
µmol	micromole

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1. *INTRODUCTION*

1.1 *MORPHOLOGY OF THE SHEEP PLACENTA*

In Grosser's classic 1909 publication on placental structure, the ruminant placenta was defined as syndesmochorial, because he considered that the uterine epithelium disappeared and fetal trophectoderm was apposed directly to the maternal uterine tissue (see Steven, 1975). However, subsequent workers demonstrated that the uterine epithelium persists, albeit in all altered form, and therefore reclassified the ruminant placenta as epitheliochorial, i.e. the chorionic trophoblast is apposed to the uterine epithelium (Ludwig, 1962; Steven, 1975; Ramsey, 1982). In the last decade considerable evidence has accumulated to show that a unique feature of the ruminant placenta is a population of fetal chorionic binucleate cells (BNC), which are formed throughout pregnancy from mononuclear trophoblast cells. They then migrate through the tight junctions on the apical surface of the trophoblast layer to fuse with uterine epithelial cells. This is associated with the conversion of the epithelium from a cellular to a syncytial layer. BNC appear to be directly involved in this modification of the uterine epithelium, which begins at implantation and continues until term (Wooding, 1982, 1983; Wooding et al, 1986). Another important function of BNC is to produce and deliver protein and steroid hormones to the ewe and fetus. A placental lactogen is measurable in maternal and fetal circulations through the latter two-thirds of pregnancy in sheep (Chan et al, 1978; Martal and Lacroix, 1978). BNC are the sole source of this protein hormone (Forsyth, 1986). Moreover, BNC isolated from sheep placenta are capable of considerable progesterone production from endogenous sources and added labeled pregnenolone (Reimers et al, 1985; Ullmann and Reimers, 1989).

1.2 *METABOLIC AND ENDOCRINE FUNCTIONS OF PLACENTA*

The uteroplacenta of the pregnant sheep is a heterogeneous group of structures including myometrium, endometrium, placental cotyledons and chorion-allantoic membranes. However, the studies on the distribution of uterine blood flow, and the transplacental diffusion of glucose and oxygen indicate that placental metabolism is the major metabolic component of the uteroplacenta (Meschia et al, 1980). The uteroplacenta has a very high metabolic rate and, in late gestation consumes 45~50% of oxygen and ~67% of the glucose used by the uterus and its contents (Meschia et al, 1980; Wilkening and Meschia, 1983).

The placenta also plays an active endocrine role during pregnancy, producing various steroid and protein hormones, as well as other molecules that yet do not have a demonstrated endocrine function. There are species differences in the precise nature of the endocrine products produced by the placenta. Thus, for example is some species (e.g. human) a placental gonadotropin (e.g. HCG) is produced in large quantities, whereas in others (e.g. sheep), no such placental hormone has been demonstrated (Conley and Mason, 1994). The primary hormones produced by the sheep placenta include prostaglandins E_2 and $F_{2\alpha}$ (Liggins, 1974; Magness et al, 1990; Yoshimura et al, 1990; Kelleman et al, 1992; Thorburn, 1992; Bocking et al, 1993), various peptide and protein hormones, such as ovine placental lactogen (Hurley et al, 1977), and progestins and estrogens. As noted above, BNC appear to be of primary importance in the synthesis of placental hormones in the sheep. In this thesis, the primary discussion will be on progesterone.

1.3 PLACENTAL PROGESTERONE PRODUCTION

1.3.1 Progesterone Levels and Production Rates in Pregnancy

The unique steroid of pregnancy is progesterone and its adequate formation by the corpus luteum and/or the placenta is essential to the establishment and maintenance of pregnancy in sheep and other mammalian species (Conley and Mason, 1994). In some species, such as the goat, the secretion of progesterone by the corpus luteum is the dominant feature in the hormonal maintenance of pregnancy. In contrast, in the sheep, as well as in some other species, the corpus luteum is the principal source of progesterone in early gestation, but later the placenta assumes an increasingly important role. However, although bilateral ovariectomy can be performed in sheep without causing abortion from about day 50 after mating (Casida and Warwick, 1945), the corpus luteum continues to secrete progesterone for most of gestation (Edgar and Ronaldson, 1958; Harrison and Heap, 1968).

Plasma progesterone levels have been measured in pregnant sheep by several investigators (Bassett et al, 1969; Stabenfeldt et al, 1969; Fylling, 1970). The concentration rises steadily after about 70 days' gestation with the highest values being found during the last third of pregnancy (Neher and Zarrow, 1954; Bassett et al, 1969). Within two days prior to parturition the levels fall rapidly so that at the time of delivery, they frequently resemble those found in non-pregnant sheep during the luteal phase of the normal cycle (Thorburn et al, 1969). Linzell and Heap (1968) found a net production of progesterone by the gravid uterus of sheep by measuring a uterine veno-arterio difference of 43.3 ng/ml allowing for an estimate of progesterone output of 10 μg/min or 14 mg/day. Mattner and Thorburn (1971) reported that in single- and twin-bearing ewes lacking a

corpus luteum, the mean concentration of progesterone in utero-ovarian venous plasma increased from 18 ng/ml and 32 ng/ml, respectively, at day 100 of pregnancy to a plateau of 45~60 ng/ml and 68~80 ng/ml respectively, between days ~120 and 140 (term is ~145 days in sheep). In single-bearing ewes with a corpus luteum, the concentration fell from 115-145 ng/ml at days 100-110 to 53 ng/ml at days 135-140. A corresponding fall occurred in twin-bearing ewes with a corpus luteum: from 160-175 ng/ml at days 100-110 to 72 ng/ml at about day 130. The daily output of progesterone by the placenta in single-bearing and twin-bearing ewes was about 4 mg and 8 mg, respectively, at day 100 of pregnancy and rose to about 33 mg and 55 mg, respectively, at 5 days before parturition. In pregnant sheep, there is also a high plasma concentration of the reduced metabolite of progesterone, 20α-dihydroprogesterone (20α-diHP), similar to the concentration of progesterone (Short and Moore, 1959). *In vitro* studies have indicated that progesterone is reduced to 20α-diHP by several maternal and fetal tissues (Nancarrow and Seamark, 1968).

The marked prepartum fall in maternal circulating progesterone levels noted above is the result of a fetal cortisol-induced increase in placental 17α-hydroxylase activity, which is the primary trigger for the onset of parturition in sheep (Anderson et al, 1975, Silver, 1994). As a consequence, pregnenolone is converted to 17α-hydroxypregnenolone and then, via C-17,20 lyase, to dehydroepiandrosterone. In this manner, pregnenolone is diverted from progesterone synthesis to dehydroepiandrosterone, which in turn is converted to androstenedione (by the action of steroid 3ß-hydroxysteroid dehydrogenase) and then to estrone via aromatase (Steele et al, 1976). Progesterone formed from pregnenolone is also acted upon by 17α-hydroxylase to give 17α-hydroxyprogesterone, which also contributes to progesterone decrease (Flint et al, 1975a&b). Compared with 17α-hydroxypregnenolone, however, 17α-hydroxyprogesterone is a poor substrate for

steroid-17,20 desmolase reaction; thus, androstenedione, the immediate precursor of estrogen in the sheep placenta, arises primarily from 17α-hydroxypregnenolone via dehydroepiandrosterone and not from 17α-hydroxyprogesterone (France et al, 1988). Fetal cortisol also acts to cause a modest increase in aromatase activity, but aromatase is not the rate-limiting step in estrogen formation; rather, the supply of C-19 steroid precursors, i.e., dehydroepiandrosterone, is rate-limiting. In this coordinated manner, progesterone secretion is severely reduced at the onset of parturition, with a concomitant increase in estrogen secretion, which via its effects on myometrial gap junctions and oxytocin receptors, uterine prostaglandin production and cervical tissue constituents, caused the coordinated labour contractions and cervical dilatation (Verhoeff et al, 1985; Silver, 1990; Neuland and Breckwoldt, 1994).

Bedford et al (1971) have shown that the hormonal maintenance of the pregnancy in sheep is associated predominantly with an increased production rate of progesterone and not with any progesterone-conserving mechanism such as could be provided by a decrease in its metabolic clearance rate (MCR). The production rate of progesterone during gestation increases significantly from early pregnancy to a maximum at days 135-145. At that time, the rate is about 10 times higher than that found in non-pregnant sheep during the luteal phase. In contrast, the MCR of progesterone changes little during gestation and is only slightly greater in pregnant sheep than in non-pregnant ewes. Within 2 weeks before parturition there is a small, though statistically insignificant increase in MCR. However, MCR corrected for body weight is the same in sheep during the normal estrous cycle and in pregnancy suggesting that the change in clearance rate in late gestation is the result of alteration in some weight-related component such as total body water (Paterson and Seamark, 1968).

1.3.2 *Progesterone Biosynthesis*

Progesterone is a C-21 steroid hormone synthesized directly from pregnenolone and is of major importance as an intermediate step in the biosynthetic pathway of sex hormones. The rate-limiting step in progesterone biosynthesis is conversion of cholesterol to pregnenolone by mitochondrial cytochrome $P450_{side\text{-}chain}$ cleavage enzyme ($P450_{scc}$) (Kashiwagi et al, 1980). The enzyme functions together with its associated NADP-specific electron transport proteins, flavoprotein NADP-adrenodoxin reductase and the iron-sulphur protein, adrenodoxin (Lambeth et al, 1982). The electron transport proteins are isolated as soluble components from sonicated mitochondria, while the cytochrome $P\text{-}450_{scc}$ is an intrinsic mitochondrial inner membrane protein (Yago and Ichii, 1969). The cytochrome binds cholesterol and catalyzes 3 successive oxidations (Orme-Johnson et al, 1979). The intermediates, 22R-hydroxycholesterol and 20α, 22R-dihydroxycholesterol remain bound to the enzyme, while the final product, pregnenolone, is released (Burstein and Gut, 1976). Three moles of oxygen and NADP are required per mole of pregnenolone synthesized.

In several species, ovarian cholesterol side-chain cleavage appears to regulated by luteinizing hormone, which stimulates synthesis of the enzyme (Toaff et al, 1983; Funkenstein et al, 1984; Hedin et al, 1987) and mRNA encoding for the enzyme (Golos et al, 1987; Urban et al, 1991) in granulosa cells. Once luteinization has occurred, cytochrome $P450_{scc}$ is thought to be constitutively produced and thus less dependent upon hormonal regulation (Oonk et al, 1990). Enzyme concentrations are paralleled by levels of mRNA encoding $P450_{scc}$ in bovine and human granulosa and luteal cells (Rodgers et al, 1987; Doody et al, 1990).

In the adult bovine adrenal, adrenocoticotropin (ACTH) increases cholesterol side-chain cleavage gene transcription (John et al, 1986), the stability of $P450_{scc}$-encoding mRNA (Boggaram et al, 1989) and the amount as well as activity of the enzyme (Kramer et al, 1983). In the human fetal adrenal, ACTH has also been shown to increase most of these parameters (John et al, 1986; Ohashi et al, 1983; Di Blasio et al, 1987). Moreover, in the sheep fetus, an ACTH infusion *in vivo* strikingly increases both the amount of $P450_{scc}$-encoding mRNA (Tangalakis et al, 1990) and the adrenal cells' ability to produce pregnenolone (Durand et al, 1982). In rat adrenal cortical mitochondrial preparations, the side-chain cleavage reaction is stimulated rapidly (in less than 10 min) by treatment of adrenal cells with ACTH, and the rapid phase (first 2 min) is highly oxygen dependent, probably because of limitations in cholesterol and/or electron supply (Liddle et al, 1962; Simpson, 1979). However, the second phase (2-10 min) is essentially oxygen independent (Stevens et al, 1984). There appears to be little information on the regulation of placental cytochrome $P450_{scc}$.

1.3.3 *Regulation of Placental Progesterone Secretion*

Although much evidence has been accumulated showing that the placenta of many mammalian species synthesize and secret steroids, the mechanisms which regulate these processes remain ambiguous (Heap and Flint, 1984). Wango et al (1992) demonstrated that progesterone synthesis in binucleate cell preparations in sheep is increased by prostaglandin E_2 (PGE_2). Sheep binucleate cells also produce PGE_2 from arachidonic acid. Nordihydroguaiaracetic acid (NDGA, a lipoxygenase inhibitor) stimulated progesterone production, whereas it was inhibited by indomethacin (a cyclooxygenase inhibitor). These results suggest that in sheep the products of both the cyclooxygenase (producing PGE_2) and lipoxygenase pathways of arachidonic acid metabolism have

regulatory roles in placental steroid synthesis. In an *in vivo* study in pregnant sheep (Nathanielsz and Seamark, 1988), induction of premature delivery by cortisol caused an increase in maternal and fetal 17α-hydroxyprogesterone, dehydroepiandrosterone and estrone levels and also a rise in maternal 17α-hydroxypregnenolone concentration, with a concomitant decrease in maternal and fetal pregnenolone and progesterone levels, indicating that induction on these above enzymes was brought about by cortisol infusion *in vivo*, as occurs at normal parturition.

1.3.4 *Progesterone Effects*

Progesterone has a variety of physiological effects in pregnancy and in the post-partum period (Conley and Mason, 1994). One of the more important effects is the induction of the cyclic changes in the glandular morphology of the endometrium allowing for implantation and successful placentation and growth of the fertilized ovum. It is also responsible for the continuous maintenance of pregnancy. Progesterone is also believed to suppress uterine myometrial contractions until just prior to parturition, and as noted above, progesterone withdrawal with concomitant increased placental estrogen production is associated with onset of parturition in sheep. In addition, progesterone stimulates and prepares for lactation in the mammary gland. The biological potency of 20α-diHP is much less than that of the parent compound, and the physiological role of 20α-diHP in pregnant sheep is obscure. In the pregnant rabbit, it has been implicated in the regulation of luteinizing hormone release (Hilliard et al, 1967). However, unlike the rabbit, the ovarian secretion rate of 20α-diHP in the sheep is low, being less than 10% that of progesterone (Short et al, 1962).

1.4 *FETAL EFFECTS AND FETAL RESPONSES TO HYPOXEMIA*

Fetal hypoxia, which can be defined as a reduction in fetal tissue oxygen supply, is a common cause of fetal morbidity and mortality (Edelstone, 1984; Carter, 1989; Richardson et al, 1989; Rurak, 1994; Rurak, 1995). It normally results from a reduction in the delivery of oxygen from mother to fetus. As fetal oxygen delivery is the product of umbilical blood flow and umbilical venous O_2 content, it can be lowered by either a fall in umbilical blood flow (e.g. via cord compression) or a reduction in the umbilical venous O_2 concentration. The latter perturbation can result from a number of causes, including maternal hypoxia (e.g. from high altitude, anemia or cigarette smoking), reduced uterine or maternal placental blood flow, placental abruption or fetal anemia. The biophysical, cardiovascular, metabolic and endocrine responses to both acute and chronic fetal hypoxemia have been investigated in numerous animal studies and there are also data from the hypoxic human fetus (see Edelstone, 1984; Carter, 1989; Richardson et al, 1989; Rurak, 1994; Rurak, 1995). Acute hypoxemia (~30-60 min), whether induced by the maternal inhalation of a hypoxic gas mixture or a reduction in maternal uterine blood flow, causes a number of physiological responses. Fetal breathing movements and body movements are greatly reduced (Boddy et al, 1974; Bocking et al, 1986), and this likely serves to minimize fetal O_2 demands. In terms of fetal cardiovascular function, there is hypertension and initial bradycardia, and a redistribution of cardiac output in favor of the heart, brain and adrenal gland, at the expense of flow to other organs (Cohn et al, 1974). Sympathetic and parasympathetic mechanisms, and increased circulating catecholamine and vasopressin (AVP) concentrations appear to be involved in these cardiovascular responses (Jones et al, 1977a; Rurak, 1978; Walker et al, 1979; Lewis et al, 1980; Cohen et al, 1982; Ruess et al, 1982; Parer, 1983; Court et al, 1984). In addition to the increased fetal concentrations of catecholamines and AVP, there are also elevations in ACTH, cortisol and

PGE_2 (Boddy et al, 1974; Jones et al, 1977a; Challis et al, 1986; Bocking et al, 1986; Hooper et al, 1990; Murotsuki et al, 1995). The metabolic consequences of acute fetal hypoxemia include hyperglycemia, achieved primarily via catecholamine-induced glycogenolysis, and a progressive rise in blood lactate concentration (Jones and Ritchie, 1978b). It is this lactic acidemia that limits the fetal tolerance to hypoxia, as the resulting metabolic acidemia further reduces blood O_2 concentration via a Bohr shift on the hemoglobin oxygen dissociation curve (Rurak et al, 1990a&b).

In contrast to the fetal responses to acute hypoxemia, with a chronic reduction (> ~2 h) in fetal O_2 delivery, there is a gradual return in the frequency of fetal breathing movements to the normal levels (Bocking et al, 1988; Hooper et al, 1990; Koos et al, 1988). There is also gradual resolution of the metabolic acidemia, and a plateau in lactate levels, (Bocking et al, 1992; Boyle et al, 1992; Wilkening et al, 1993; Hooper et al, 1995). Fetal O_2 consumption is maintained with a 24 h reduction in O_2 delivery (Bocking et al, 1992). However, with a ~9 days reduction in fetal O_2 delivery, achieved by a controlled long-term decrease in umbilical blood flow, fetal O_2 uptake is reduced compared to control animals, probably as a result of a fall in fetal growth rate (Anderson et al, 1986). In terms of fetal cardiovascular function, there appears to be an initial increase in fetal cardiac output that lasts from ~1 to 3 h of the hypoxemic period, with increased blood flow to the brain, heart, placenta and adrenal, but with no decrease in perfusion to other less vital organs (Court et al, 1984; Milley, 1987; Milley, 1988; Bocking et al, 1988; Rurak et al, 1990b). In addition, plasma concentrations of ACTH decline to near control levels, but cortisol concentrations remain elevated (Challis et al, 1989; Gagnon et al, 1994). It is clear, therefore, that fetal responses to acute hypoxemia do not adequately reflect the responses to prolonged hypoxemia.

1.5 *EFFECTS OF HYPOXEMIA ON PLACENTAL FUNCTIONS*

As discussed above, there have been many studies conducted on the fetal responses to hypoxemia. However there has been much less investigation of the effects of reduced oxygenation on the placenta. Placental O_2 supply clearly could be reduced by a fall in O_2 delivery from the mother, i.e. by a maternal hypoxia or a decrease in uterine blood flow. However, it is also possible that a reduction in oxygen delivery from the fetus to placenta could also affect the placenta, if the tissues of the fetal components of the placenta (which are perfused with fetal blood delivered by the umbilical arteries) derive oxygen from this source. However, this has not been yet demonstrated for placental O_2 usage, although Hay et al (1984) have found that ~40% of the glucose utilized by the sheep uteroplacenta is derived from fetal blood. Moreover, if fetal glucose concentrations are increased, the proportion of uteroplacental glucose supply supplied by the fetus rises (Simmons et al, 1979). Furthermore, when uterine blood flow is reduced acutely in pregnant sheep to decrease uteroplacental glucose supply, there is placental uptake of lactate from the fetal circulation (i.e the reverse of the normal situation), and this is sufficient to make up for the fall in placental glucose consumption (Gu et al, 1985; Hooper et al, 1995). In sheep, it is also possible that placental progesterone production could be indirectly reduced by hypoxia via the increase in fetal ACTH and cortisol levels that occurs with hypoxemia, with the latter response leading to activation of placental 17α-hydroxylase. However, this process would likely take considerably longer than a direct effect of hypoxia on the placenta (Jones et al, 1977a; Clapp et al, 1982a; Gagnon et al, 1994).

There have been several *in vitro* studies which indicate that steroidogenesis is oxygen dependent. As discussed previously, the cholesterol side-chain cleavage reaction in rat adrenal mitochondrial preparations is oxygen dependent when the supply of either

substrate (cholesterol) or reducing equivalents is low (Stevens et al, 1984). And a recent study of cytochrome $P450_{scc}$ activity in human placental trophoblast cells indicates that the cholesterol supply is limiting (Tuckey et al, 1994), so that an oxygen-dependence of the side-chain cleavage reaction may also be present in the placenta. The O_2 dependence of cytochrome $P450_{scc}$ is similar to that seen with the cytochrome P-450s involved in many phase 1 drug detoxification reactions (Jones et al, 1989). Aw et al (1985) examined the oxygen dependence of estrogen production (from androgens via aromatase) in human placental microsomal preparations and in cultured choriocarcinoma cells (BeWo line). Oxygen dependence was demonstrated in both preparations, with the effect being more pronounced in the intact BeWo cells. Thus, the *in vitro* data suggest that reactions involved in placental steroidogenesis could be impaired by reduced O_2 supply.

There are limited *in vivo* data on the oxygen dependence of placental steroidogenesis. Several of the studies which have examined the effects of reduced O_2 delivery on chronically instrumented fetal lambs have also measured the circulating levels of some of the endocrine products of the placenta. A consistent finding has been a rise in fetal plasma PGE_2 concentrations (Hooper et al, 1990; Sue-Tang et al, 1992; Murotsuki et al, 1995). It is likely that the placenta is the source of this PGE_2 (Kelleman et al, 1992; Murotsuki et al, 1995), and there is also evidence that the rise in fetal circulating levels of the compound is important in minimizing the fetal hyperglycemia and lactic acidemia that occur in response to hypoxemia (Hooper et al, 1992; Thorburn, 1992). Challis et al (1989) measured maternal and fetal arterial progesterone concentrations in experiments involving a 48 h reduction in uterine blood flow. Although arterial plasma progesterone levels increased transiently in the first 1-2 h in the fetus, there were no significant differences in comparison to a control group of animals. However, measurement of arterial progesterone concentration alone provides little information of uteroplacental

production. Keller-Wood and Wood (1991) measured progesterone levels in both maternal arterial and uterine venous blood before and during a 30 min hypoxic period (achieved by lowering maternal inspired O_2 concentration). A rise in uterine venous progesterone concentrations occurred in 7/10 experiments at 20 and 30 min of hypoxia. However, as uterine blood flow was not measured, actual uteroplacental progesterone output could not be estimated. In addition, the duration of hypoxia (30 min) may not have been sufficient to elicit an effect. This study also found no evidence for placental production of ACTH or corticotropin-releasing factor (CRF) under either normoxic or hypoxic conditions and similar results have been obtained by Sue-Tang et al (1992) in a study involving a 24 h reduction in uterine blood flow. In studies on pregnant sheep carried out by Clapp et al. (1981, 1982a), which involved microembolization of the uterine circulation over 13 days to limit the rise in uterine blood flow in late gestation, there was a progressive rise in fetal in cortisol level, and this was followed in the post-embolization period by a decrease in uterine venous progesterone concentration, and perhaps uteroplacental progesterone secretion rate into the maternal circulation (Clapp et al, 1982a). In some of the animals, delivery occurred prematurely, and in these, the reciprocal changes in fetal cortisol and uterine venous progesterone occurred simultaneously, likely reflecting the cortisol effect on 17α-hydroxylase that normally operates at term. In pregnant baboons, as in the human, there is a high level of estrogen production from androgenic steroid precursors. Fritz et al (1985) conducted acute studies in anesthetized pregnant baboons, and found that with experimental reductions in uterine blood flow, the rate of conversion of maternal dehydroepiandrosterone to estradiol was linearly related to uterine perfusion, suggesting an O_2 dependence of placental aromatase. In a subsequent study (Fritz et al, 1986), maternal plasma estradiol levels were decreased in association with the reduced placental clearance of maternal androgen precursors. However, this only occurred in animals where there was no evidence of fetal distress during the period of reduced uterine perfusion. When fetal

distress appeared to be present, maternal estradiol concentration appeared to be increased, and it was hypothesized that this was due to an increased supply of androgen precursors from fetus to placenta. This hypothesis has recently been confirmed; a transient reduction in uterine blood flow that resulted in fetal hypoxemia and acidemia was associated with an increased fetal production rate of dehydroepiandrosterone and elevated estrogen concentrations in maternal plasma (Shepherd et al, 1992). These studies in baboons suggest that, in conditions involving fetal hypoxemia, placental steroidogenesis, at least for estrogens, can be affected by factors other than O_2 supply.

1.6 *RATIONALE*

From the previous discussion, the following factors are apparent:

1. During pregnancy, the placenta has a very high metabolic rate and consumes significant proportion of the oxygen and glucose delivered to the uterus and its contents. It is also possible that some of the oxygen used by the placenta is supplied from fetal blood.

2. The placenta is an important endocrine organ which produces large amounts of steroid and protein hormones which are released into the maternal and fetal circulation. This synthetic activity requires metabolic energy, the bulk of which is likely provided via oxidative phosphorylation and hence requires oxygen.

3. Progesterone is one of the most important hormones synthesized by the placenta as it is essential for pregnancy maintenance.

4. *In vitro* studies of steroidogenic pathways have demonstrated that some steps, such as cholesterol side-chain cleavage and aromatase, are oxygen dependent.

5. The limited number of *in vivo* studies on the effects of hypoxemia on placental hormone production have not provided evidence for oxygen-dependent processes for progesterone or other hormones. However, the appropriate measurements (i.e. uteroplacental progesterone output) have not been made.

1.7 *OBJECTIVES*

The objective of this study is to determine the effects of short-term maternal hypoxemia on uteroplacental progesterone output into the maternal circulation. In addition, a number of other physiological and metabolic parameters were measured, including maternal and fetal blood gas status, acid-base balance; hemoglobin concentration, blood oxygen saturation; uteroplacental oxygen delivery and consumption, uteroplacental glucose uptake and lactate flux, and fetal plasma cortisol concentration.

1.8 *SPECIFIC AIMS*

To examine the effect of maternal short-term acute hypoxemia on uteroplacental progesterone production in chronically instrumented pregnant sheep.

1.9 *HYPOTHESIS*

Short term maternal hypoxemia decreases placental progesterone production via a reduction in placental oxygen supply from the mother and/or fetus.

2 *EXPERIMENTAL METHODS*

2.1 *ANIMAL PREPARATION*

A. *Breeding*

Sheep have an estrous cycle which lasts ~17 days. The ewes (Dorset and Suffolk breeds) used in the current study were time-mated using estrous synchronization. This was accomplished with intravaginally implanted Veramix Sheep Sponges (Tuco Products Co., Orangeville, Ont.), which release medroxyprogesterone acetate, a progestin, to suppress spontaneous ovulation. After removal of the sponge 14 days later, ovulation was induced by intramuscular injection of 250-500 I.U. Pregnant Mares' Serum Gonadotropin (Ayerst Laboratories,). Ovulation normally occurs 24-48 h later (Whyman et al, 1979), and over this time a ram was placed with the ewes. To improve the conception rate, the ewes were kept with the ram for a further 2 weeks, i.e. until the next ovulation, if conception did not occur with the first ovulation. Pregnancy was assessed by measurement of plasma progesterone concentration in the ewe at ~19 days after pessary removal, and confirmed later in gestation (>50 days) by ultrasound examination.

B. *Surgical Procedures*

Surgery was performed on the pregnant ewes at 121~127 days gestation (term is ~145 days). Ewes were fasted for ~18 h prior to surgery, but had access to water. On the day of surgery, atropine sulfate (6 mg) was administrated via the maternal jugular vein to control salivation. Approximately 10 min later, anesthesia was induced with an injection of

sodium pentothal (1 g) given via a maternal jugular vein. The ewe was then intubated and anesthesia maintained by ventilation with 1.0-1.5% halothane and 60% nitrous oxide in oxygen. A slow infusion of 5% glucose solution in water (500 ml) was given to the ewe by an i.v. drip. The ewe's abdomen was then shaved, and washed with povidone-iodine antiseptic solution. The remaining areas of the ewe were covered with sterile sheets.

Sterile procedures were employed throughout the surgery. A lower midline abdominal incision was made to expose the uterus. Then a small incision was made in an area of uterus over the fetal head and neck and free from placental cotyledons and major blood vessels. The fetal head was exteriorized and a sterile silicone rubber catheters (Dow Corning, Midland, Ml) were implanted in the fetal trachea and a carotid artery. A catheter was also implanted into the amniotic cavity and sutured to the fetal skin. The head was then returned and the uterine incision closed and oversewn. A second uterine incision was made to gain access to the lower body of the fetus and the fetal hindquarters were exteriorized. Silicone rubber catheters were implanted in both femoral arteries and lateral tarsal veins, and also in the common umbilical vein (using a non-occlusive technique, Rurak et al, 1990a). A second amniotic catheter was also implanted. The fetal hindquarters were then returned to the uterus and ~1,500 ml of sterile irrigation saline was added to replace amniotic fluid lost during surgery. The uterine incision was then closed and oversewn. The main uterine vein of the horn containing the fetus was then identified and a small branch was exposed at the ovarian end. A silicone rubber catheter was implanted in this branch and advanced ~10 cm so that the tip lay in the main uterine vein. Finally, a type 6R Transonic transit-time blood flow transducer (Transonics Corp., Itheca, NY) was placed around the middle uterine artery of the horn containing the operated fetus. All catheters and cables were tunneled subcutaneously in the maternal abdomen to emerge from an incision on the ewe's flank. The abdominal incision was then closed in layers,

and silicone rubber catheters were implanted in a maternal femoral artery and vein. All catheters are then capped and stored in a denim pouch on the ewe's flank, along with the blood flow transducer cable. In the 5 initial animals studied, a non-occlusive, silicon rubber catheter (0.080 in outside diameter) was implanted in the maternal trachea below the larynx for tracheal infusion of nitrogen and other gases (Gleed et al, 1986). This catheter did not interfere normal breathing by the ewe. At the end of the surgery, 500 mg ampicillin and 40 mg gentamicin were injected intramuscularly to the ewe, and these doses were repeated for the first 4 post-surgical days. The fetus received 500 mg ampicillin and 10 mg gentamicin via the tarsal vein post-surgery, while ampicillin (500 mg) and gentamicin (20 mg) were administered to the amniotic cavity on the daily basis for the duration of the preparation.

C. *Post-Surgical Maintenance*

Following surgery, the ewe was kept in holding pen with other sheep and allowed free access to food and water. Catheter patency was maintained by daily flushing with ~2 ml of heparinized (12 I.U./ml) sterile normal saline. In order to monitor fetal condition, arterial and umbilical venous blood samples (~0.8 ml) were collected daily for measurement of fetal blood gas and acid-base status, hemoglobin concentration and glucose and lactate levels. The animals were allowed to recover for a minimum of 3 days post-surgery before monitoring and experimental procedures commenced. Then the sheep was transferred to a monitoring pen adjacent to and in full view of the holding pen and companion sheep.

2.2 *DESIGN OF CATHETERS*

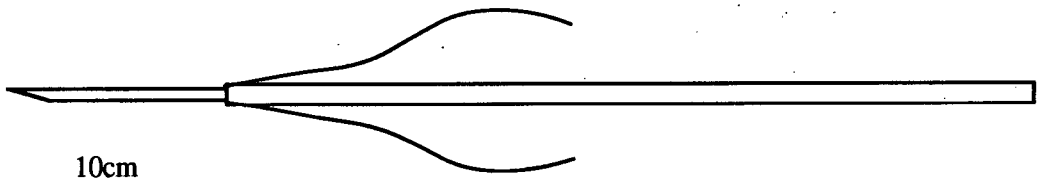
The basic design of the maternal and fetal catheters were composed of 125 cm lengths of silicone rubber tubing (Silastic^R medical grade tubing, Dow Corning Corporation, Medland, Michigan) with a 30 cm long, 3-0 silk suture (Davis & Geck Cyanamid Canada Inc., Montreal, Quebec) tied to one end of the tubing and secured in placed with silastic medical adhesive (Silastic^R medical adhesive silicone type A; Dow Corning Corporation). Depending on the type of catheter, the tubing's diameter and the suture position varied. Given in Fig. 1 and Fig. 2 are the specifications for each type of catheter.

2.3 *EXPERIMENTAL PROTOCOL*

A. *Hypoxemia Protocol*

Experiments were performed at 125-136 days gestation. The total duration of experiments was 6 h, including a 2h normoxic control period, a 2 h period of maternal and fetal hypoxemia and 2 h recovery period (Fig. 3). In the initial 5 experiments hypoxemia was achieved by infusion of nitrogen (9-13 l/min) via the maternal tracheal catheter to result in moderate hypoxemia (maternal and fetal Pao_2 reduced by 26-35% and 16-24%, respectively). In 4 subsequent experiments, more severe hypoxemia (maternal and fetal arterial Pao_2 reduced by 41-58% and 25-33%, respectively) was achieved by delivering a low oxygen mixture (~9% O_2, 1% CO_2, balance N_2 at 40 l/min) to a plexiglass chamber in the front of the monitoring pen (Rurak et al., 1990a). In both situations, the ewe was able to eat and drink as usual. Prior to the experiment, 21 ml of maternal blood was collected for subsequent transfusion to the fetus via the tarsal vein to replace the fetal blood lost by

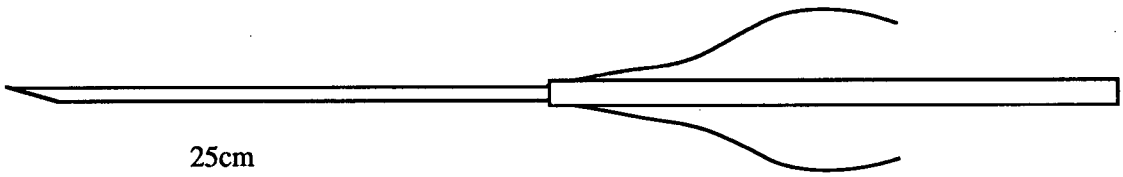
Maternal venous Catheter



10cm

Inside Diameter (I.D.) 0.040inch
Outside Diameter (I.D.) 0.085inch

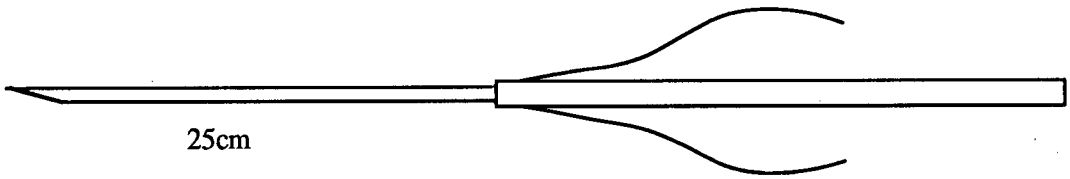
Maternal Arterial Catheter



25cm

I.D. 0.04inch
O.D. 0.085inch

Uterine Venous Catheter



25cm

I.D. 0.04inch
O.D. 0.085inch

Figure 1. Catheters used in the chronic maternal sheep preparation (not drawn to scale).

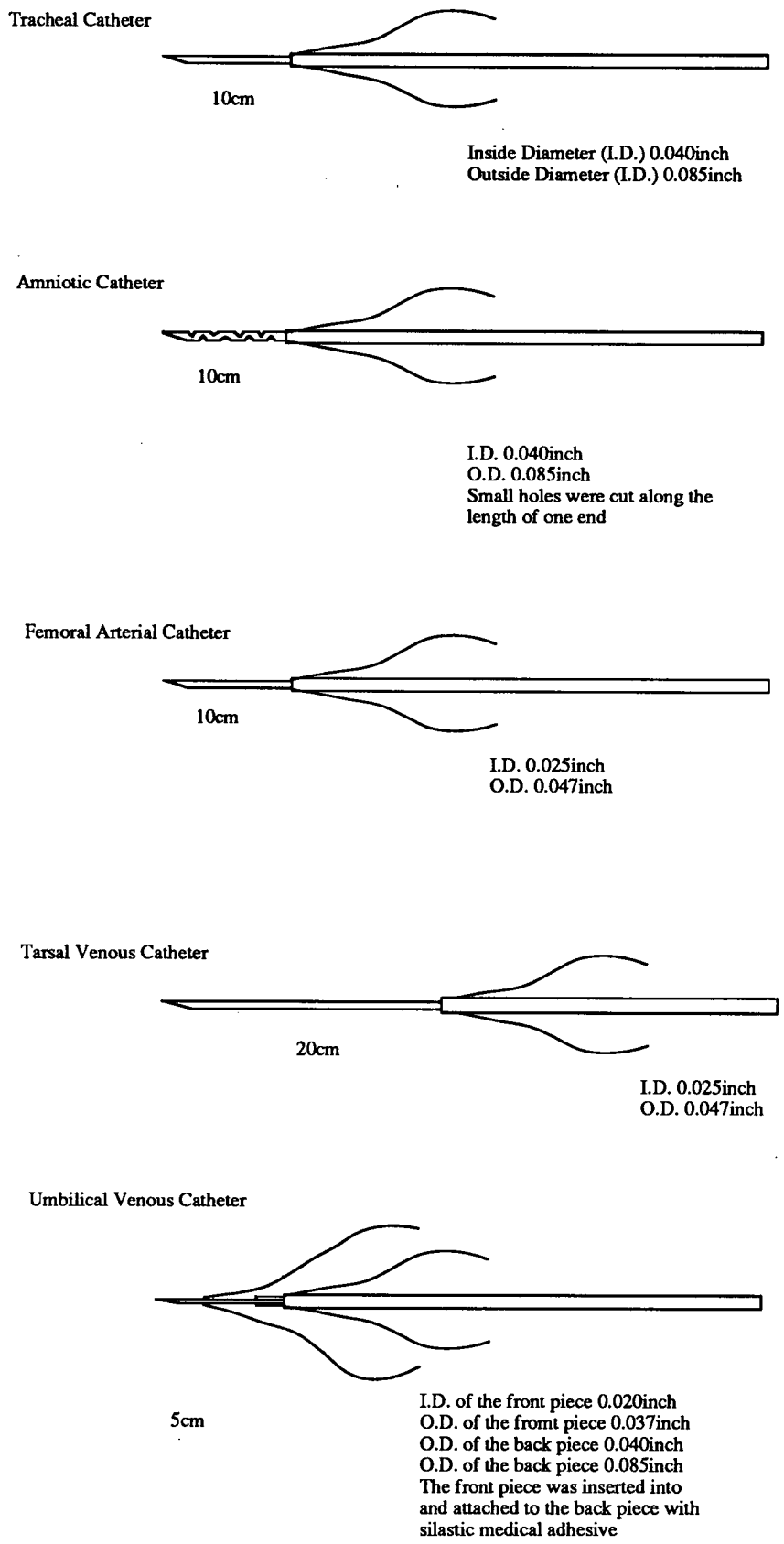


Figure 2. Catheters used in the chronic fetal sheep preparation (not drawn to scale).

Experimental Protocol

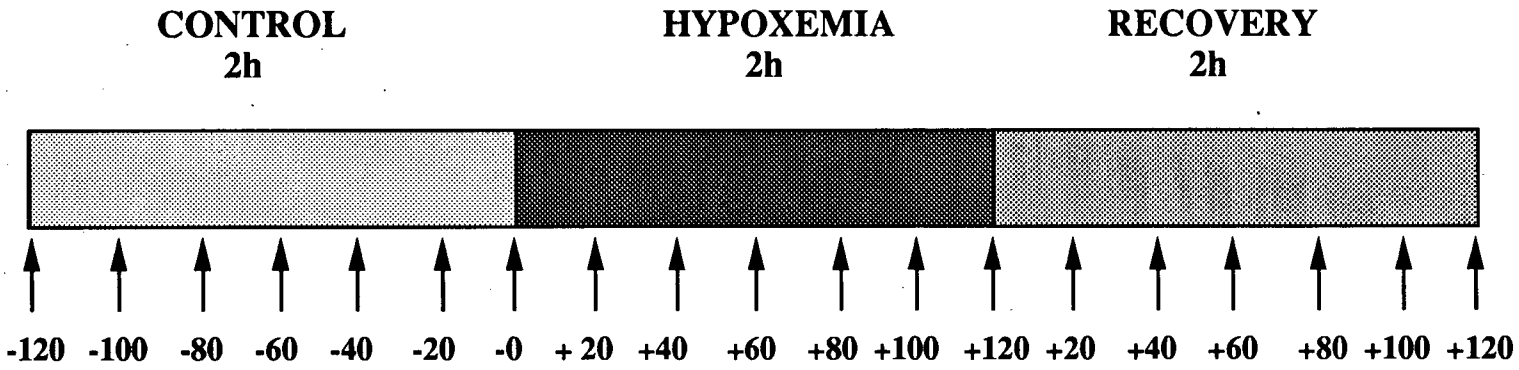


Figure 3 Experimental protocol.

sampling. During the experiment samples (3 ml) were collected at 20-min intervals from maternal femoral arterial (MA) and uterine venous (UtV) catheters for measurement of progesterone concentration. At 0 and 120 min of the control period, 20, 80 120 min of the hypoxemia interval and 20 and 120 min of the recovery period, MA, UtV, fetal arterial (FA) and umbilical venous blood (UV) samples (0.8 ml) were collected for measurement of Po_2, Pco_2, pH, O_2 saturation, hemoglobin concentration and glucose and lactate levels. FA and UV samples (3 ml) were also collected at the these times for measurement of cortisol concentrations. After each fetal blood sample, the total volume collected was replaced with an equal amount of the maternal blood collected prior to the experiment.

B. *Control Protocol*

Control experiments were performed using the same duration and sampling regimen as for the hypoxemia experiments. The only difference between the 2 protocols was that in the control studies, the ewe breathed a normoxic gas mixture for the entire 6 h.

2.4 *MONITORING TECHNIQUES*

During the experiment, the following variables were recorded continuously on a 12 channel polygraph recorder (Sensormedics R711, Sensormedics, Anaheim, CA) using appropriate Beckman or Sensormedic input couplers: fetal arterial, tracheal, and amniotic pressures, maternal arterial and uterine venous pressure, maternal and fetal heart rates, and uterine blood flow. The hydrostatic pressures were measured with Gould DTX disposable transducers (Spectramed Inc., Oxnard, CA) connected to type 9872 strain-gage couplers (Sensormedics). Maternal and fetal heart rates were determined from the maternal and fetal arterial pulse with type 9875 cardiotachometers (Sensormedics). Uterine blood flow was

measured with Transonic model T201 transit-time flow meter (Transonic Systems). The analog signals from amniotic and arterial pressures, heart rates and uterine blood flow were also digitized and processed on-line (Kwan, 1989). The computerized data acquisition system comprised an Apple IIe computer system (Apple Computer Inc., Cupertino, CA) containing an analog to digital conversion board (AI-13 Analog Input System, Daisi Electronics Inc., Newton Square, PA). The digitized samples for each variable were averaged and displayed at 10 sec intervals, and the 1 min averaged values were stored on floppy diskettes. Fetal arterial pressure was corrected for intrauterine pressure by the computer program.

2.5 *MEASUREMENT TECHNIQUES*

A. *Blood Gas and Acid-Base Parameters*

Samples for blood gas analysis were collected into preheparinized blood gas syringes (Marquest Medical Products, Englewood, CO), which are then capped and placed on ice until analysis, usually within 5-30 min Po_2, Pco_2, pH, base excess/deficit, and bicarbonate concentration were estimated using an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milano, Italy) with temperature corrected to 39° C for maternal samples and 39.5° C for fetal samples. Blood O_2 saturation and hemoglobin concentration were measured in triplicate using an OSM-2 hemoximeter (Radiometer, Copenhagen).

B. *Glucose and Lactate*

Whole blood glucose and lactate concentrations were determined in triplicate using membrane-bound glucose oxidase and D-lactate dehydrogenase enzymes, respectively, with a Stat Glucose Lactate Analyzer (Model 23A, Yellow Springs Instruments, Yellow Springs, OH).

C. *Progesterone and Cortisol*

Blood samples for progesterone and cortisol were collected in chilled plastic syringes and centrifuged at 3000 g for 25 min at 4° C. The plasma was the transferred to 200 µl vials and stored at -70° C until assayed.

Progesterone concentration was measured using a commercial radioimmunoassay kit (Diagnostic System Lab Inc., Webster, TX). This kit provided 6 progesterone standard concentrations (0, 0.3, 1, 5, 20 and 60 ng/ml), and assay tubes coated with rabbit anti-progesterone immunoglobulin. The samples and standards were incubated with ^{125}I-labeled progesterone at 35~37° C for 60-70 minutes. After incubation, the fluid in the assay tubes was aspirated from all tubes, except for the total count tubes. Then the tubes were counted in a gamma scintillation counter (Searle Analytical, Des Plaines, IL) for 1 min. The lowest detectable level of progesterone that could be distinguished from background was 0.12 ng/ml at the 95% confidence limit. The intra-assay coefficient of variation ranges from 3.2±0.5% to 8.5±1.4% (Mean = 5.6±0.3%) and the inter-assay coefficient of variation is 7.3±1.9%.

Cortisol was also measured using a commercial radioimmunoassay kit (Diagnostic Products Corp., Los Angeles. CA.). The assay included 5 cortisol standard concentrations (1, 5, 10, 20, and 50 μg/dl), and assay tubes that were coated with anti-cortisol serum. The standard and unknown samples were incubated with ^{125}I labeled cortisol tracer at 35~37° C for 45 min. After incubation, the tube contents were aspirated (except for the total counts tube) and the tubes counted by a gamma counter for 1 min. The lowest detectable level of cortisol that can be distinguished from background was 0.2 μg/dl. The intra-assay coefficient of variation ranges from 9.1±1.3% to 10.6±1.4% (Mean = 9.8±1.0%) and the inter-assay coefficient of variation is 3.5±1.8%.

2.6 *ANALYSIS*

A. *Calculations*

The following parameters were calculated from the measured variables:

i **Oxygen Content**

O_2 content = (0.616 x [Hb] x O_2 saturation) x 100

ii **Uteroplacental Oxygen Delivery**

Uteroplacental O_2 delivery = $[O_2]_{MA} \times Q_{ut}$

iii **Uteroplacental Oxygen Consumption**

Uteroplacental O_2 consumption = $([O_2]_{MA} - [O_2]_{UtV}) \times Q_{ut}$

iv **Uteroplacental Oxygen Extraction**

Uteroplacental O_2 extraction = (Uterine O_2 consumption/uterine O_2 delivery) x 100

v **Uteroplacental Glucose Uptake**

Uteroplacental glucose uptake = $([Glu]_{MA} - [Glu]_{UtV}) \times Q_{ut}$

vi **Uteroplacental Lactate Flux**

Uteroplacental lactate flux = $([Lac]_{UtV} - [Lac]_{MA}) \times Q_{ut}$

vii **Uteroplacental Progesterone Secretion**

Uteroplacental progesterone secretion = $([P4]_{UtV} - [P4]_{MA}) \times Q_{ut}$

Note that total uterine blood flow was not measured in the studies, as the flow transducer was implanted on only one of the paired middle uterine arteries. Thus the estimate of progesterone secretion is also not a total estimate, but the rate from the horn of the uterus containing the operated fetus.

B. *Estimation of Fetal Weight in utero*

The weight of the operated fetuses and unoperated twins at the time of experimentation was estimated from the birth weight using an equation for the normal growth curve in fetal lambs determinated by Koong et al (1975):

$$\text{Log weight}_{\text{in utero}} = \log \text{weight}_{\text{birth}} + 0.000165\ (2 \times d \times GA + d^2) - 0.0556d$$

where d is the number of days between birth and the *in utero* day and GA is the gestational age *in utero*. This equation predicts an average rate of fetal growth (~2%/day) that is not different from the value determined in our labatory using fetal blood volume estimates of weight *in utero* (Kwan et al, 1995).

C. *Statistics*

Changes in the measured variables were tested for statistically significance using 2 way analysis of variance for repeated measures, with time and animals being the parameters tested. When a statistically significant F value was obtained for the time results ($p<0.05$), then Fisher's least significant difference test for multiple comparisons was used to compare individual means. Values are expressed as mean ± sem.

3. *RESULTS*

3.1 *ANIMALS STUDIED*

A total of 13 animals were surgically prepared for study. Of these, 7 animals were successful preparations and hypoxia and/or normoxia experiments were performed on them (Table 1). The remaining 6 were not used because of catheter failure, preterm labour or fetal death *in utero* (Table 2).

3.2 *EXPERIMENTAL DETAILS*

At the time of surgery, the average gestational age was 124.5±0.5 days (range 121-127 days). Hypoxemia studies were performed at 131.0±1.2 days, 6.9±0.7 days after surgery. Fetal weight at the time of experimentation averaged 2.94±0.18 kg, with the singleton fetuses weighing 3.34±0.19 kg and the twin fetuses weighing 2.48±0.18 kg. The twins unoperated weighed 3.28±0.20 kg at birth and had an estimated weight of 2.81±0.17 kg at the time of experimentation. A total of 4 normoxia experiments, 5 moderate hypoxemia experiments and 4 severe hypoxemia studies were carried out (Table 1). At least 48 h was allowed to elapse between successive experiments in animals subjected to more than one study.

Table 1. Details on animals studied.

Ewe No.	G.A. at surgery (days)	G.A. at Experiment (days)	Weight at Experiment (kg)	Type of Experiment	# of Fetuses	Sex of Fetus	G.A. at Delivery (day)	Birth Weight (kg)	Maternal Weight (kg)
1142	122	130	1.71	control	2	op F non-op M	139	2.11 3.10	74.5
1119	125	130 132 136	3.41 3.61 4.00	moderate hypoxemia control moderate hypoxemia	1	op M	143	4.67	72.6
1250	125	130 132	2.32 2.46	control moderate hyoxemia	2	op M non-op M	142	3.12 3.84	74.0
971	125	131 133	2.64 2.79	moderate hypoxemia control	2	op M non-op M	139	3.23 3.86	98.5
2216	126	132 134	3.42 3.61	control severe hpoxemia	1	op F	139	4.08	79.0
158	121	125 127	2.57 2.74	severe hypoxemia severe hypoxemia	1	op F	134*	3.35	70.3
2175	127	134	2.96	severe hypoxemia	2	op F non-op M	141	3.49 3.51	93.52

* Fetus died *in utero*,: op F, operated fetus; non-op F, non-operated fetus; GA, gestational age.

Table 2. Details on unsuccessful animals preparations.

Ewe No.	G. A. at Surgery (days)	Reason for failure
20	123	Preterm labor
239	127	Maternal infection
145	125	Catheter failure
55	125	Fetus died *in utero*
1122	125	Fetus died *in utero*
4132	125	Preterm labor

GA, gestational age.

3.3 *NORMAL PROGESTERONE CONCENTRATIONS, UTERINE BLOOD FLOW AND UTEROPLACENTAL PROGESTERONE OUTPUT*

In Figures 4 and 5, the data obtained in the pre-experimental (control) period are plotted for the 3 ewes with a single fetus and for the 4 ewes with twins. Maternal arterial progesterone concentration averaged 4.27±0.13 ng/ml in ewes with a single fetus and 6.53±0.19 ng/ml in ewes with twins (Fig. 4). In the uterine vein, progesterone concentration was 17.82±1.17 ng/ml in ewes with a single fetus and 21.05±0.56 ng/ml in ewes carrying twins (Fig 4). Uterine arterial blood flow was 339.6±6.2 ml/min in ewes with a single fetus and 549.5±17.9 ml/min in ewes with twins (Fig. 5). Uteroplacental progesterone output averaged 4720±243 ng/min in ewes with a single fetus and 8156±426 ng/ml in the twin bearing sheep (Fig. 5). Thus the ewes with twins fetuses have slightly higher values for all the above variables, and the differences were statistically significant (unpaired t-test, $p<0.05$).

3.4 *NORMOXIA AND HYPOXEMIA EXPERIMENTS*

3.4.1 *Maternal Blood Gas Status, Glucose and Lactate Levels*

A. *Normoxia Experiments*

Four experiments involving normoxia were performed on 4 animals. The normoxia experiments followed the same experimental protocol as with hypoxemia, with the exception that a normoxic gas mixture was administrated for entire 6-h period.

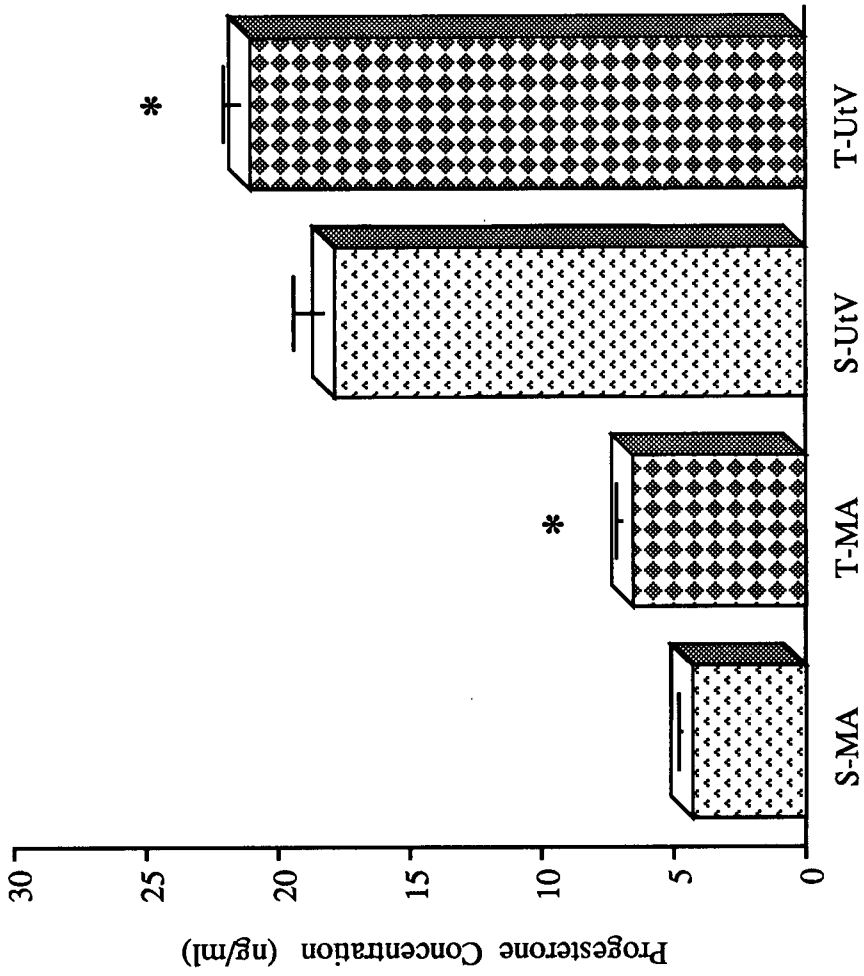


Figure 4 Normal progesterone concentration in singleton and twins pregnancies in the pre-experimental period.
S-MA: Single (Maternal artery)
T-MA: Twin (Maternal artery)
S-UtV: Single (Maternal uterine vein)
T-UtV: Twin (Maternal uterine vein)
* significant difference ($P < 0.05$), unpaired t-test

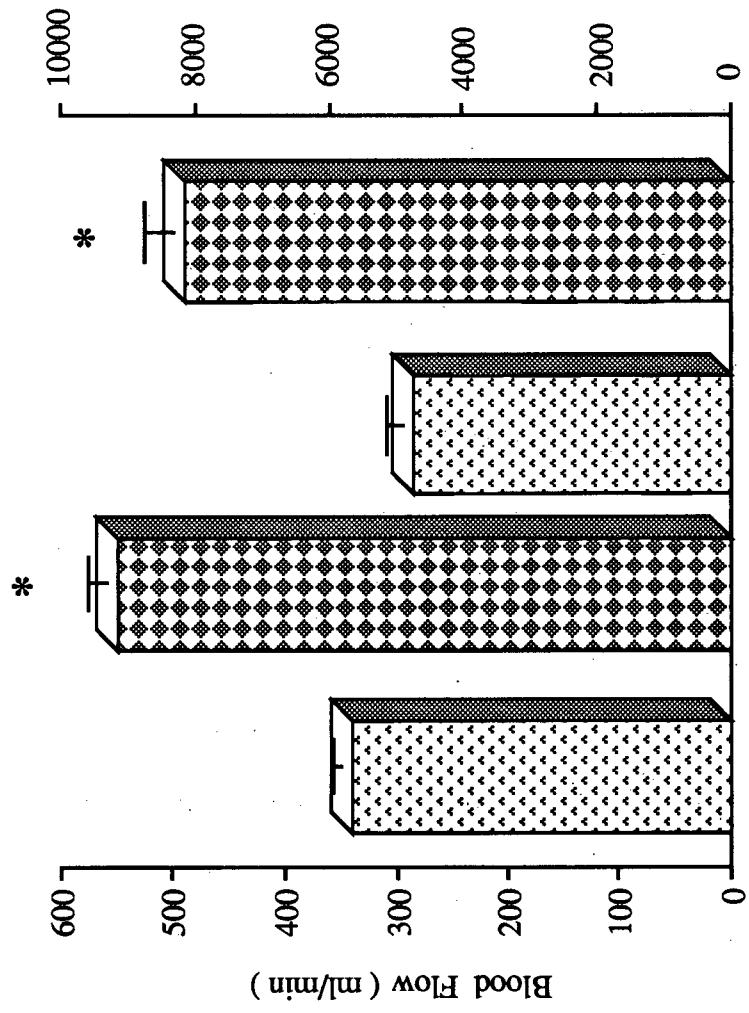


Figure 5 Uterine arterial blood flow and uteroplacental progesterone output in singleton and twin pregnancies in the pre-experimental perod.
S-Qp4: Single (Blood flow)
T-Qp4: Twin (Blood flow)
S-VP4: Single (Progesterone output)
T-Vp4: Twin (Progesterone output)
* significant difference ($P < 0.05$), unpaired t-test

Maternal arterial and uterine venous blood gas, pH, glucose and lactate values are presented in Table 3 and Table 4, and in Figures 6-8. There were no significant changes from pre-experimental values for any of the variables.

B. *Moderate Hypoxemia Experiments*

Five experiments involving maternal moderate hypoxemia experiments on 4 animals, maternal arterial Po_2 was lowered by 26-35% (mean = 30.7±1.8%) or 38.9±4.7 mmHg. The protocol involved infusion of nitrogen gas (9-13 l/min) via the maternal tracheal catheter. Mean values of maternal arterial and uterine venous blood gas, pH, glucose and lactate values are presented in Table 5 and Table 6 and in Figures 6-8. There was a significant decrease in maternal arterial Po_2 (Fig. 6), and a slight, but significant fall in O_2 saturation (Fig.7). However, O_2 content was not significantly altered (Fig. 8), nor were there alterations in Pco_2 and pH (Table 5). Uterine venous Po_2 and O_2 saturation were also significantly reduced during the hypoxia period (Table 6), but there again there was no significant change in O_2 content. Uterine Pco_2 and pH were unchanged and there were also no consistent changes in arterial and uterine venous glucose and lactate concentrations (Tables 5 and 6).

C. *Severe Hypoxemia Experiments*

Four experiments involving maternal severe hypoxemia experiments on 3 animals , maternal arterial Po_2 fell by 41-58% (mean = 48.5±3.5%) or 59.4±6.6 mmHg. The protocol involved delivery of a low oxygen gas mixture (9-10%) to a plexiglass chamber that enclosed the ewe's head and neck.

Table 3. Maternal arterial blood gas parameters and glucose and lactate levels during the control, normoxia experiments. Values are mean ± SE. Data from E1119, E1250, E971 and E2216.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	130.9 ± 4.2	138.0 ± 16.4	136.3 ± 13.8	129.3 ± 14.8	132.5 ± 3.3	130.0 ± 9.3
Pco_2 (mmHg)	34.5 ± 0.8	32.6 ± 1.6	33.5 ± 1.5	31.5 ± 2.9	33.3 ± 1.2	34.0 ± 0.9
pH	7.505 ± 0.010	7.514 ± 0.018	7.509 ± 0.016	7.523 ± 0.032	7.501 ± 0.010	7.517 ± 0.010
Base Excess (mEq/l)	5.5 ± 0.8	4.9 ± 0.9	5.2 ± 0.8	4.7 ± 1.1	4.6 ± 1.2	6.1 ± 0.6
HCO_3 (mmol/l)	27.1 ± 0.7	26.1 ± 0.9	26.5 ± 0.8	25.4 ± 1.2	25.9 ± 1.3	27.4 ± 0.7
TCO_2 (mmol/l)	28.0 ± 0.8	27.0 ± 1.0	27.5 ± 0.8	26.3 ± 1.3	26.9 ± 1.4	28.3 ± 0.7
Hemoglobin (g/100ml)	9.8 ± 0.4	9.9 ± 0.5	10.1 ± 0.5	10.2 ± 0.5	9.9 ± 0.6	9.7 ± 0.6
O_2 Saturation (%)	98.0 ± 0.4	98.0± 1.0	98.3 ± 0.8	98.2 ± 0.9	97.7 ± 0.9	98.6 ± 0.6
Oxygen Content (mM)	5.9 ± 0.3	6.0 ± 0.4	6.1 ± 0.4	6.2 ± 0.4	6.0 ± 0.4	5.9 ± 0.4
[Lactate] (mmol/l)	0.47 ± 0.06	0.47 ± 0.08	0.48 ± 0.07	0.45 ± 0.07	0.50 ± 0.07	0.58 ± 0.15
[Glucose] (mmol/l)	3.17 ± 0.10	3.33 ± 0.18	3.15 ± 0.14	3.01 ± 0.06	3.14 ± 0.10	3.23 ± 0.16

Table 4. Maternal uterine venous blood gas parameters and glucose and lactate levels during the control, normoxia experiments. Values are mean ± SE. Data from E1119, E1250, E971 and E2216.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	58.7 ± 1.5	59.0 ± 1.1	59.0 ± 2.7	58.0 ± 1.1	60.3 ± 0.9	59.0 ± 1.7
Pco_2 (mmHg)	37.7 ± 0.4	38.7 ± 1.1	37.5 ± 0.6	38.3 ± 1.4	38.3 ± 0.8	38.4 ± 0.6
pH	7.476 ± 0.009	7.457 ± 0.010	7.468 ± 0.006	7.460 ± 0.022	7.463 ± 0.015	7.473 ± 0.012
Base Excess (mEq/l)	5.2 ± 0.7	4.6 ± 1.0	4.9 ± 0.6	4.5 ± 1.3	4.7 ± 1.1	5.5 ± 0.7
HCO_3 (mmol/l)	27.46 ± 0.62	27.2 ± 1.0	27.4 ± 0.7	27.1 ± 1.1	27.3 ± 1.1	28.0 ± 0.6
TCO_2 (mmol/l)	28.57 ± 0.61	28.3 ± 1.1	28.4 ± 0.7	28.2 ± 1.1	28.4 ± 1.1	29.1 ± 0.6
Hemoglobin (g/100ml)	9.9 ± 0.4	10.2 ± 0.5	10.2 ± 0.6	10.5 ± 0.4	10.1 ± 0.5	9.8 ± 0.5
O_2 Saturation (%)	78.3 ± 1.5	77.1 ± 3.2	76.3 ± 1.8	74.3 ± 2.9	77.6 ± 2.4	79.0 ± 2.5
Oxygen Content (mM)	4.8 ± 0.3	4.9 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4
[Lactate] (mmol/l)	0.54 ± 0.06	0.56 ± 0.07	0.51 ± 0.07	0.50 ± 0.06	0.51 ± 0.06	0.61 ± 0.14
[Glucose] (mmol/l)	2.88 ± 0.09	3.05 ± 0.19	2.88 ± 0.11	2.82± 0.08	2.84 ± 0.07	2.94 ± 0.16

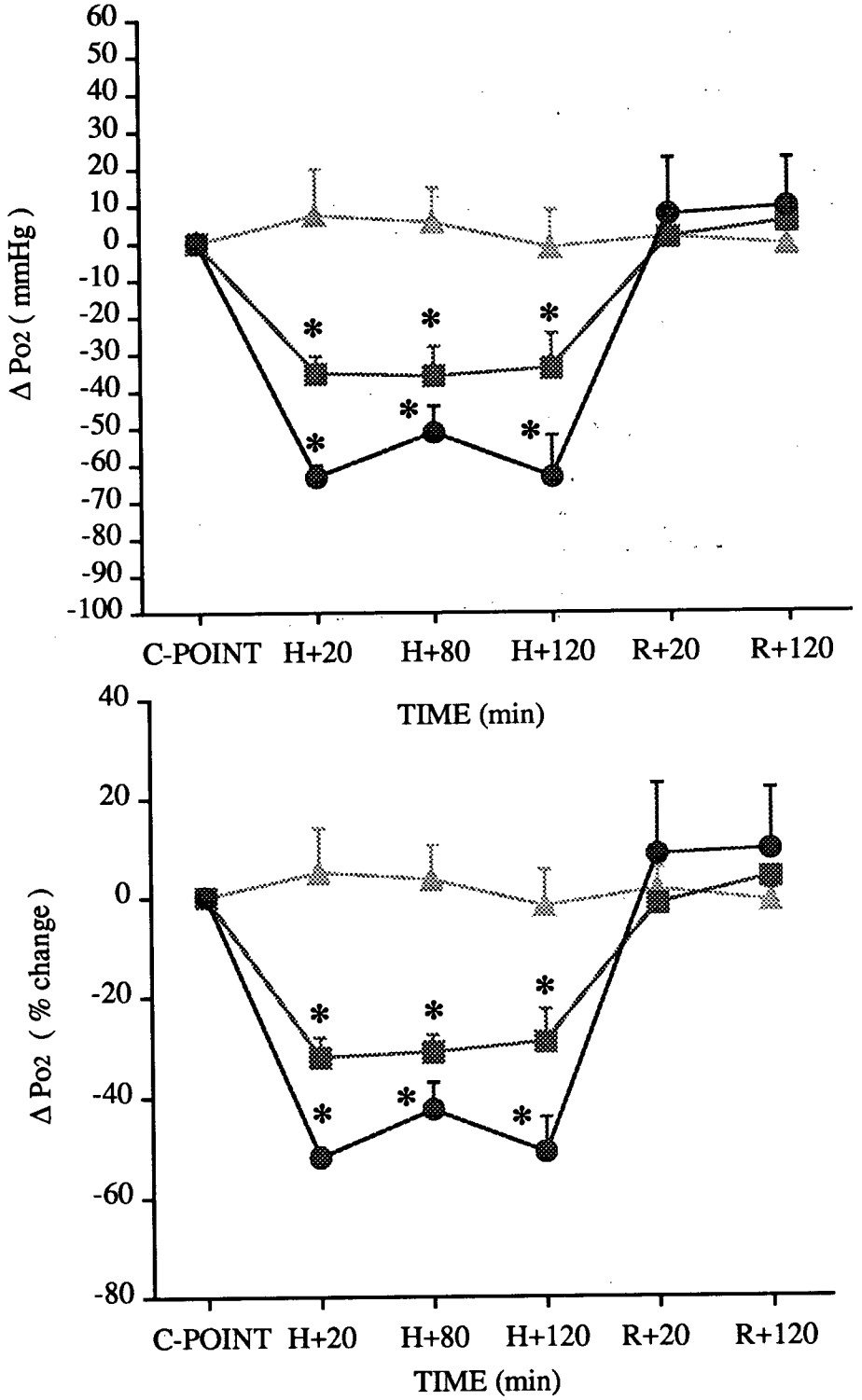


Figure 6 Maternal arterial oxygen tension during the control, moderate and severe hypoxia experiments.
The upper panel plots maternal Po2 as the change from the control value.
The lower panel plots maternal Po2 as the % change from control value.
△ Maternal nornoxia experiments
▩ Maternal moderate hypoxemia experiments
● Maternal severe hypoxemia experiments
* significant difference from control value ($p < 0.05$)

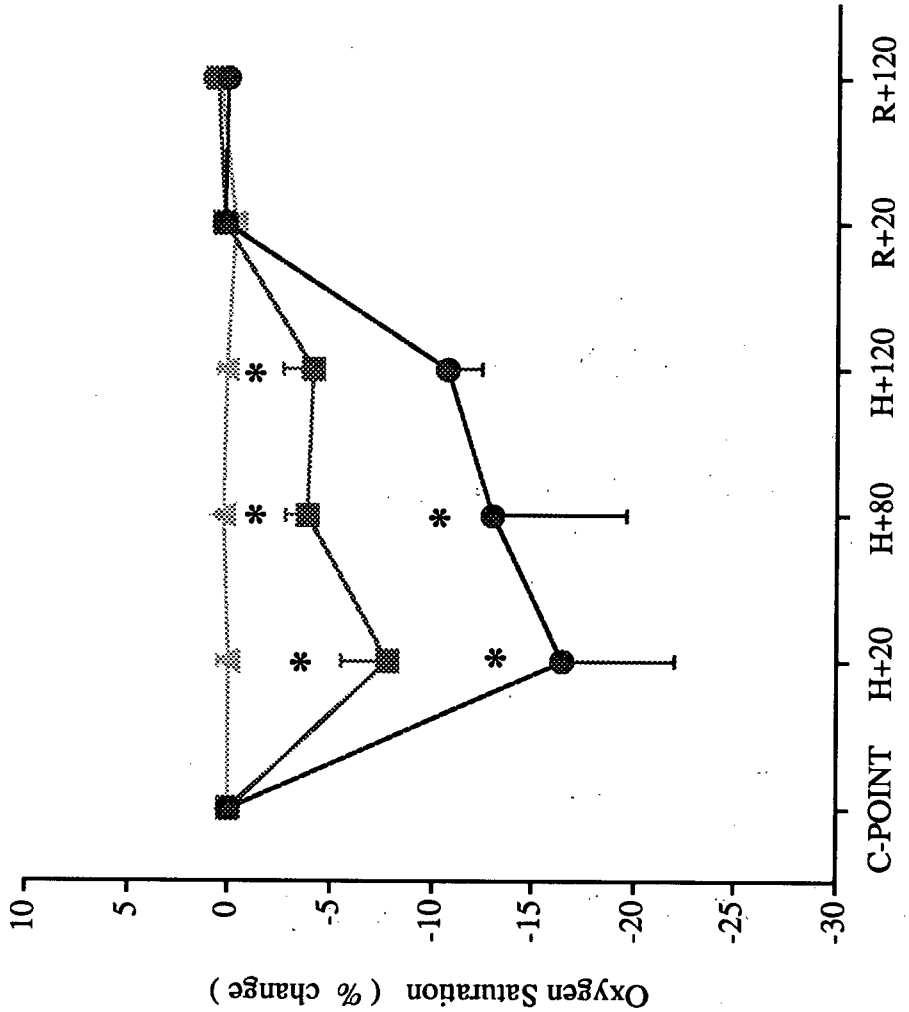


Figure 7 Maternal arterial oxygen saturation during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia
- * significant difference from control value ($p < 0.05$)

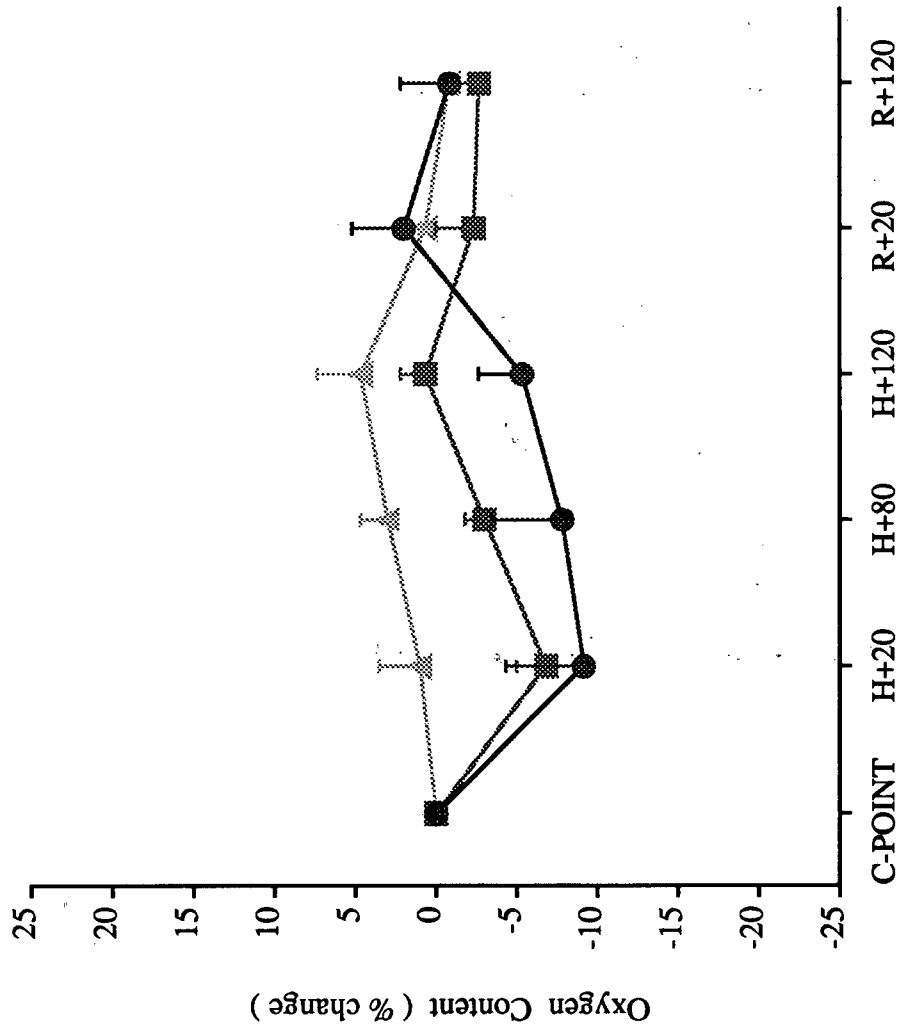


Figure 8 Maternal arterial oxygen content during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

Table 5. Maternal arterial blood gas parameters and glucose and lactate levels during the moderate hypoxemia experiments. Values are mean ± SE. Data from E1142, E1119H1, E1119H2, E1250 and E971.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	5	5	5	5	5	5
Po_2 (mmHg)	125.9 ± 7.1	86.8 ± 12.9 †	85.8 ± 2.8 †	88.4 ± 7.1 †	124.6 ± 12.1	130.6 ± 8.6
Pco_2 (mmHg)	33.2 ± 1.5	32.4 ± 1.9	31.6 ± 1.0	31.7 ± 0.9	31.8 ± 1.1	33.5 ± 0.8
pH	7.512 ± 0.011	7.519 ± 0.024	7.498 ± 0.010	7.514 ± 0.007	7.516 ± 0.012	7.504 ± 0.009
Base Excess (mEq/l)	5.0 ± 0.4	5.1 ± 0.7	3.2 ± 0.6	4.4 ± 0.6	4.6 ± 0.7	4.9 ± 0.9
HCO_3 (mmol/l)	26.2 ± 0.6	26.1 ± 0.7	24.4 ± 0.6	25.4 ± 0.7	25.6 ± 0.8	26.3 ± 1.0
TCO_2 (mmol/l)	27.2 ± 0.7	27.0 ± 0.7	25.3 ± 0.7	26.3 ± 0.7	26.5 ± 0.8	27.2 ± 1.0
Hemoglobin (g/100ml)	9.9 ± 0.2	10.0 ± 0.3	10.0 ± 0.1	10.4 ± 0.5	9.6 ± 0.3	9.4 ± 0.3
O_2 Satuation (%)	97.4 ± 0.5	89.8 ± 2.8 †	93.6 ± 1.5	93.5 ± 1.9	97.7 ± 0.6	97.9 ± 0.5
Oxygen Content (mM)	5.93 ± 0.13	5.5 ± 0.2	5.7 ± 0.1	6.0 ± 0.2	5.8 ± 0.2	5.7 ± 0.2
[Lactate] (mmol/l)	0.580 ± 0.041	0.55 ± 0.04	0.72 ± 0.14	0.59 ± 0.05	0.65 ± 0.06	0.74 ± 0.13
[Glucose] (mmol/l)	3.37 ± 0.13	3.28± 0.10	3.11 ± 0.13	3.12 ± 0.11	3.17 ± 0.17	3.32 ± 0.20

† = significant change from control period ($p < 0.05$)

Table 6. Maternal uterine venous blood gas parameters and glucose and lactate levels during the moderate hypoxemia experiments. Values are mean ± SE. Data from E1142, E1119H1, E1119H2, E1250 and E971.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	5	5	5	5	5	5
Po_2 (mmHg)	56.4 ± 1.0	48.8 ± 1.5 †	49.6 ± 1.4 †	50.4 ± 1.6	57.6 ± 0.9	56.6 ± 0.7
Pco_2 (mmHg)	38.3 ± 0.8	37.2 ± 1.3	37.1 ± 0.9	36.1 ± 1.3	37.1 ± 1.0	37.6 ± 0.7
pH	7.459 ± 0.003	7.469 ± 0.015	7.449 ± 0.003	7.467 ± 0.011	7.464 ± 0.010	7.464 ± 0.012
Base Excess (mEq/l)	4.4 ± 0.5	4.5 ± 0.7	3.1 ± 0.6	3.8 ± 0.7	4.1 ± 0.8	4.4 ± 1.0
HCO_3 (mmol/l)	26.9 ± 0.6	26.8 ± 0.6	25.6 ± 0.7	25.9 ± 0.8	26.4 ± 0.9	26.8 ± 1.0
TCO_2 (mol/l)	28.0 ± 0.6	27.8 ± 0.6	26.6 ± 0.7	26.9 ± 0.8	27.5 ± 0.9	27.9 ± 1.0
Hemoglobin (g/100ml)	9.8 ± 0.2	9.9 ± 0.3	9.9 ± 0.2	10.3 ± 0.4	9.6 ± 0.3	9.6 ± 0.3
O_2 Saturation (%)	75.3 ± 0.9	66.4 ± 3.3 †	68.6 ± 1.7	69.4 ± 4.0	76.0 ± 2.6	75.7 ± 1.5
Oxygen Content (mM)	4.56 ± 0.10	4.05 ± 0.16	4.20 ± 0.13	4.40 ± 0.24	4.49 ± 0.15	4.5 ± 0.1
[Lactate] (mmol/l)	0.62 ± 0.04	0.57 ± 0.04	0.73 ± 0.13	0.64 ± 0.05	0.68 ± 0.06	0.75 ± 0.12
[Glucose] (mmol/l)	3.12 ± 0.13	3.04 ± 0.07	2.76 ± 0.16	2.86 ± 0.13	2.88 ± 0.20	3.10 ± 0.19

† = significant change from control period ($p < 0.05$)

Maternal arterial and uterine venous blood gas, pH, glucose and lactate levels are presented in Table 7 and Table 8 and Figures 6-8. There were significant decreases in maternal arterial Po_2 (Fig. 6) and O_2 saturation (Fig. 7). Arterial O_2 content decreased slightly but the change was not statistically significant (Fig. 8). As with moderate hypoxia, there were no changes in arterial Pco_2 and pH, and although base excess decreased progressively during the experiment, the change was not significant (Table 7). In uterine venous blood, Po_2 tended to fall, but not significantly, and there were no changes in O_2 saturation and content and Pco_2, pH and base excess (Table 8). There were no significant alterations in arterial and uterine venous glucose and lactate levels (Tables 7 and 8).

3.4.2 *Fetal Blood Gas Status, Glucose and Lactate Levels*

A. *Normoxia Experiments*

Fetal arterial and umbilical venous blood gas, pH, glucose and lactate values are presented in Table 9 and Table 10, respectively. There were no significant changes from pre-experimental values for any of the variables.

B. *Moderate Hypoxemia*

Fetal arterial Po_2 significantly was decreased (by 4.1±0.5 mm Hg or 19.7±2.4%) during the hypoxia period (Table 11 and Fig. 9), and, in contrast to the situation in the ewe, there were similar reductions in O_2 saturation (Fig. 10) and content (Fig. 11). Umbilical venous oxygen variables were also lowered (Table 12), although the changes were not significant for O_2 saturation. As illustrated in Fig. 12, fetal arterial pH was maintained during the experiment. However, lactate concentration increased during and

Table 7. Maternal arterial blood gas parameters and glucose and lactate levels during the severe hypoxemia experiments. Values are mean ± SE. Data from E2216, E158H1 , E158H2 and E2175.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	121.4 ± 4.1	58.0 ± 2.9†	69.8 ± 7.5 †	58.3 ± 5.0 †	128.8 ± 9.5	130.8 ± 6.9
Pco_2 (mmHg)	34.6 ± 0.9	34.5 ± 0.8	34.6 ± 1.0	33.5 ± 0.9	32.6 ± 1.0	31.8 ± 0.9
pH	7.513 ± 0.007	7.512 ± 0.011	7.500 ± 0.014	7.508 ± 0.017	7.511 ± 0.012	7.517 ± 0.004
Base Excess (mEq/l)	6.1 ± 0.4	6.1 ± 1.0	5.2 ± 0.5	5.2 ± 1.0	4.7 ± 0.4	4.8 ± 0.6
HCO_3 (mmol/l)	27.6 ± 0.5	27.6 ± 1.0	26.8 ± 0.4	26.5 ± 0.8	25.8 ± 0.4	25.7± 0.7
TCO_2 (mmol/l)	28.6 ± 0.5	28.6 ± 1.0	27.8 ± 0.4	27.4 ± 0.8	26.8 ± 0.5	26.6 ± 0.8
Hemoglobin (g/100ml)	10.1 ± 0.7	10.8 ± 0.8	10.6 ± 0.9	10.6 ± 0.9	10.3 ± 1.4	9.9± 0.9
O_2 Saturation (%)	98.4 ± 0.5	82.4 ± 6.0 †	85.8 ± 7.0 †	87.9 ± 2.0	98.7 ± 0.4	98.5 ± 0.6
Oxygen Content (mM)	6.1 ± 0.5	5.6 ± 0.8	5.7 ± 0.9	5.8 ± 0.6	6.3 ± 0.8	6.0 ± 0.6
[Lactate] (mmol/l)	0.47 ± 0.07	0.53 ± 0.14	0.46 ± 0.10	0.44 ± 0.08	0.43 ± 0.07	0.48 ± 0.14
[Glucose] (mmol/l)	2.89 ± 0.14	3.00 ± 0.17	3.11 ± 0.19	3.11 ± 0.20	3.17 ± 0.19	3.16 ± 0.14

† = significant change from control period ($p < 0.05$)

Table 8. Maternal uterine venous blood gas parameters and glucose and lactate levels during the severe hypoxemia experiments. Values are mean ± SE. Data from E2216, E158H1 , E158H2 and E2175.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	53.0 ± 2.0	43.0 ± 1.0	43.0 ± 3.5	43.0 ± 3.2	55.3 ± 5.7	53.8 ± 6.5
Pco_2 (mmHg)	38.91 ± 0.60	37.5 ± 1.6	38.1 ± 1.0	37.7 ± 0.8	37.5 ± 1.1	36.6 ± 1.1
pH	7.467 ± 0.004	7.472 ± 0.003	7.463 ± 0.012	7.470 ± 0.010	7.459 ± 0.004	7.467 ± 0.003
Base Excess (mEq/l)	5.34 ± 0.36	6.4 ± 0.7	4.6 ± 0.6	5.0 ± 0.8	3.9 ± 0.5	4.0 ± 0.4
HCO_3 (mmol/l)	27.9 ± 0.4	28.5 ± 0.9	27.1 ± 0.5	27.3 ± 0.7	26.4 ± 0.7	26.2 ± 0.6
TCO_2 (mmol/l)	29.0 ± 0.4	29.6 ± 1.0	28.2 ± 0.5	28.3 ± 0.7	27.5 ± 0.7	27.3 ± 0.7
Hemoglobin (g/100ml)	10.2 ± 0.8	11.3 ± 0.9	10.8 ± 0.9	10.8 ± 1.0	10.3 ± 1.2	10.1 ± 0.9
O_2 Saturtion (%)	76.5 ± 3.0	68.2 ± 8.9	64.7 ± 8.2	65.4 ± 6.7	75.5 ± 2.4	73.9 ± 4.2
Oxygen Content (mM)	4.9 ± 0.5	4.8 ± 1.0	4.4 ± 0.9	4.4 ± 0.8	4.8 ± 0.7	4.7 ± 0.6
[Lactate] (mmol/l)	0.52 ± 0.07	0.47 ± 0.04	0.54 ± 0.08	0.52 ± 0.08	0.53 ± 0.08	0.53 ± 0.13
[Glucose] (mmol/l)	2.68 ± 0.15	2.71 ± 0.23	2.89 ± 0.19	2.83 ± 0.17	2.98 ± 0.22	2.98 ± 0.14

† = significant change from control period ($p < 0.05$)

Table 9. Fetal arterial blood gas parameters and glucose and lactate levels during the control, normoxia experiments. Values are mean ± SE. Data from E1119, E1250, E971 and E2216.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	20.4 ± 1.3	20.8 ± 2.1	20.3 ± 2.6	22.8 ± 1.3	22.3 ± 1.7	22.8 ± 1.5
Pco_2 (mmHg)	49.0 ± 0.8	48.8 ± 1.3	48.8 ± 0.7	49.0 ± 0.7	49.5 ± 0.8	48.7 ± 0.5
pH	7.349 ± 0.008	7.351 ± 0.010	7.349 ± 0.019	7.348 ± 0.014	7.343 ± 0.010	7.346 ± 0.016
Base Excess (mEq/l)	1.8 ± 0.6	1.8 ± 1.0	1.7 ± 1.2	1.8 ± 1.0	1.5 ± 1.0	1.5 ± 1.1
HCO_3 (mmol/l)	26.6 ± 0.6	26.6 ± 1.0	26.5 ± 0.9	26.6 ± 0.8	26.5 ± 1.0	26.3 ± 1.0
TCO_2 (mmol/l)	28.0 ± 0.6	27.4± 0.8	27.8 ± 0.9	27.9 ± 0.8	27.9 ± 1.0	27.6 ± 1.0
Hemoblobin (g/100ml)	9.89 ± 0.21	10.02 ± 0.34	9.88 ± 0.42	9.84 ± 0.41	9.75 ± 0.38	9.8 ± 0.3
O_2 Saturation (%)	49.9 ± 3.3	49.6 ± 5.8	46.6 ± 6.9	50.6 ± 5.1	51.5 ± 5.2	52.8 ± 4.6
Oxygen Content (mM)	3.0 ± 0.2	3.1 ± 0.4	2.8 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	2.8 ± 0.5
[Lactate] (mmol/l)	1.33 ± 0.15	1.22 ± 0.25	1.42 ± 0.21	1.43 ± 0.19	1.48 ± 0.20	1.25 ± 0.18
[Glucose] (mmol/l)	0.84 ± 0.14	0.96 ± 0.35	0.64 ± 0.15	0.57 ± 0.14	0.55 ± 0.16	0.89 ± 0.42

Table 10. Umbilical venous blood gas parameters and glucose and lactate levels during the control, normoxia experiments. Values are mean ± SE. Data from E1119, E1250, E971 and E2216.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	3	3	3	3	3	3
Po_2 (mmHg)	30.0 ± 2.7	30.3 ± 3.2	32.0 ± 6.0	32.3 ± 2.7	34.0 ± 2.6	33.7 ± 3.8
Pco_2 (mmHg)	45.7 ± 1.1	45.3 ± 0.2	45.3 ± 0.7	45.0 ± 1.2	44.5 ± 1.0	46.2 ± 1.0
pH	7.356 ± 0.009	7.355 ± 0.011	7.354 ± 0.013	7.360 ± 0.017	7.350 ± 0.013	7.349 ± 0.017
Base Excess (mEq/l)	0.8 ± 0.6	0.6 ± 0.8	0.5 ± 0.7	0.8 ± 1.0	-0.1 ± 1.3	0.5 ± 0.9
HCO_3 (mmol/l)	25.2 ± 0.6	26.6 ± 1.5	24.8 ± 0.4	25.0 ± 0.8	24.2 ± 1.2	25.1 ± 0.7
TCO_2 (mmol/l)	26.5 ± 0.7	26.9 ± 1.4	26.1 ± 0.4	26.2 ± 0.8	25.5 ± 1.2	26.3 ± 0.7
Hemoblobin (g/100ml)	9.9 ± 0.2	10.1 ± 0.4	10.0 ± 0.4	10.0 ± 0.4	9.9 ± 0.4	9.9 ± 0.2
O_2 Saturation (%)	69.7 ± 3.6	70.7 ± 4.1	70.5 ± 5.0	72.8± 4.8	72.8 ± 5.6	73.4 ± 4.8
Oxygen Content (mM)	4.2 ± 0.2	4.4 ± 0.3	4.4 ± 0.5	4.5 ± 0.4	4.4 ± 0.4	4.5 ± 0.3
[Lactate] (mmol/l)	1.43 ± 0.14	1.50 ± 0.22	1.43 ± 0.23	1.44 ± 0.20	1.40 ± 0.19	1.45 ± 0.21
[Glucose] (mmol/l)	0.79 ± 0.08	0.78 ± 0.12	0.74 ± 0.19	0.69 ± 0.18	0.62 ± 0.15	0.65 ± 0.19

Table 11. Fetal arterial blood gas parameters and glucose and lactate levels during the moderate hypoxemia experiments. Values are mean ± SE. Data from E1142, E1119H1, E1119H2, E1250 and E971.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	5	5	5	5	5	5
Po_2 (mmHg)	20.8 ± 0.5	17.0 ± 0.8 †	16.6 ± 1.2 †	16.6 ± 1.2 †	20.4 ± 0.9	19.6 ± 0.5
Pco_2 (mmHg)	49.2 ± 0.6	48.4 ± 1.5	47.5 ± 1.0	47.8 ± 0.7	48.1 ± 1.0	49.8 ± 1.6
pH	7.350 ± 0.009	7.349 ± 0.015	7.339 ± 0.016	7.332 ± 0.016	7.332 ± 0.021	7.338 ± 0.015
Base Excess (mEq/l)	1.9 ± 0.7	1.5 ± 1.0	0.4 ± 1.0	0.3 ± 1.2	0.2 ± 1.3	1.3 ± 1.0
HCO_3 (mmol/l)	26.8 ± 0.6	26.3 ± 1.0	25.2 ± 0.9	25.0 ± 1.1	25.1 ± 1.0	26.4 ± 0.9
TCO_2 (mmol/l)	28.1 ± 0.7	27.6 ± 1.0	26.5 ± 0.9	26.3 ± 1.1	26.4 ± 1.0	27.7 ± 1.0
Hemoglobin (g/100ml)	9.8 ± 0.3	9.9 ± 0.5	9.8 ± 0.6	9.9 ± 0.4	9.4 ± 0.4	9.8 ± 0.4
O_2 Saturation (%)	49.3 ± 2.3	37.0 ± 3.6 †	35.2 ± 4.4 †	36.4 ± 4.2 †	46.7 ± 4.4	43.6 ± 3.3
Oxygen Content (mM)	2.9 ± 0.1	2.2 ± 0.2 †	2.1 ± 0.2†	2.2 ± 0.2 †	2.7 ± 0.2	2.6 ± 0.2
[Lactate] (mmol/l)	1.47 ± 0.09	2.05 ± 0.10	2.57 ± 0.43 †	2.36 ± 0.13	2.81 ± 0.54 †	2.26 ± 0.40
[Glucose] (mmol/l)	0.80 ± 0.09	0.80 ± 0.17	0.65 ± 0.14	0.68 ± 0.12	0.66 ± 0.13	0.66 ± 0.17

† = significant change from control period ($p < 0.05$)

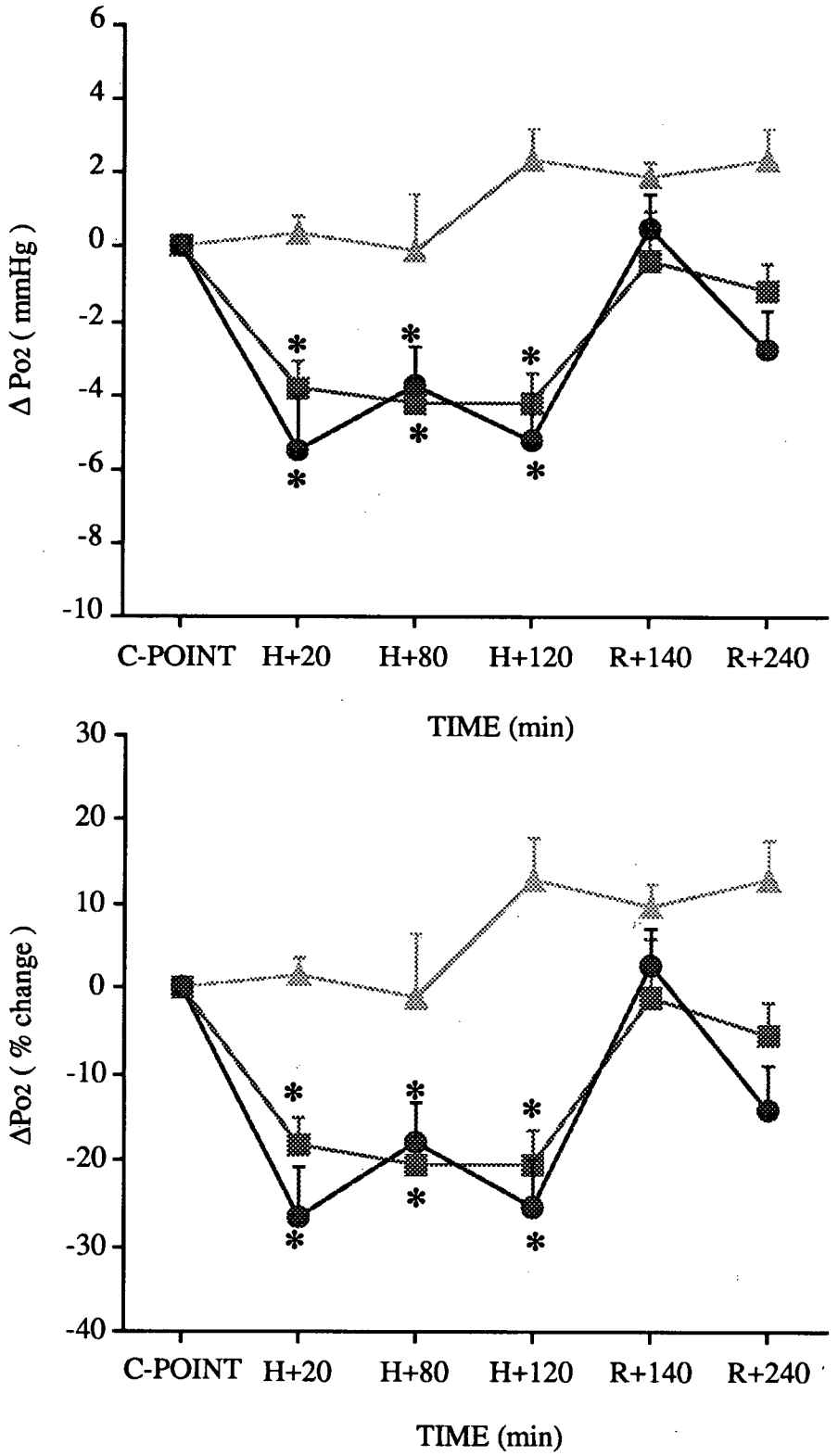


Figure 9 Fetal arterial oxygen tension during the control, moderate and severe hypoxia experiments.
The upper panel plots fetal P_{O_2} as the change from the control value.
The lower panel plots fetal P_{O_2} as the % change from control value.
△ Maternal nornoxia experiments
▩ Maternal moderate hypoxemia experiments
● Maternal severe hypoxemia experiments
* significant difference from control value ($p < 0.05$)

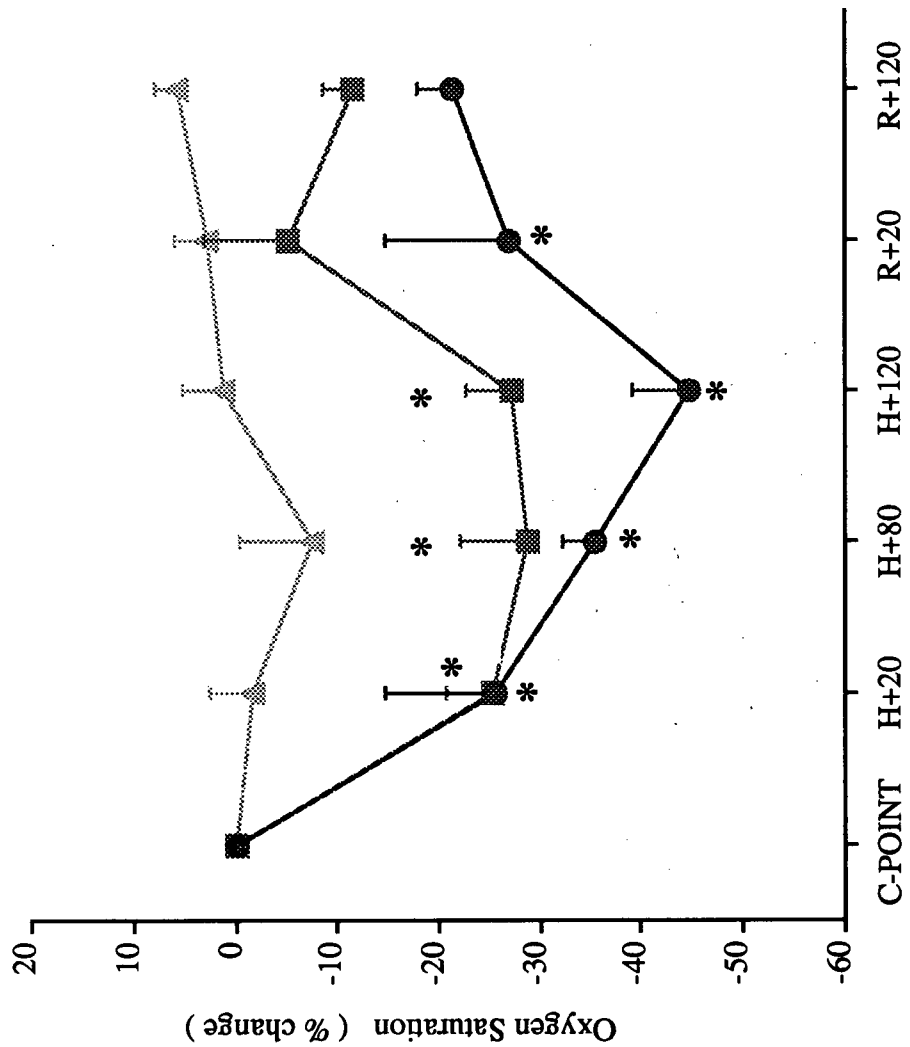


Figure 10 Fetal arterial oxygen saturation during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia
- * significant difference from control value ($p < 0.05$)

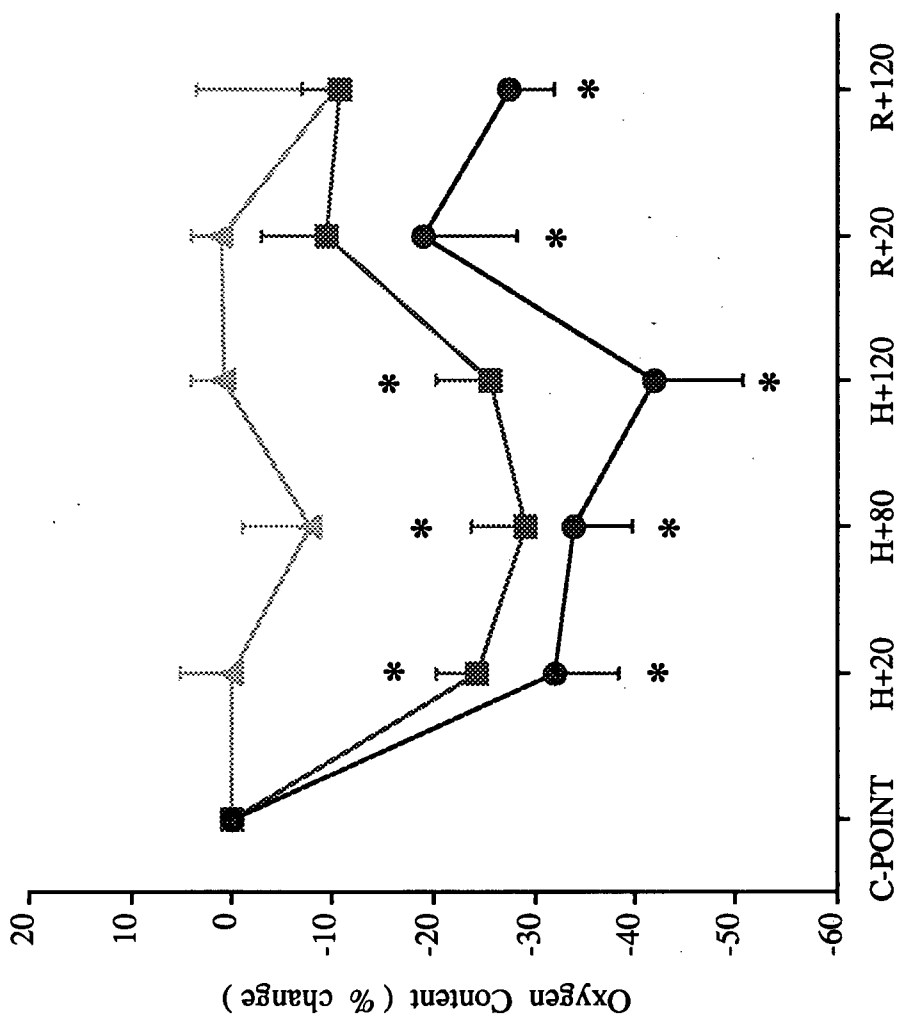


Figure 11 Fetal arterial oxygen content during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

▲ normoxia
■ moderate hypoxia
● severe hypoxia
* significant difference from control value ($p < 0.05$)

Table 12. Umbilical venous blood gas parameters and glucose and lactate levels during the moderate hypoxemia experiments. Values are mean ± SE. Data from E1142, E1119H1, E1119H2, E1250 and E971.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	3	3	3	3	3	3
Po_2 (mmHg)	32.0 ± 2.4	23.0 ± 2.1	24.0 ± 3.1	21.7 ± 3.8 †	32.7 ± 1.2	32.7 ± 3.8
Pco_2 (mmHg)	45.0 ± 0.8	44.0 ± 0.1	43.7 ± 1.0	43.5 ± 2.5	43.8 ± 1.6	44.5 ± 1.3
pH	7.350 ± 0.009	7.347 ± 0.013	7.338 ± 0.012	7.333 ± 0.020	7.324 ± 0.017	7.341 ± 0.005
Base Excess (mEq/l)	0.1 ± 0.7	-0.1 ± 1.0	-1.3 ± 0.7	-1.7 ± 0.6	-2.2 ± 0.7	-0.8 ± 0.5
HCO_3 (mmol/l)	24.5 ± 0.7	24.4 ± 1.0	23.1 ± 0.7	22.7 ± 0.5	22.4 ± 0.5	23.7 ± 0.7
TCO_2 (mmol/l)	25.8 ± 0.7	25.6 ± 1.0	24.3 ± 0.7	23.9 ± 0.5	23.6 ± 0.5	24.9 ± 0.7
Hemoblobin (g/100ml)	9.9 ± 0.6	9.8 ± 0.7	9.7 ± 0.8	9.8 ± 0.6	8.8 ± 0.8	9.4 ± 0.6
O_2 Saturation (%)	73.3 ± 5.4	56.8 ± 8.6	58.6 ± 8.9	61.0 ± 9.6	73.4 ± 7.4	71.9 ± 8.5
Oxygen Content (mM)	4.4 ± 0.1	3.4 ± 2.1 †	3.8 ± 0.3 †	3.6 ± 0.3 †	3.9 ± 0.2	4.1 ± 0.2
[Lactate] (mmol/l)	1.48 ± 0.10	2.08 ± 0.14	2.98 ± 0.73	3.42 ± 0.77 †	3.32 ± 0.87	2.58 ± 0.66
[Glucose] (mmol/l)	0.79 ± 0.13	0.81 ± 0.29	0.71 ± 0.26	0.71 ± 0.25	0.71 ± 0.20	0.66 ± 0.29

† = significant change from control period ($p < 0.05$)

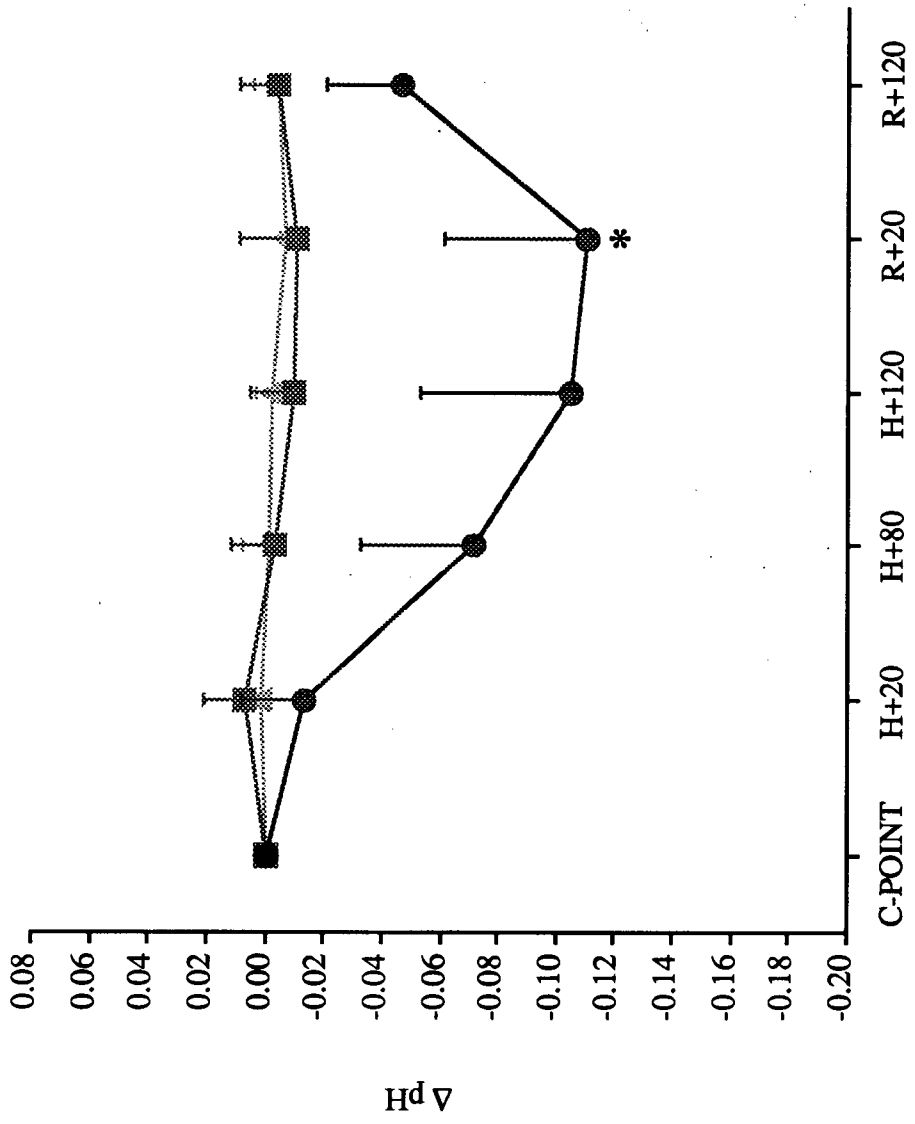


Figure 12 Fetal arterial pH during the control, moderate and severe hypoxia experiments plotted as the change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia
- * significant difference from control value ($p < 0.05$)

following hypoxia, from 1.47±0.09 mM in the control period to a maximum of 2.81±0.54 mM at R+20 (Table 11 and Fig. 13). Umbilical venous lactate concentration also increased (Table 12), but the rise was slightly greater than in arterial blood, with the result that the umbilical veno-arterial lactate difference also increased in those animals with paired arterial and umbilical venous samples, from 0.31±0.21 mM to 0.42±0.27 mM. However, the change was not statistically significant. Fetal arterial and umbilical venous Pco_2 and base excess tended to decrease during hypoxia, but the changes were not significant (Tables 11 and 12). Fetal glucose concentrations were unaltered.

C. *Severe Hypoxemia*

Fetal arterial blood gas, pH, glucose and lactate values for the severe hypoxia experiments are presented in Table 13, while umbilical venous blood gas, pH, glucose and lactate values are presented in Table 14 (n=1 only). Arterial Po_2 was significantly decreased (by 4.8±1.0 mm Hg or 28.6±2.0%) during the hypoxia period (Fig. 9), and this was associated with comparable falls in O_2 saturation (Fig. 10) and O_2 content. In the 1 animal with a working umbilical vein catheter, there were also marked decreases in the blood oxygen variables (Table 14). In contrast to the situation with moderate hypoxia, fetal arterial pH fell progressively with severe hypoxia (Fig. 12). However, because of inter-animal variability, only the value at R+20 was significantly different from the control value. There was also a fall in umbilical venous pH (Table 14). Arterial and umbilical venous lactate concentrations were elevated during and following hypoxia and the magnitude of the increase tended to be greater than with moderate hypoxia (Fig. 13, Tables 12 and 14).

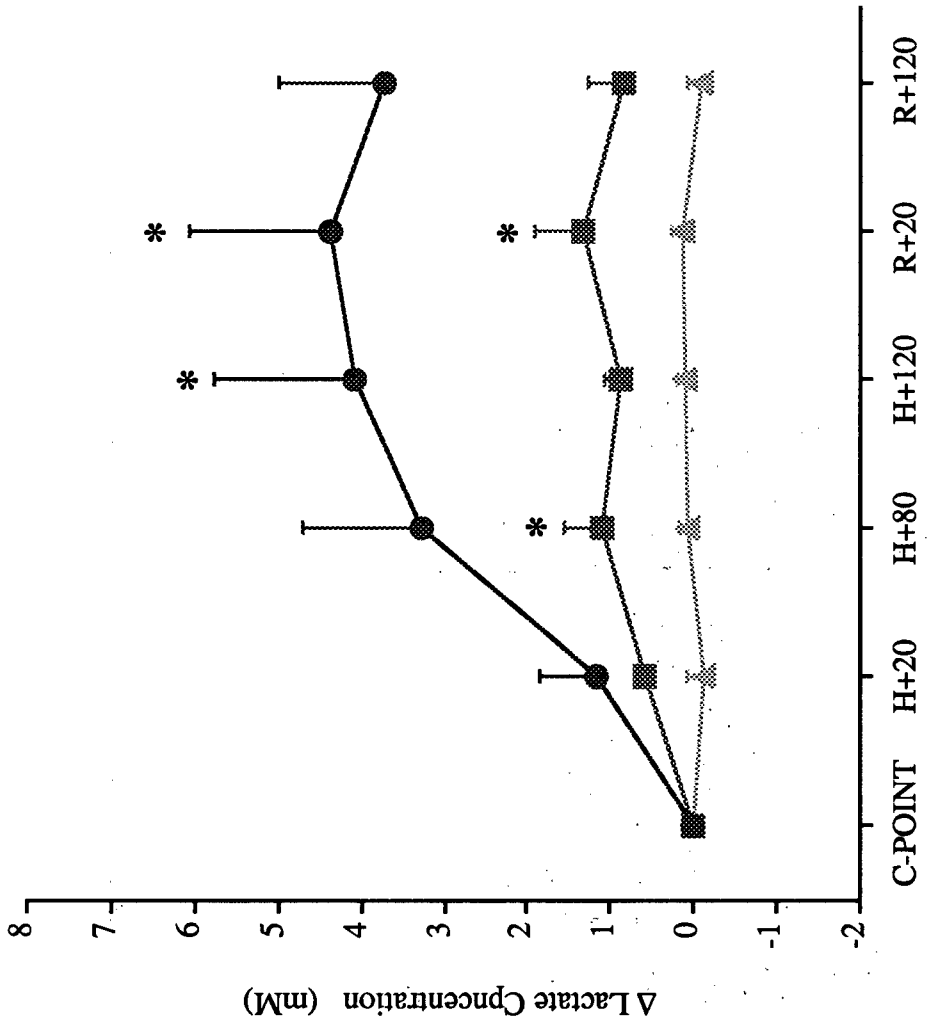


Figure 13 Fetal arterial lactate concentration during the control, moderate and severe hypoxia experiments plotted as the change from the control value.

◭ normoxia
■ moderate hypoxia
● severe hypoxia
* significant difference from control value ($p < 0.05$)

Table 13. Fetal arterial blood gas parameters and glucose and lactate levels during the severe hypoxemia experiments. Values are mean ± SE. Data from E2216, E158H1, E158H2 and E2175.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Po_2 (mmHg)	20.0 ± 1.0	14.5 ± 0.9 †	16.3 ± 0.9 †	14.8 ± 1.1 †	20.5 ± 1.7	17.3 ± 2.0
Pco_2 (mmHg)	48.36 ± 0.56	47.3 ± 1.6	46.9 ± 0.7	46.9 ± 1.3	45.2 ± 1.8	45.8 ± 0.9
pH	7.334 ± 0.006	7.304 ± 0.026	7.242 ± 0.046	7.230 ± 0.057	7.233 ± 0.055	7.308 ± 0.027
Base Excess (mEq/l)	0.44 ± 0.36	-2.1 ± 1.2	-6.0 ± 2.5	-6.6 ± 2.9 †	-7.1 ± 2.7 †	-2.3 ± 1.7
HCO_3 (mmol/l)	25.4 ± 0.3	23.1 ± 0.8	20.1 ± 1.8	19.6 ± 2.0 †	18.9 ± 1.8 †	22.7 ± 1.4
TCO_2 (mmol/l)	26.7 ± 0.3	24.4 ± 0.8	21.3 ± 1.8	20.8 ± 2.0 †	20.2 ± 1.8 †	24.0 ± 1.3
Hemoglobin (g/100ml)	12.2 ± 0.5	12.9 ± 0.6	12.3 ± 0.9	12.6 ± 1.0	11.6 ± 0.9	11.8 ± 1.1
O_2 Saturation (%)	43.4 ± 3.3	27.7 ± 3.7	26.5 ± 3.4 †	22.3 ± 2.5 †	36.2 ± 4.4	31.2 ± 5.8
Oxygen Content (mM)	3.3 ± 0.3	2.2 ± 0.4	2.0 ± 0.4	1.7 ± 0.3 †	2.6 ± 0.5	2.6 ± 0.6
[Lactate] (mmol/l)	1.40 ± 0.19	3.15 ± 0.71	4.90 ± 2.00	5.62 ± 2.36 †	5.94 ± 1.69 †	4.63 ± 1.33
[Glucose] (mmol/l)	0.71 ± 0.10	1.01 ± 0.32	1.05 ± 0.35	1.09 ± 0.22	0.90 ± 0.22	0.80 ± 0.16

† = significant change from control period ($p < 0.05$)

Table 14. Umbilical venous blood gas parameters and glucose and lactate levels during the severe hypoxemia experiments.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	1	1	1	1	1	1
Po_2 (mmHg)	32.5	21	24	25	42	33
Pco_2 (mmHg)	47.9	46.9	44.9	45.8	42.9	42.5
pH	7.321	7.257	7.142	7.088	7.080	7.238
Base Excess (mEq/l)	-0.7	-5.2	-12.2	-14.7	-15.8	-7.8
HCO_3 (mmol/l)	24.4	20.5	15	13.5	12.4	17.8
TCO_2 (mmol/l)	25.7	21.8	16.2	14.8	13.6	19
Hemoglobin (g/100ml)	10.3	11	10	9.7	9.4	8.5
O_2 Saturation (%)	65.8	38	41	40.3	56.07	59.1
Oxygen Content (mM)	4.2	2.7	2.6	2.4	3.3	3.1
[Lactate] (mmol/l)	1.23	4.26	8.47	10.1	10.06	7.8
[Glucose] (mmol/l)	1.07	1.74	1.83	1.63	1.4	1.08

However, as with moderate hypoxia, the rise in umbilical venous lactate was greater than that in arterial blood, with the result that the mean veno-arterial lactate difference rose from 0.025 mM in the control period to 0.077 mM during hypoxia. Fetal arterial and umbilical venous Pco_2 tended to decrease and this in conjunction with the fall in pH resulted in a marked reduction in base excess (Tables 13 and 14). Glucose levels tended to increase, but the changes were not significant.

3.4.3 *Fetal Cortisol Concentration*

Fetal arterial cortisol concentrations in 3 experimental groups are presented in Table 15 and Fig. 14. In the normoxia experiments, there was a transient and slight, but statistically significant, decrease in fetal cortisol level of 3.12±1.11 ng/ml. In contrast with both moderate and severe hypoxemia, the cortisol level were significantly elevated, and the rise was greater in the latter experiments (26.19±10.01 versus 17.35±2.44 ng/ml) (Fig. 14).

3.4.4 *Uterine Blood Flow, O_2 Delivery, O_2 Consumption, O_2 Extraction and Uteroplacental Lactate Output and Glucose Uptake*

A. *Normoxia Experiments*

Uterine blood flow remained stable during the duration of 6-h normoxia experiments (Table 16, Fig. 15). Likewise, there were no significant changes in uterine O_2 delivery (Fig. 16), O_2 extraction, O_2 consumption (Fig. 17), lactate output (Fig. 18) and glucose uptake.

Table 15. Fetal cortisol concentrations during the control (C), moderate hypoxia (MH) and severe hypoxia (SH) experiments. Values are mean ± SE.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
C (n=4)						
Cortisol (ng/ml)	6.48 ± 0.97	4.15 ± 1.13	3.36 ± 0.32 †	4.02 ± 0.82	5.90 ± 1.19	8.34 ± 1.60
MH (n=5)						
Cortisol (ng/ml)	7.36 ± 1.05	14.27 ± 4.25	14.48 ± 3.60	16.58 ± 4.04 †	15.73 ± 3.78 †	7.93 ± 2.05
SH (n=4)						
Cortisol (ng/ml)	20.04 ± 5.13	41.18 ± 11.75 †	35.73 ± 4.83	37.00 ± 3.99	38.44 ± 6.35	20.04 ± 7.73

† $P < 0.05$ for the change from the control value.

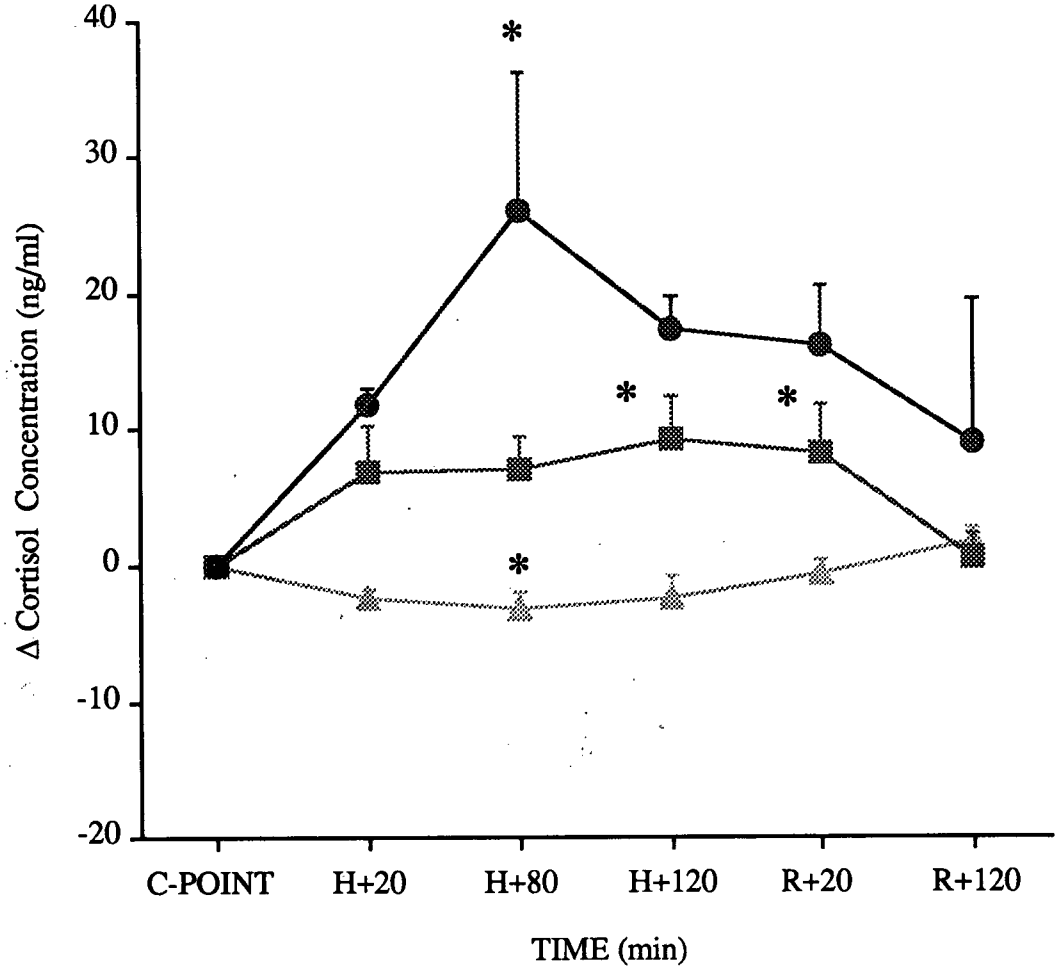


Figure 14 Fetal arterial cortisol concentration during the control, moderate and severe hypoxia experiments plotted as the change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia
- * significant difference from control value ($p < 0.05$)

Table 16. Uterine blood flow, O_2 delivery, consumption and extraction, lactate output and glucose uptake during the control normoxic experiments. Values are mean ± SE. Data from E1119, E1250, E971 and E2216.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Blood Flow (ml/min)	453.7 ± 69.9	448.6 ± 106.2	484.4 ± 131.2	437.1 ± 123.7	485.0 ± 104.8	482.6 ± 108.7
A-V O_2 Content (mM)	1.16 ± 0.05	1.14 ± 0.07	1.30 ± 0.04	1.38 ± 0.07	1.14 ± 0.09	1.11 ± 0.11
O_2 Delivery (µmol/min)	2700 ± 413	2678 ± 621	2942 ± 752	2688 ± 719	2930 ± 667	2863 ± 664
O_2 Consumption (µmol/min)	543 ± 100	520 ± 149	635 ± 185	596 ± 162	581 ± 169	569 ± 181
O2 Extraction (%)	19.7 ± 1.2	19.4 ± 2.2	21.5 ± 1.5	22.5 ± 1.9	19.3 ± 1.7	18.9 ± 1.8
Lactate Output (µmol/min)	23 ± 6	37± 11	14 ± 2	21 ± 8	6± 5	12 ± 9
Glucose Uptake (µmol/min)	134 ± 24	126 ± 34	132 ± 32.29	72 ± 12	159 ± 58	140 ± 27

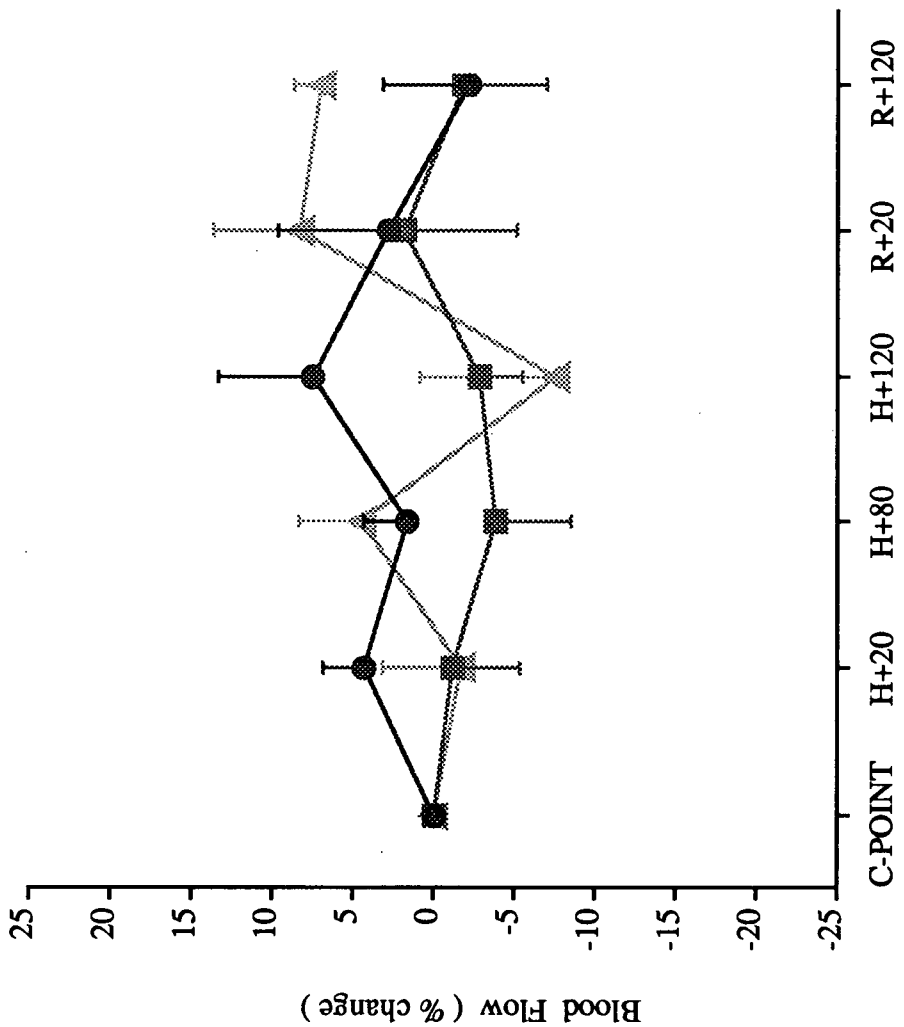


Figure 15 Uterine blood flow during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

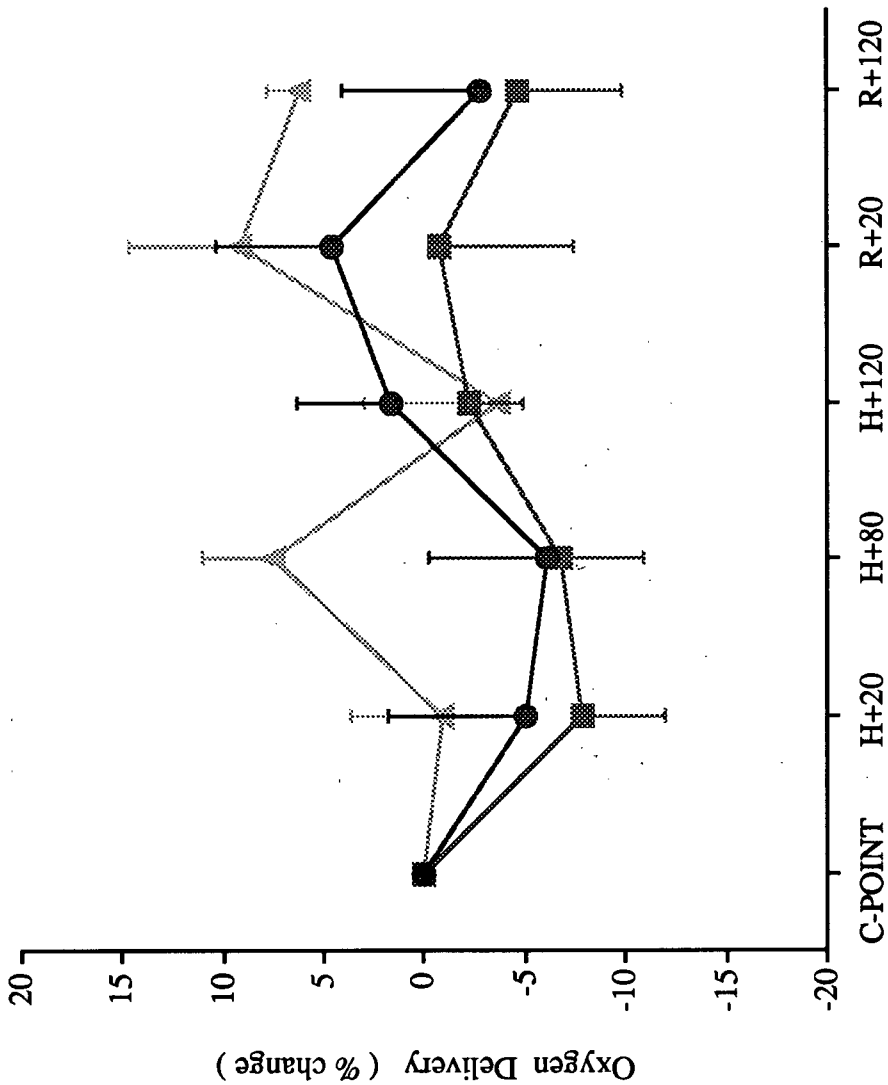


Figure 16 Uterine oxygen delivery during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

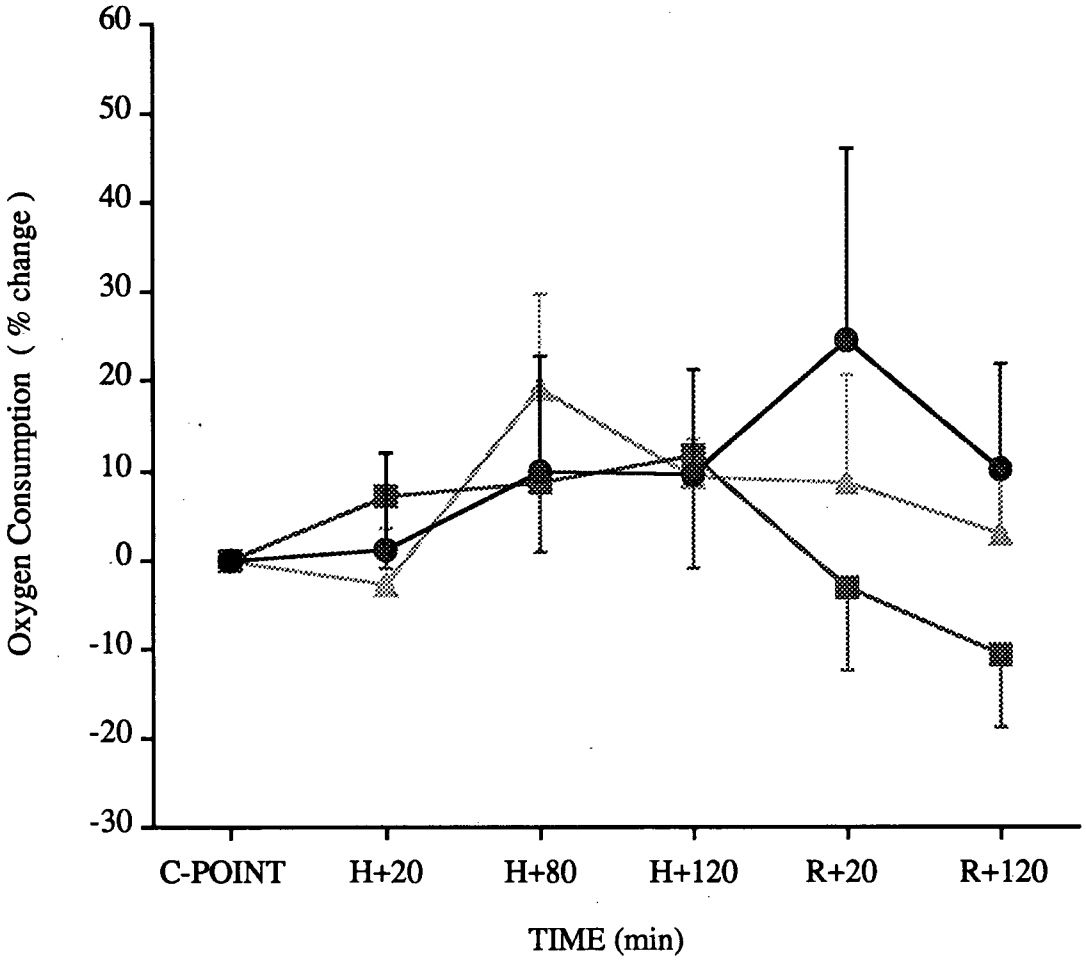


Figure 17 Uteroplacental oxygen consumption during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia

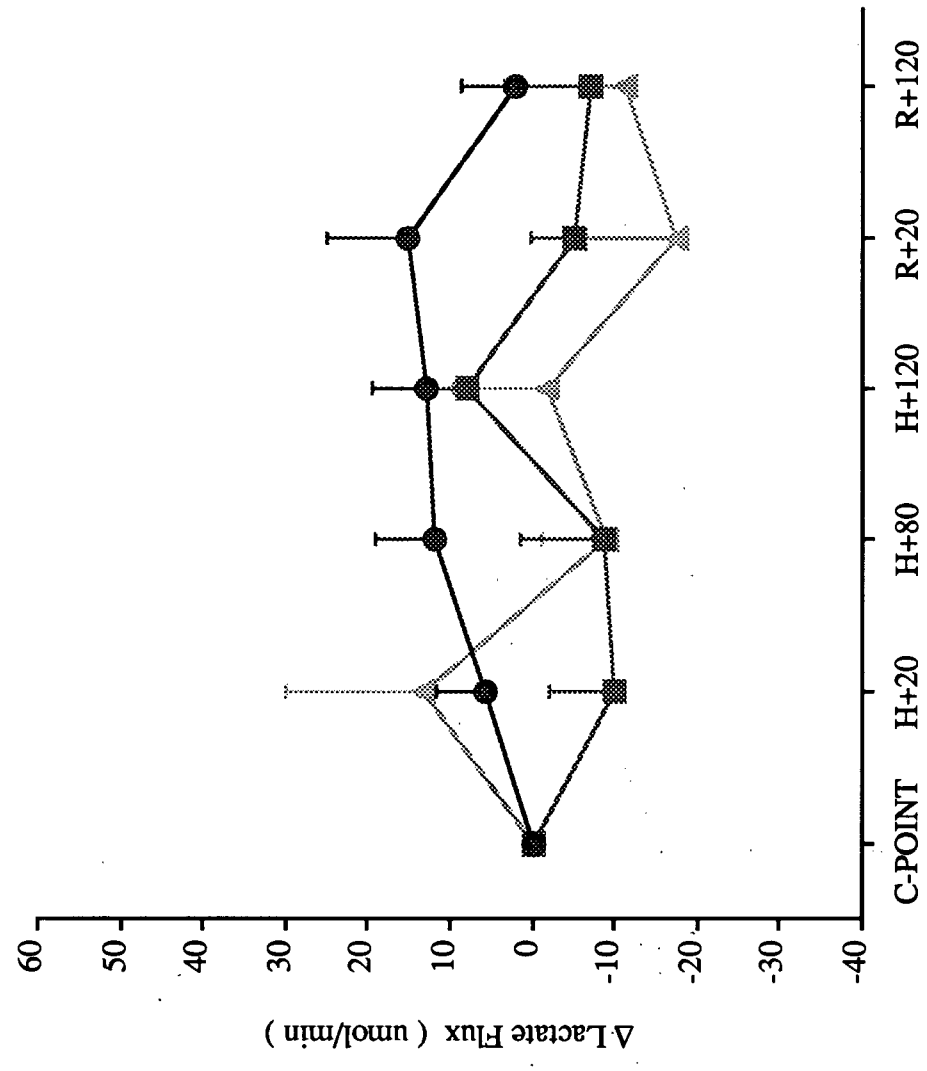


Figure 18 Uteroplacental lactate flux during the control, moderate and severe hypoxia experiments plotted as the change from the control value.

B. *Moderate Hypoxemia Experiments*

In the moderate hypoxemia experiments, there was a tendency for uterine blood flow to fall during the hypoxia period, and this taken with the slight fall in arterial O_2 content (Fig. 8) resulted in a slight reduction in uterine O_2 delivery (Fig. 16). However, none of these changes were statistically significant. Likewise there were no consistent alterations in uteroplacental O_2 consumption (Fig. 17). O_2 extraction tended to increase during the hypoxia period, as a consequence of the fall in O_2 delivery and maintained O_2 consumption, but the change was not statistically significant (Table 17). There were no consistent changes in lactate output (Fig. 18) or glucose uptake (Table 17).

C. Severe Hypoxemia

Uterine blood flow tended to increase during the hypoxemia period, but this was not statistically significant (Table 18, Fig. 15). However, this compensated for the slight fall in arterial O_2 content (Fig. 8) so that uterine O_2 delivery was only slightly and non-significantly reduced during the hypoxia interval (Fig. 16) and there was no change in O_2 consumption (Fig. 17). As with the moderate hypoxia experiments, uterine O_2 extraction increased, but again the change was not statistically significant (Table 18). In contrast to the moderate hypoxia experiments, uteroplacental lactate output increased progressively during hypoxia, from 19±3 μmol/min in the control period to 35±8 μmol/min at R+20 (Table 18, Fig. 18). There was also a trend for glucose uptake to increase (Table 18). However, neither of these changes were statistically significant.

Table 17. Uterine blood flow, O_2 delivery, consumption and extraction, lactate output and glucose uptake during the moderate hypoxemia experiments. Values are mean ± SE. Data from E1142, E1119H1, E1119H2, E1250 and E971.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	5	5	5	5	5	5
Blood Flow (ml/min)	461.4 ± 74.0	449.6 ± 98.7	430.4 ± 85.2	436.8 ± 89.4	455.4 ± 92.3	441.0 ± 91.8
A-V Oxygen Content (mM)	1.37 ± 0.09	1.48 ± 0.13	1.55 ± 0.15	1.58 ± 0.22	1.30 ± 0.13	1.21 ± 0.10
O2 Delivery (µmol/min)	2726 ± 433	2436 ± 474	2468 ± 481	2605 ± 546	2617 ± 522	2498 ± 515
O2 Consumption (µmol/min)	634 ± 112	635 ± 114	683± 171	720 ± 241	591 ± 150	539 ± 135
O2 Extraction (%)	23.0 ± 1.3	26.6 ± 1.7	26.9 ± 2.3	26.3 ± 3.3	22.4 ± 1.9	21.2 ± 1.3
Lactate Output (µmol/min)	19 ± 7	9 ± 7	2 ± 8	27± 12	14 ± 8	3 ± 7
Glucose Uptake (µmol/min)	113 ± 21	109± 36	162 ± 52	102 ± 18	128 ± 27	101 ± 28

Table 18. Uterine blood flow, O_2 delivery, consumption and extraction, lactate output and glucose uptake during the severe hypoxemia experiments. Data from E2216, E158H1 , E158H2 and E2175.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
n	4	4	4	4	4	4
Blood Flow (ml/min)	380.0 ± 32.6	399.1 ± 57.0	385.4 ± 48.7	400.9 ± 36.3	386.0 ± 47.6	366.6 ± 39.0
A-V Oxygen Content (mM)	1.24 ± 0.11	1.17 ± 0.11	1.30 ± 0.10	1.32 ± 0.29	1.44 ± 0.15	1.36 ± 0.12
O_2 Delivery (µmol/min)	2331± 262	2288 ± 496	2236 ± 497	2330 ± 361	2378 ± 321	2234 ± 388
O_2 Consumption (µmol/min)	473 ± 64	480 ± 98	502 ± 75	532 ± 140	556 ± 83	504 ± 74
O_2 Extraction (%)	21.6 ± 3.1	22.3 ± 3.6	24.2 ± 3.8	24.4 ± 6.5	23.5 ± 1.8	23.5 ± 3.9
Lactate Output (µmol/min)	19 ± 3	25 ± 8	31 ± 9	32 ± 6	35 ± 8	22 ± 8
Glucose Uptake (µmol/min)	72 ± 17	92 ± 27	87 ± 17	107 ± 17	66 ± 10	69 ± 18

3.4.5 *Maternal Arterial and Uterine Venous Progesterone Concentrations and Uteroplacental Progesterone Output*

The mean values for maternal arterial and uterine venous progesterone concentrations and uteroplacental progesterone output for the 3 types of experiments are given in Table 19, while the mean changes from the control values are plotted in figures 19-21. Maternal arterial progesterone concentration was not altered in the normoxia and moderate hypoxia experiments. With severe hypoxia, it tended to decrease progressively throughout the entire experimental period, but this change was not statistically significant (Fig. 19). There was no changes in uterine venous progesterone concentration with normoxia and severe hypoxia, whereas with moderate hypoxia it was increased during most of the hypoxia interval from the control value of 19.7±2.3 ng/ml to a maximum value of 25.5±5.5 ng/ml at H+80. The increase occurred in 4 of the 5 experiments, and the average increase during the entire hypoxic interval, expressed as a percentage of the control mean was 13.7±4.3%. This change was of borderline statistical significance ($p<0.10$, paired t-test). As a consequence of the elevated uterine venous P_4 concentration, uteroplacental progesterone output also tended to increase during moderate hypoxia, and again this occurred in 4 of 5 experiments. The mean % increase during hypoxia was 18.7±6.4, which was not statistically significant ($p<0.10$, paired t-test). These changes were not observed with severe hypoxia or normoxia (Fig. 21).

Table 19. Maternal progesterone (P_4) concentrations and uteroplacental progesterone output (V_{P4}) during the contol (C), Moderate hypoxia (MH) and severe hypoxia (SH) experiments.

	CONTROL	INTERVENTION			RECOVERY	
	C-POINT	H+20	H+80	H+120	R+20	R+120
C (n=4)						
Blood Flow (ml/min)	454.7 ± 72.5	467.1 ± 121.5	455.7 ± 116.0	453.5 ± 129.3	482.3 ± 103.8	467.8 ± 93.6
Arterial [P_4] (ng/ml)	5.83 ± 0.72	6.04 ± 1.11	5.80 ± 0.52	6.95 ± 0.69	5.98 ± 0.89	5.79 ± 0.93
Uterine Venous [P_4] (ng/ml)	21.41 ± 2.55	21.28 ± 0.89	20.32 ± 1.64	19.25 ± 1.39	20.51 ± 1.69	23.43 ± 3.63
Utv-Ma [P_4] (ng/ml)	15.59 ± 2.26	15.24 ± 0.87	14.52 ± 1.48	12.30 ± 1.48	14.53 ± 1.01	17.64 ± 3.02
V_{P4} (ng/min)	7397 ± 1997	6911 ± 1465	6528 ± 1484	5180 ± 1028	7234 ± 1897	8837 ± 2894
MH (n=5)						
Blood Flow (ml/min)	462.9 ± 76.0	448.5 ± 97.7	426.6 ± 78.9	451.6 ± 86.8	465.8 ± 90.0	440.2 ± 81.4
Arterial [P_4] (ng/ml)	5.72 ± 0.79	5.61 ± 0.95	5.67 ± 0.73	6.07 ± 1.10	6.02 ± 1.07	5.92 ± 1.00
Uterine Venous [P_4] (ng/ml)	19.74 ± 2.25	20.78 ± 3.39	25.52 ± 5.53	24.60 ± 6.19	20.57 ± 4.47	20.27 ± 2.35
Utv-Ma [P_4] (ng/ml)	14.03 ± 1.98	15.17 ± 2.82	19.84 ± 4.80	18.52 ± 5.54	14.55 ± 3.56	14.35 ± 1.48
V_{P4} (ng/min)	6279 ± 1144	7263 ± 2300	9598 ± 4057	8476 ± 2768	7439 ± 2656	6494 ± 1586
SH (n=4)						
Blood Flow (ml/min)	376.7 ± 34.0	394.6 ± 58.4	396.0 ± 49.2	388.1 ± 33.1	388.9 ± 53.0	367.0 ± 39.7
Arterial [P_4] (ng/ml)	4.47 ± 0.69	4.17 ± 0.71	3.90 ± 0.73	3.54 ± 0.78	4.16 ± 0.70	3.37 ± 0.57
Uterine Venous [P_4] (ng/ml)	17.14 ± 2.50	14.89 ± 1.97	15.26 ± 2.17	16.65 ± 3.33	15.15 ± 1.37	14.58 ± 1.36
Utv-Ma [P_4] (ng/ml)	12.68 ± 1.99	10.73 ± 1.41	11.36 ± 1.71	13.11 ± 2.72	11.00 ± 1.00	11.20 ± 1.14
V_{P4} (ng/min)	5027 ± 1109	4248 ± 975	4503 ± 982	5239 ± 1347	4327 ± 861	4020 ± 408

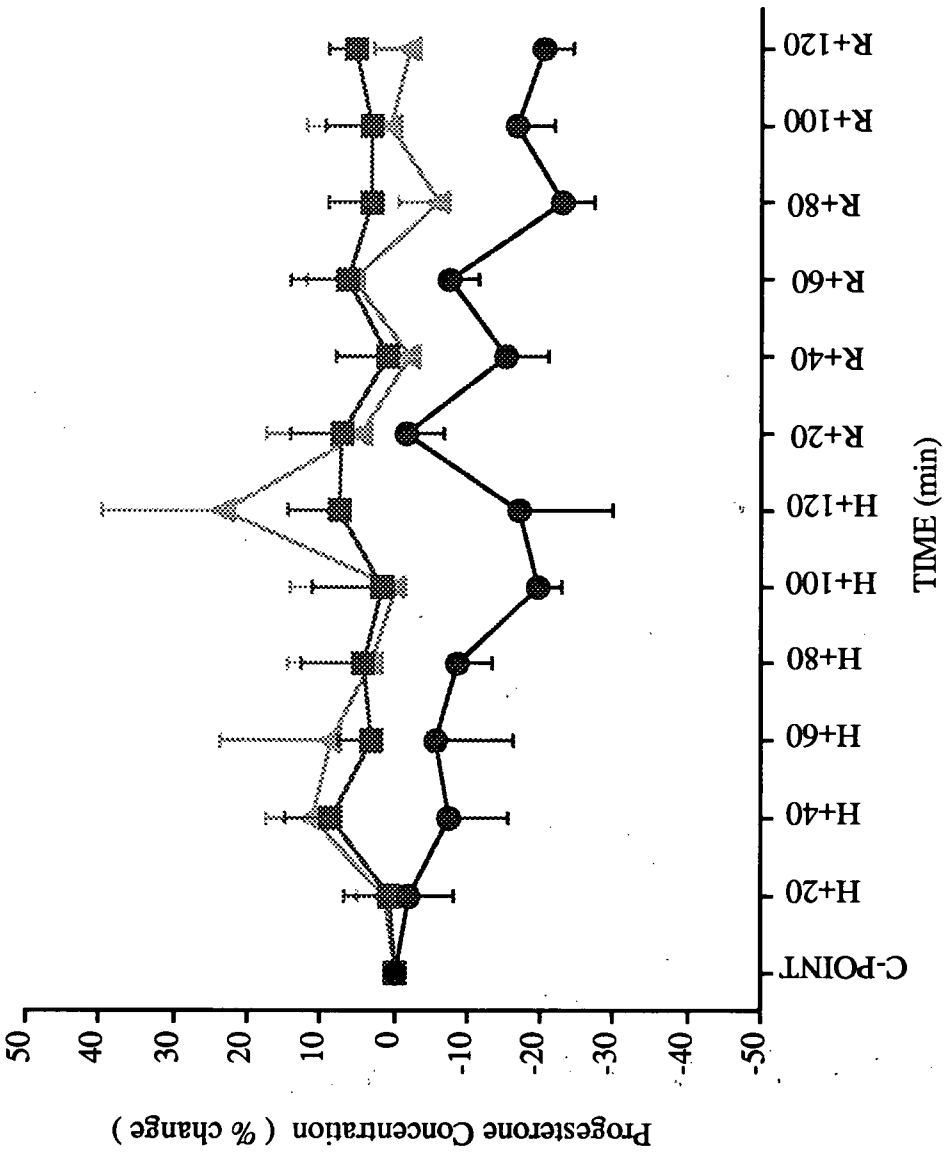


Figure 19 Matrnal arterial progesterone concentration during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia

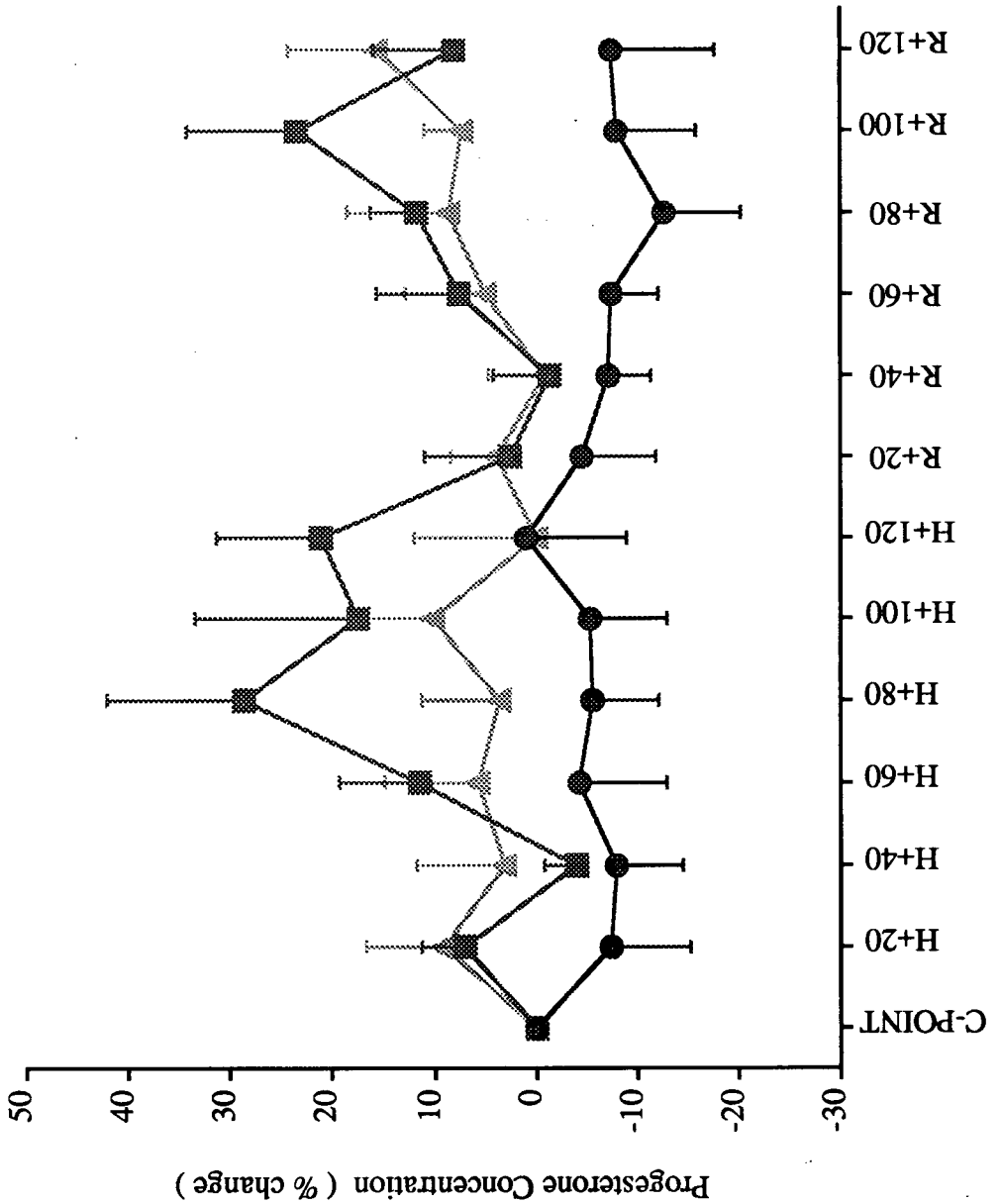


Figure 20 Matrnal uterine venous progesterone concentration during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

△ normoxia
▩ moderate hypoxia
● severe hypoxia

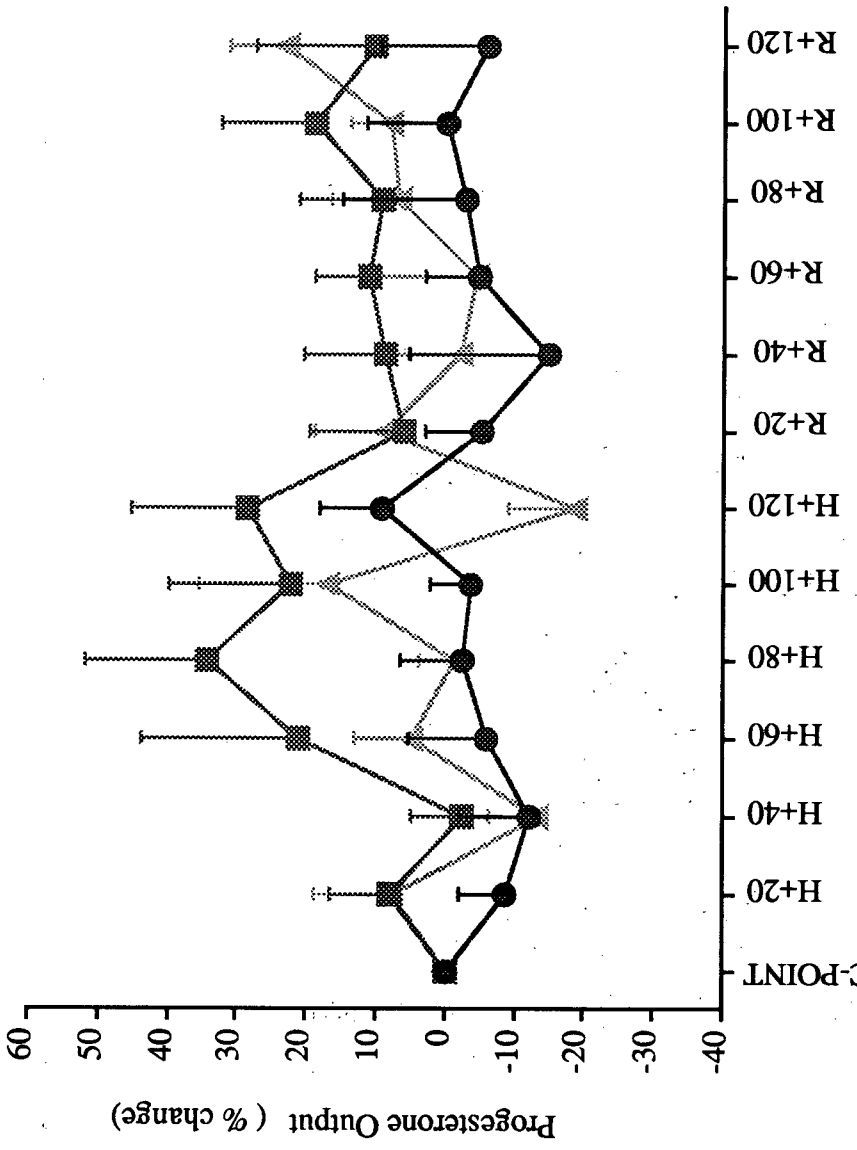


Figure 21 Uteroplacental progesterone output during the control, moderate and severe hypoxia experiments plotted as the % change from the control value.

- normoxia
- moderate hypoxia
- severe hypoxia

3.4.6 *Maternal Arterial Pressure, Uterine Vein Pressure and Heart Rate*

Mean values over 10 min for maternal arterial pressure and heart rate are presented in figures 22-24. In the normoxia and moderate hypoxia experiments, there was a trend for heart rate to increase during the experiments, but the changes were not significant (Figs. 22,23). In contrast, with severe hypoxia, there was maternal tachycardia that lasted for the duration of the hypoxia interval (Fig. 24). Arterial pressure was not altered in any of the experimental protocols. Likewise, uterine venous pressure, which in the control period averaged 14.8±0.5, 14.2±0.3 and 11.6±0.3 mm Hg for the normoxia, moderate hypoxia and severe hypoxia experiments, respectively, was not changed.

3.4.7 *Fetal Arterial Pressure, Umbilical Venous Pressure and Fetal Heart Rate*

Mean values over 10 min for fetal arterial pressure and heart rate are presented in figures 25-27. In the moderate hypoxemia experiment, there was a trend for heart rate to increase during hypoxia, but the changes was not significant. In contrast, with severe hypoxia, there was fetal bradycardia that lasted for the duration of the hypoxia interval followed by a significant increase in the recovery period. Arterial pressure was not altered in normoxia and moderate hypoxia. It tended to increase during severe hypoxia, however, the change was not significant.

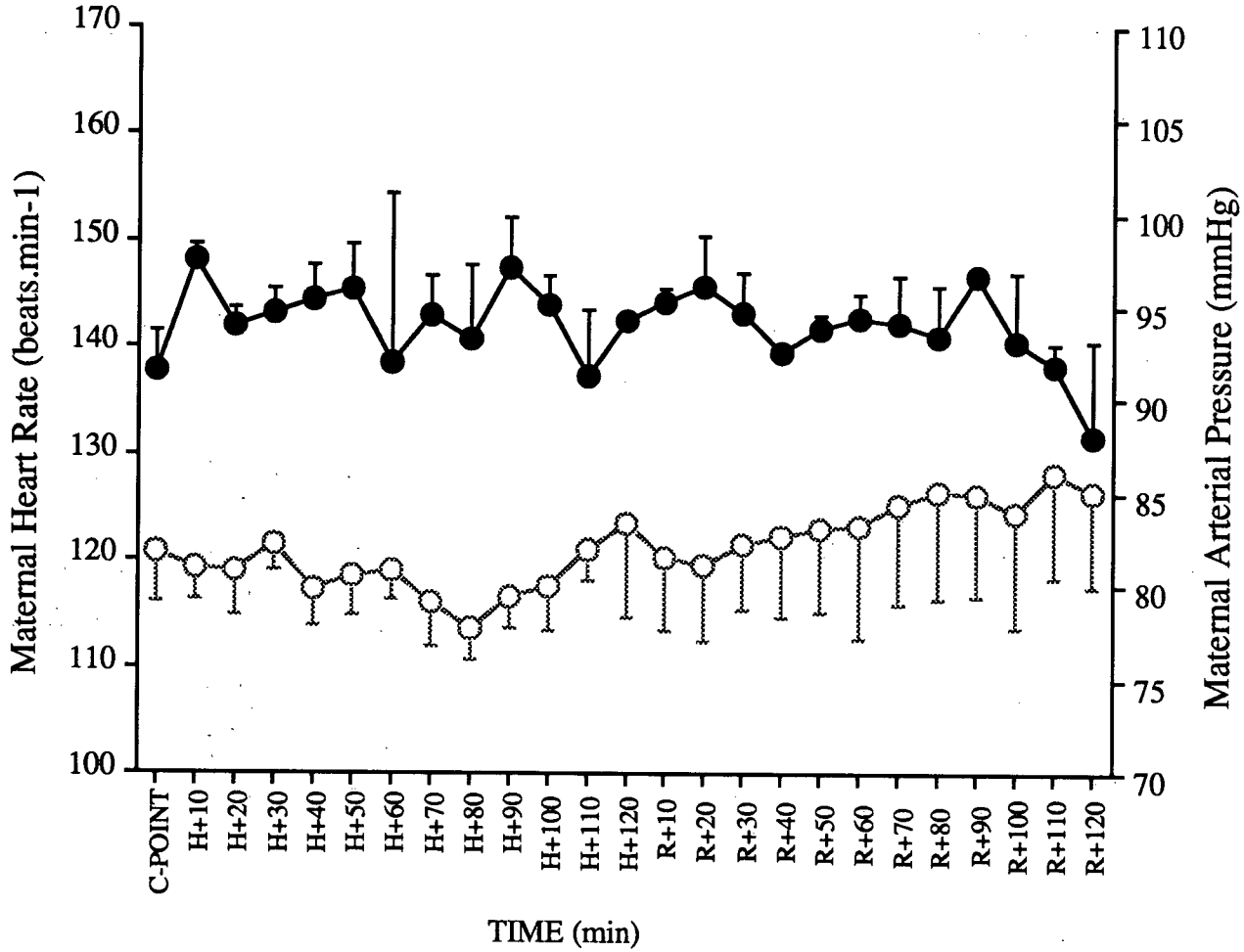


Figure 22 Maternal heart rate and maternal arterial pressure during the normoxia experiments.

○ Maternal heart rate

● Maternal arterial pressure

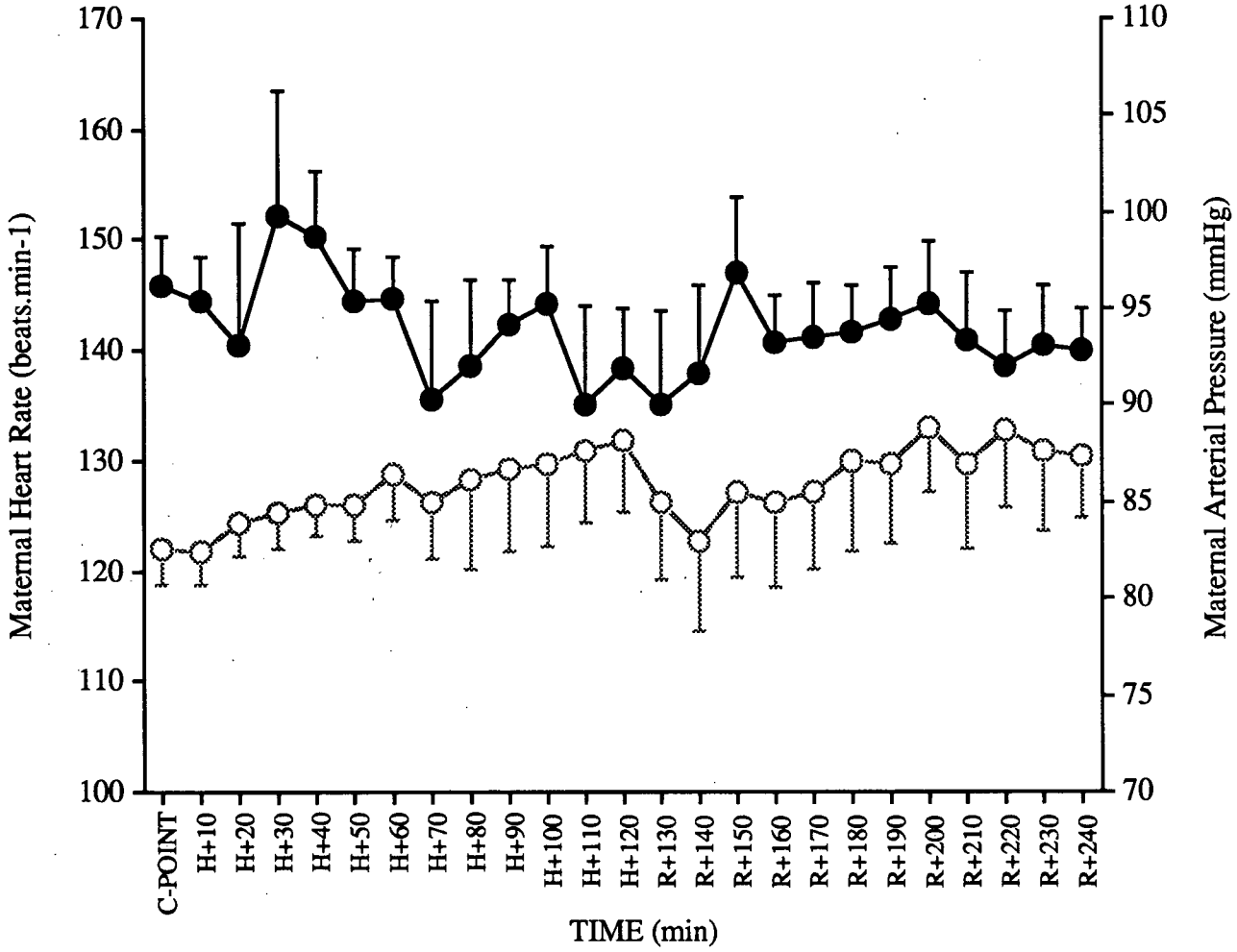


Figure 23 Maternal heart rate and arterial pressure during the moderate hypoxemia experiments.

○ Maternal heart rate

● Maternal arterial pressure

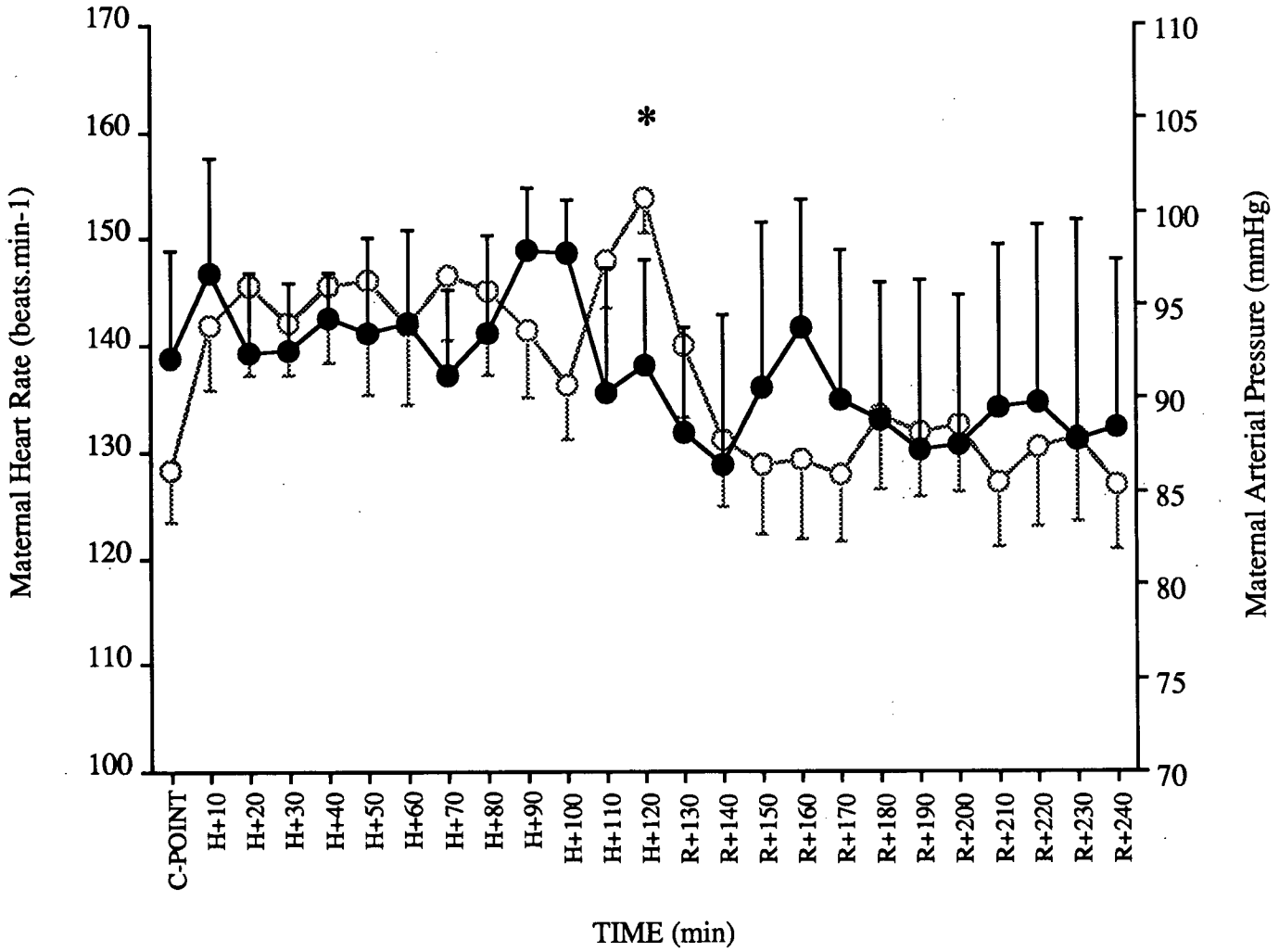


Figure 24 Maternal arterial pressure and heart rate during the severe hypoxemia experiments.

○ Maternal heart rate

● Maternal arterial pressure

* significant difference from control value ($p < 0.05$)

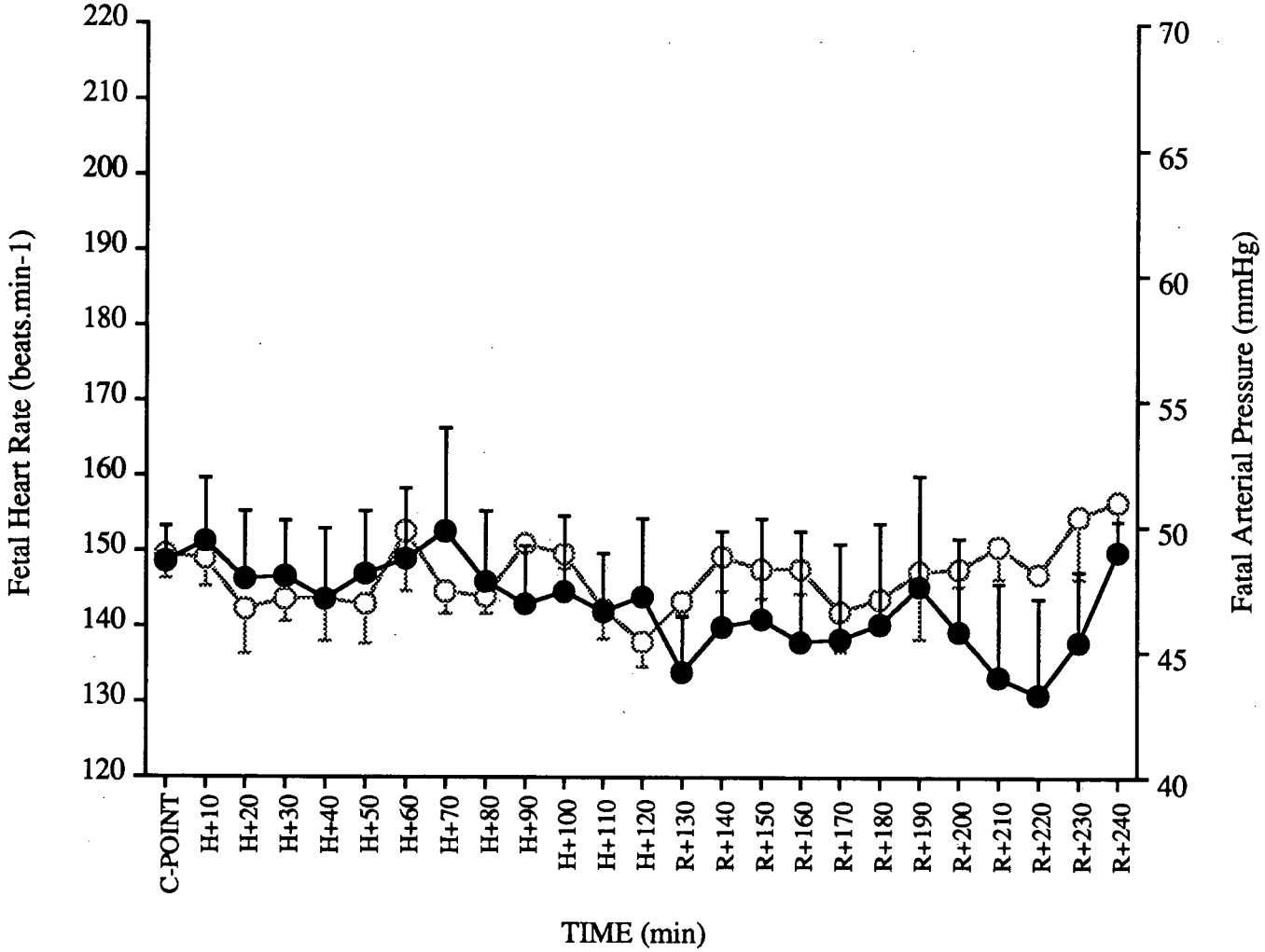


Figure 25 Fetal heart rate and arterial pressure during the normoxia experiments.

○ Fetal heart rate

● Fetal arterial pressure

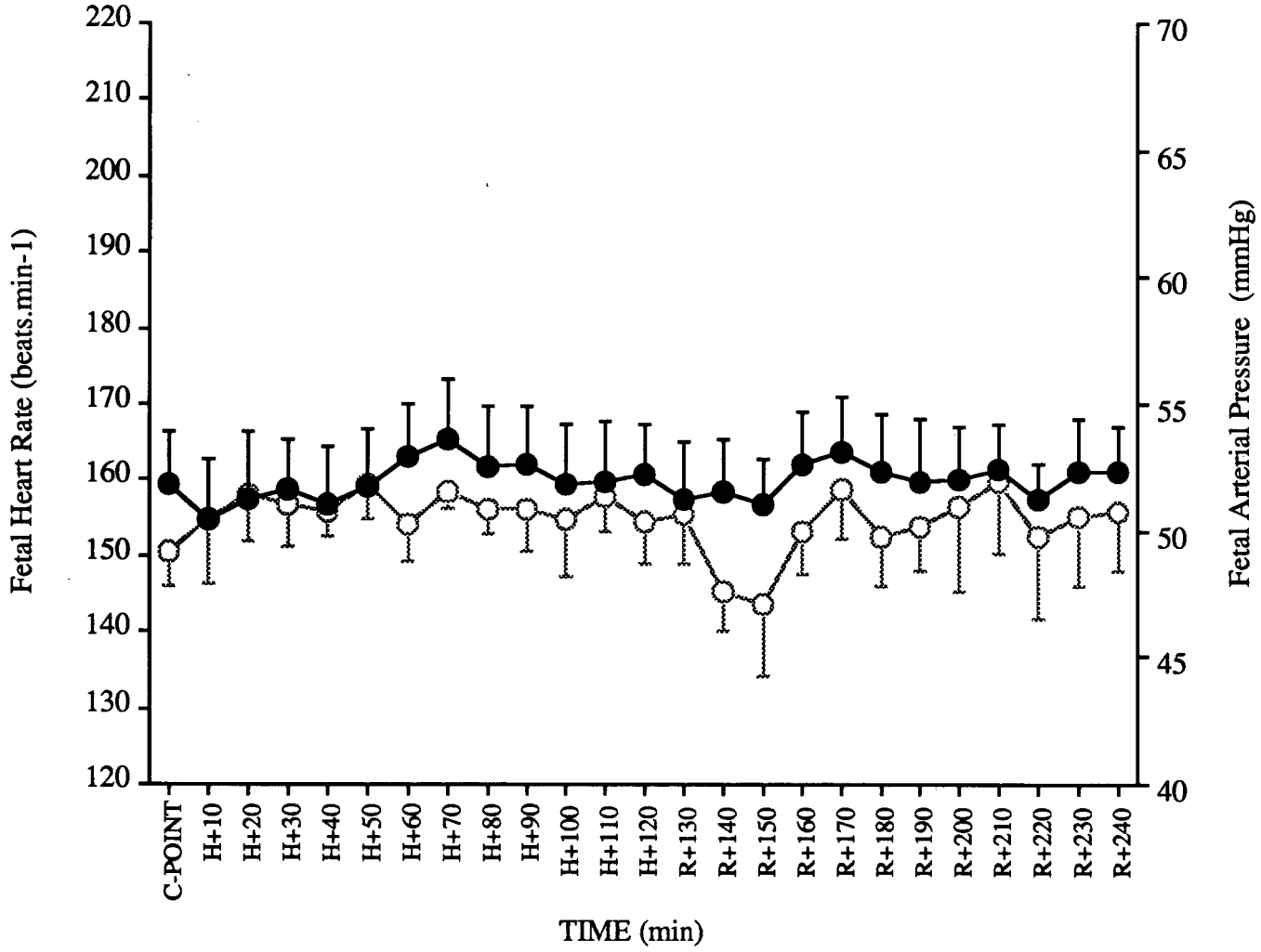


Figure 26 Fetal heart rate and arterial pressure during the moderate hypoxemia experiments.

○ Fetal heart rate

● Fetal arterial pressure

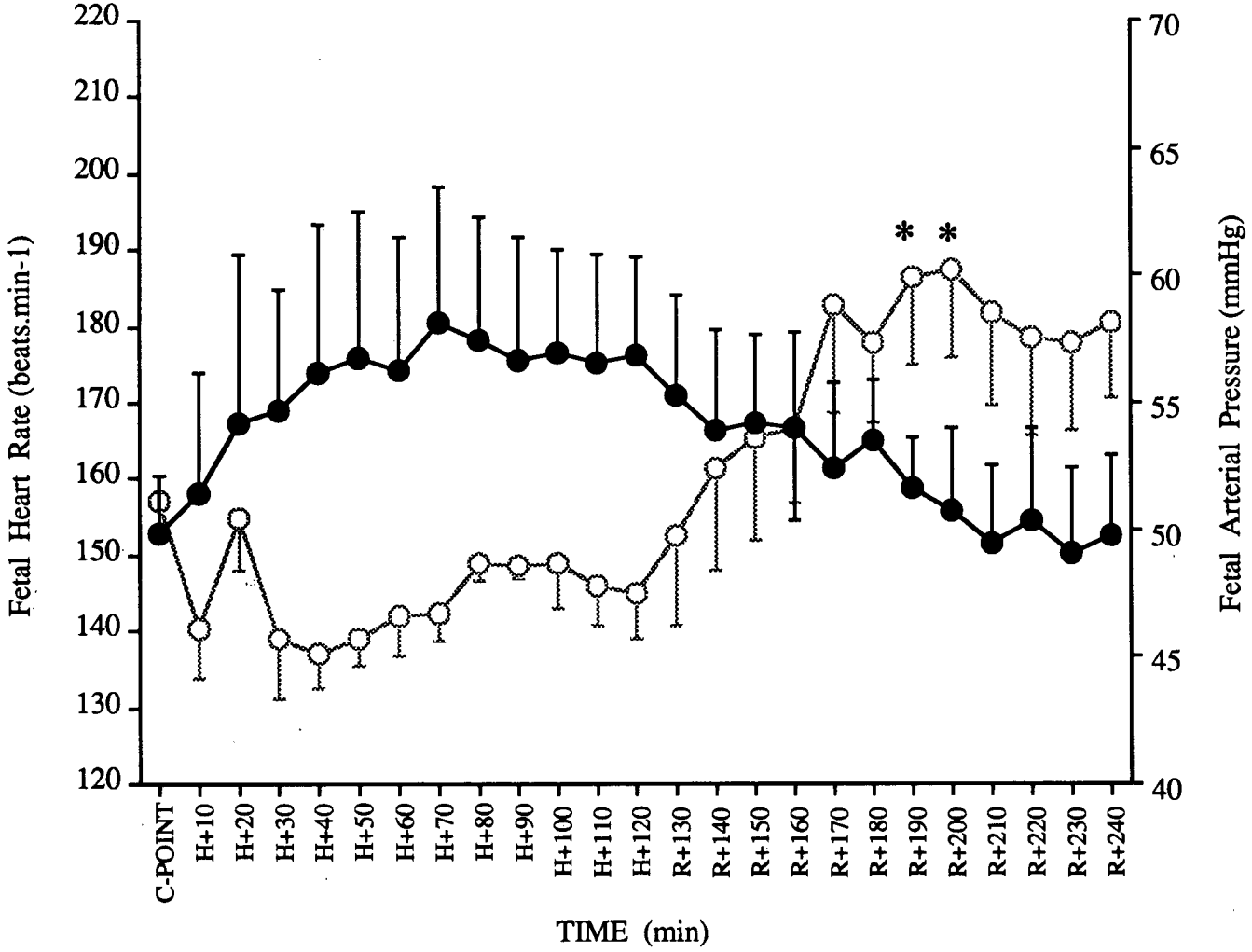


Figure 27 Fetal heart rate and arterial pressure during the severe hypoxemia experiments.

○ Fetal heart rate

● Fetal arterial pressure

* significant difference from control value ($P < 0.05$)

4. DISCUSSION

4.1 UTERINE BLOOD FLOW

As is the case in other mammalian species, the blood supply to the uterus of sheep is primarily provided by paired arteries (main uterine arteries) which arise from the distal end of the descending aorta (Fuller et al, 1975). Each main uterine artery divides into middle and dorsal branches. The middle uterine artery runs along the lesser curvature of the uterine horn, and sends branches (arcuate arteries) towards the greater curvature. It is these branches that mainly supply the components of the uterus including the maternal portions (caruncles) of the placenta. The dorsal uterine artery supplies the cervical end of the uterus and also sends branches to the cervix. Other potential sources of uterine perfusion include small arteries in the cervix which originate from the external iliac artery, and a branch of the ovarian artery which supplies the tip of the uterine horn and anastomoses with an arcurate branch of the middle uterine artery. However, in late pregnancy, the bulk of uterine blood flow is supplied by the latter artery. Fuller et al (1975) found that in anesthetized, near-term pregnant ewes carrying single fetuses, flow through each middle uterine artery, averaged 323±44 ml/min, while total flow to each horn averaged 396±41 ml/min, so that middle uterine arterial flow comprised 82% of total flow. They also found no significant difference in uterine blood flow between the horn containing the fetus and the empty horn (mean difference = -14±88 ml/min), and similar findings were obtained in conscious sheep by Wilkening (1986). This is a reflection of the fact that in even in singleton pregnancies the placental cotyledons are distributed throughout both horns. It is the cotyledons that receive the bulk of uterine blood flow in late pregnancy. Makowski et al (1968) utilized radioactive microspheres to estimate the

distribution of uterine blood flow in pregnant sheep from 83 days gestation to term. The cotyledons received 82.7±2.0% of total flow, while the endometrium and myometrium received 13.4±16% and 3.9±0.7%, respectively. They also found that cotyledonary flow was linearly related to fetal weight, and thus gestational age, increasing from ~300 ml/min at 83 days gestation to ~1100 ml/min at term. This was not the case with either endometrial or myometrial flow.

In the current study, total uterine blood flow was not measured; rather flow in the middle uterine artery of the uterine horn containing the operated fetus was estimated. Flow in the ewes with a single fetus averaged 339.6±6.2 ml/min in the pre-experimental period, which is very similar to the value of 323±44 ml/min obtained by Fuller et al (1975). Using their findings that middle uterine arterial flow comprises 82% of total flow to the horn and flow in the pregnant and nonpregnant horns is not different, a total uterine flow value of 828 ml/min can be calculated for the singleton ewes in the current study, or 248 $ml \cdot min^{-1} \cdot kg^{-1}$ fetal weight. Middle uterine artery flow in the ewes carrying twins averaged 549.5±17.9 ml/min in the pre-experimental period, a value significantly higher than that for the singleton ewes. A total uterine flow of 1340 ml/min can be calculated for the twin bearing ewes or 253 $ml \cdot min^{-1} \cdot kg^{-1}$ total fetal weight. Thus the ewes with twins were able to provide about the same rate of uterine blood flow/kg fetal weight as those carrying single fetuses. However this is only because the average birth weight in the twin pregnancies (3.28±0.20 kg) is substantially lower than with the two singleton lambs that were born alive (4.38 kg), indicating some additional constraints upon fetal growth in the twin pregnancies. Reduced fetal growth in twin (and triplet) pregnancy in sheep has been reported by others (Barcroft, 1946, Bassett et al, 1969; Stegeman, 1974), and this first becomes apparent at ~120 days gestation. The overall average pre-experimental value for middle uterine arterial blood flow (singleton and twin ewes) is 437±11 ml/min, or ~1066

ml/min total flow. This value is similar to in other estimates of uterine blood flow in the late gestation pregnant sheep, which range from ~900-1400 ml/min (Makowski et al, 1968; Huckabee et al, 1972; Rankin and Phernetton, 1976; Clapp et al, 1982b; Sunderji et al, 1984; Longo et al, 1986; Wilkening; 1986, Kitanaka et al, 1989; van der Weyde et al, 1992).

4.2 MATERNAL PROGESTERONE CONCENTRATIONS AND UTEROPLACENTAL PROGESTERONE OUTPUT UNDER NORMAL CONDITIONS

Measurement of the progesterone concentration in ovarian venous blood of pregnant sheep has demonstrated that ovarian secretion of the hormone continues throughout pregnancy at a rate comparable to that during the luteal phase of the estrous cycle in nonpregnant ewes (Edgar and Ronaldson, 1958). However, as pregnant ewes can be ovariectomized after the 50th day of gestation without pregnancy termination (Casid and Warwick, 1945), and as progesterone has been identified in placental tissue from intact and ovariectomized ewes (Short and Moore, 1959), it is evident that the placenta is able to secrete sufficient progesterone for the maintenance of pregnancy during the latter two-thirds of gestation.

In the current study, the pre-experimental maternal arterial and uterine venous concentrations averaged 4.27±0.13 ng/ml and 17.82±1.17 ng/ml, respectively in the ewes with a single fetus, and 6.53±0.19 ng/ml and 21.05±0.56 ng/ml in the ewes carrying twins. Higher maternal progesterone concentrations in twin pregnancies has been reported in numerous previous studies (e.g. Bassett et al, 1969; Mattner and Thorburn, 1971; Stabenfeldt et al, 1972; Thompson and Wagner, 1974). This difference is not present in

early pregnancy however, and first becomes obvious at around 55 days (Robertson and Sarda, 1971; Stabenfeldt et al 1972). As was found in the current and previous studies (e.g. Mattner and Thorburn, 1971), the higher progesterone concentrations in twin bearing ewes is associated with a higher rate of progesterone production. In the present study, pre-experimental uteroplacental progesterone output averaged 4,720±242 and 8,156±426 ng/min in the singleton and twin ewes, respectively. However, as total uterine blood flow was not measured, these values are underestimates of total progesterone output. Using the same correction method as described above for total uterine blood flow, total progesterone output can be estimated as 11.5 μg/min for the singleton ewes and 19.9 μg/min for the twin bearing ewes, which is equivalent to daily progesterone production rates of 16.6 and 28.6 mg respectively. These values are lower than the estimates of ~33 and 55 mg obtained by Mattner and Thorburn (1971). In this latter report, uterine venous progesterone levels were also higher than those in the present study. This could be due differences in progesterone assay methods, or breed of sheep studied. The higher progesterone output in twin pregnancy is very likely due to the greater total placental mass with twins, and the first appearance at ~55 days gestation of the progesterone difference between singleton and twin pregnancies is probably a reflection of the switch that occurs at this time from the ovary to the placenta as the main source of progesterone synthesis. Bedford et al (1972) found that the progesterone production rate was higher in pregnancies where birth weight was greater than 4 kg, compared to pregnancies with lower birth weights. They suggested that this difference could be due to the larger placentas in the former group since fetal and placental weights are highly correlated (Dawes, 1968).

4.3 HYPOXIA EXPERIMENTS

4.3.1 Method of Achieving Hypoxemia

Two methods were employed to achieve maternal and fetal hypoxemia: infusion of nitrogen via a non-occlusive maternal tracheal catheter for moderate hypoxia (Gleed et al, 1986) and delivery of a low oxygen gas mixture to a plexiglass chamber in the front of the monitoring pen for severe hypoxia (Rurak et al, 1990a). With both methods, the ewe has access to food and water during the entire experimental period, which is in contrast to another commonly employed method, namely placing the ewe's head into a clear plastic bag into which the desired gas mixture is delivered (e.g. Boddy et al, 1974). The tracheal gas infusion uses less nitrogen than does the chamber, and this is particularly advantageous in studies of long term hypoxia (e.g. Towell et al, 1987). For this reason, and also because the initial placement of the chamber in the monitoring pen causes some disturbance to the ewe, it was the original intention to use the tracheal catheter for both the moderate and severe hypoxia experiments. However, it was not found possible to achieve severe hypoxia with this method on a consistent basis. This is probably because, with an increasing rate of nitrogen infusion via the catheter, a point is reached where additional nitrogen passes up the airway to the environment, rather than reaching the alveoli. Thus the plexiglass chamber was used for the severe hypoxia study.

4.3.2 Maternal and fetal blood gas values and pH, and glucose and lactate concentrations

In the moderate hypoxemia experiments, maternal arterial Po_2 was reduced by ~31%, or by ~39 mm Hg, whereas in the severe hypoxia protocol, the fall in Po_2 was ~49% or ~59 mm Hg. However, these substantial reductions in oxygen tension resulted in

much more modest decreases in blood O_2 saturation and content (Tables 5 and 7). This is because the adult operates at the upper, flat portion of its hemoglobin-oxygen dissociation curve, and maternal oxygen tension has to fall markedly to achieve large decreases in O_2 saturation and content (Rurak, 1994). The situation is different in the fetus, which operates on the steep portion of its hemoglobin-oxygen dissociation curve. Thus a fall in fetal oxygen tension will result in more or less equivalent reductions in O_2 saturation and content. During moderate hypoxia, fetal Po_2 fell by 4.1 mm Hg or ~20%, whereas with severe hypoxia, the decrease was 4.8 mm Hg or 29%. Thus although the two protocols resulted in different degrees of maternal hypoxemia, the effects on fetal oxygen tension were similar. In comparison to previous studies (e.g. Koos et al, 1987; Towell et al, 1987; Akagi and Challis, 1990), both protocols involved modest reductions in fetal Po_2. However, in the severe hypoxia experiments, even though the Po_2 fall was modest, there was the development of significant metabolic acidemia, and this was not observed with moderate hypoxia. There were also greater increases in lactate and cortisol concentrations with severe hypoxia. Fetal metabolic acidemia has been observed in many other studies of acute hypoxemia (e.g. Koos et al, 1987; Bocking et al, 1988; Milley, 1988; Rurak et al, 1990a; Bocking et al, 1992; Boyle et al, 1992; Wilkening et al, 1993). This usually requires that fetal arterial Po_2 decrease below ~15 mm Hg, which did happen in the severe hypoxia experiments, but not in the moderate hypoxia protocol (Table 13). If fetal Pao_2 drops below ~12 mm Hg and O_2 content below 1 mM, the blood lactate concentration rises progressively to very high levels. This is associated with marked acidemia, and when arterial pH falls below ~6.90 cardiovascular collapse occurs (Rurak et al, 1990a). However, when Pao_2 during hypoxemia is ≥12 mm Hg, the metabolic acidemia is temporary, with a return to near normal values after 12-24 h (Bocking et al, 1988; Bocking et al, 1992; Boyle et al, 1992; Wilkening et al, 1993; Hooper et al, 1995). This very likely would have occurred with severe hypoxia in the current study. None the less, the initial

perturbation in fetal oxygenation and acid-base status in this group was significant. Arterial O_2 content had fallen by 48% by the end of the hypoxemic period, and oxygen delivery to fetal tissues and organs would have fallen to the same degree unless there was a compensatory increase in tissue and organ blood flow. This undoubtedly occurred for the heart, brain, and adrenal gland and the rise in blood flow to these organs would likely be more than sufficient to compensate for the fall in oxygen concentration (Rurak et al, 1990b). Umbilical blood flow may also have increased (Milley, 1988; Rurak et al, 1990a; Hooper et al, 1995), but the probable magnitude of the rise (~20%) would have been less than the % fall in O_2 content. Thus delivery of oxygen to the placenta from the umbilical arterial blood would have been reduced.

Both maternal and fetal Pco_2 fell slightly during moderate and severe hypoxia, although the changes were not statistically significant. Maternal and fetal hypocapnia has been observed in many other hypoxia studies (e.g. Gleed et al, 1986, Rurak et al, 1990a), even when CO_2 is added to the gas mixture breathed by the ewe. This is very likely due to maternal hyperventilation in response to the induced hypoxemia. There were no significant changes in maternal or fetal glucose concentrations during the experiments, although there was a tendency for fetal glucose levels to increase during severe hypoxia. Fetal hyperglycemia has been frequently observed in hypoxia studies (e.g. Milley, 1988; Rurak et al, 1990a), and this results from catecholamine-elicited hepatic glycogenolysis, with release of hepatic glucose into the fetal circulation (Bristow et al, 1983; Rudolph et al, 1989; Apatu and Barnes, 1991).

In the pre-experimental period, fetal lactate concentration was higher than that in the ewe (Tables 3-14). This is normally the case in pregnant sheep (e.g. Rurak et al, 1990a), but the higher fetal lactate level does not indicate that the fetus is hypoxic or has a higher

rate of anaerobic metabolism. The fetus normally receives lactate from the placenta, and this is reflected in a positive umbilical veno-arterial lactate concentration difference, as was observed in the current study. Under normal circumstances, umbilical lactate uptake accounts for ~30% of fetal lactate turnover (Sparks et al, 1982), and ~72% of fetal lactate utilization is accounted for by lactate oxidation (Hay et al, 1983). Thus under normoxic conditions, lactate serves as a catabolic substrate in the fetus. As was discussed above, fetal blood lactate concentration rose during both moderate and severe hypoxia, with the increase being greater in the latter experiments. In both protocols, this was associated with a tendency for the umbilical veno-arterial lactate difference to increase, although the changes were not statistically significant. However, the data suggest an increase in placental lactate production during hypoxia and this is similar to the trend for increased lactate output into the maternal uteroplacental circulation (Fig. 18). Certainly there was no evidence for a reversal of the gradient, which would indicate net fetal lactate production from hypoxic tissues. A similar finding was obtained during 8-24 h ritodrine infusion to fetal lambs, where there is also modest fetal hypoxemia and lactic acidemia (van der Weyde et al, 1990). However, with severe fetal hypoxemia and acidemia, a reduction or reversal of the umbilical veno-arterial lactate difference does occur, indicating net fetal lactate production by the fetus (Gu et al, 1985; Milley, 1988; Boyle et al, 1992; Hooper et al, 1995). In this situation, the placenta may become important in regulating circulating fetal lactate concentration. It has been suggested that during the severe, but non-lethal fetal hypoxemia, increased lactate production by some organs is counterbalanced in increased lactate metabolism by the placenta, thereby allowing the fetus to attain a stable or decreasing blood lactate level (Boyle et al, 1992). The fetal kidney is also a significant site of fetal lactate clearance during prolonged hypoxemia, induced by reducing uterine blood flow (Cock et al, 1994). However, precise estimates of fetal lactate utilization/production in relation to umbilical lactate flux have not yet been obtained. Moreover, it may not be

possible to do this, because of the non-steady state conditions that seem to exist for lactate during fetal hypoxemia (Boyle et al, 1992).

4.3.3 Fetal Cortisol Concentration

Hennessy et al (1982) showed that prior to 120 days gestation, the majority of cortisol in the fetal circulation is of maternal origin. However, with advancing gestational age, a progressively smaller proportion of fetal plasma cortisol was derived via transplacental transfer. Thus the rise in fetal cortisol concentration during moderate and severe hypoxia was very likely the result of increased secretion of cortisol from the fetal adrenal cortex, this in turn being the result of a rise in fetal plasma ACTH levels (Challis et al, 1986; Akagi and Challis, 1990). Although plasma ACTH concentrations were not measured in the current study, many other studies have demonstrated that acute hypoxemia results in an initial increase in the fetal plasma concentrations of ACTH (e.g. Challis et al, 1986; Challis et al, 1989; Akagi and Challis, 1990; Keller-Wood and Wood, 1991; Sue-Tang et al, 1992). The mechanism of the increased ACTH release during hypoxemia is likely multifactorial. Sue-Tang et al (1992) demonstrated that the temporal pattern of ACTH concentration change is similar to that of AVP (Hooper et al, 1990), which is a corticotropin-releasing hormone in fetal sheep (Norman and Challis, 1987). Fetal catecholamine concentrations are also elevated during hypoxia (Jones and Robinson, 1975; Cohen et al, 1982; Hooper et al, 1990), and these may contribute to release of ACTH, since this response is attenuated by the α-adrenergic blockade (Jones and Ritchie, 1976). With prolonged fetal hypoxemia, fetal cortisol concentrations remain elevated, whereas ACTH concentrations return to basal levels after ~12 h (Challis et al, 1989; Sue-Tang et al, 1992). The sustained cortisol response is associated with a continued rise in adrenal blood flow (Challis et al, 1986; Bocking et al, 1988), and may be due to activation of fetal

adrenal function by the initial, prolonged elevation in plasma ACTH levels, via increases in adrenal 17α-hydroxylase activity and ACTH receptor adenylate cyclase coupling (Challis et al, 1989). It is also possible that the increase in fetal plasma PGE_2 concentrations during hypoxia may contribute to the prolonged elevation of cortisol concentrations (Hooper et al, 1990; Sue-Tang et al 1992). The sustained elevation in fetal cortisol concentration could result in activation of placental 17α-hydroxylase activity to increase estrogen synthesis and decrease progesterone production, ultimately resulting in the initiation of parturition (Jones et al, 1977b; Challis et al, 1989). However, this could not have occurred in the present study, given the acute nature of the hypoxia and resulting fetal cortisol rise.

4.3.4 Maternal and Fetal Arterial Pressure and Heart Rate

The main functional characteristics of the fetal cardiovascular system are a high cardiac output and organ blood flows, a high heart rate, low arterial pressure and low vascular resistance. These characteristics are effective in counteracting the low fetal arterial Po_2 and O_2 content, thereby permitting adequate rates of O_2 delivery to fetal tissues (Rurak, 1994). Under normal conditions, fetal heart rate is under the influence of both the sympathetic and parasympathetic systems, as well as circulating catecholamines (Vapaavouri et al, 1973; Nuwayhid et al, 1975; Walker et al, 1978). Sympathetic control is effective as early as 60 days gestation, and as gestation continues, the parasympathetic system exerts an increasing influence on the fetal heart rate, via increased vagal tone. This results in a progressive fall in heart rate during the latter half of gestation (Vapaavouri et al, 1973; Boddy et al, 1974; Nuwayhid et al, 1975; Walker et al, 1978).

The pre-experimental values of fetal heart rate and fetal arterial pressure during the moderate and severe hypoxemia experiments averaged 150.5±1.5 and 157.2±1.8 beats per

minute and 51.8±0.6 and 49.9±0.7 mm Hg, respectively. These values are within the normal range found in fetal lambs during late gestation (Boddy et al, 1974). During moderate hypoxia, there were no significant changes in either variable, although a slight increase in heart rate (~5 bpm) persisted for most of the hypoxemic period (Fig. 26). This could be due to a rise in fetal catecholamine levels (Jones and Ritchie, 1978a). In contrast, during severe hypoxia, there was a tendency for bradycardia and hypertension during the hypoxemic period, followed by tachycardia in the recovery period. This pattern of response has been observed in many studies of acute hypoxemia in the fetal lamb (e.g. Boddy et al, 1974; Rurak, 1978; Cohn et al, 1974; Cohen et al, 1982; Bocking et al, 1988). The bradycardia is likely chemoreflex and baroreflex in origin, as it is abolished by bilateral denervation carotid sinus (Giussani et al, 1990). The efferent arm of the response involves increased vagal tone, since it is abolished by bilateral vagotomy (Rurak, 1978). The post-hypoxia increase in fetal heart rate is due to a ß-adrenergic stimulation from the elevated in plasma catecholamine levels (Jones and Robinson, 1975; Jones and Ritchie, 1978b; Cohen et al, 1982; Jensen et al, 1987; Martin et al, 1987; Jones et al, 1988; Perez et al, 1989). AVP is secreted in large amounts during fetal hypoxemia and acidemia (Rurak, 1978; Devane et al, 1982; Daniel et al, 1983; Wood, 1989; Wood and Chen, 1989) and has been reported to reduce fetal heart rate (Rurak, 1978; Iwamoto et al, 1979; Courtice et al, 1984; Tomita et al, 1985; Dunlap and Valego, 1989; Irion et al, 1990). It also is a potent hypertensive agent and vasocontrictor in the fetus (Rurak, 1978; Tomita et al, 1985; Irion et al, 1990). Studies involving the use of vasopressin antagonists have indicated that AVP could be involved in the fetal heart rate, arterial pressure and blood flow responses to hypoxemia (Perez et al, 1989; Piacquadio et al, 1990). Thus the cardiovascular responses to fetal hypoxemia could involve a number of factors.

Although, there have been very many studies of the fetal cardiovascular responses to acute hypoxemia in pregnant sheep, information on the maternal cardiovascular responses is very limited. In both the moderate and severe hypoxemia experiments, maternal heart rate tended to increase during the hypoxia interval, and this was most obvious with severe hypoxia. Kitanaka et al (1989) observed an initial maternal tachycardia (~18% increase) in a study of long term (21 d) hypoxia in pregnant sheep, and they also recorded a ~8% increase in arterial pressure. No increase in maternal arterial pressure was noted in the current study, but the degree of hypoxia was somewhat less than that employed by Kitanaka et al (1989). In non-pregnant ewes subjected to 96 h of severe hypoxia (Pao_2 ~40 mm Hg), there is tachycardia, but no change in arterial pressure (Krasney et al, 1984; Kitakana et al, 1989). Cardiac output was elevated for the first 24 h, and this was associated with increases in cerebral and coronary blood flows, and decreased perfusion to the abdominal viscera. Surprisingly, adrenal blood flow was not changed, which is in marked contrast to the findings in fetal sheep subjected to acute or chronic hypoxemia (see above). Catecholamines concentrations were elevated in the hypoxemic ewes, and a nonsignificant trend for this was also found in the study of Kitakana et al (1989) in pregnant sheep. A striking finding in the non-pregnant sheep was a sustained 48% fall in total body oxygen consumption but this was associated with only a trivial increase in blood lactate concentration, whereas in the fetus, a hypoxia-induced decrease in Vo_2 is accompanied by massive lactic acidemia (e.g. Rurak et al, 1990a). The fall in Vo_2 in the adult sheep appears to be an adaptive response that is also found in certain other species (Krasney et al, 1984). Whether such a response occurs in pregnant ewes during hypoxia does not appear to have been determined.

4.3.5 Uterine blood flow, O_2 delivery and consumption

In the current study, no consistent changes in uterine blood flow were noted with either moderate or severe hypoxia. This is similar to the results of Makowski et al, (1973) in pregnant sheep subjected to acute hypoxia. With 21 day hypoxia, Kitakana et al (1989) found no change in uterine blood flow in the first 24 h, but there was a decrease for the next ~24 h, followed by a sustained increase. The mechanism(s) underlying these changes is obscure. In the present study, the lack of change in uterine blood flow during hypoxia was accompanied by a small decrease in maternal arterial O_2 content, so that uterine O_2 delivery was only minimally decreased, even with severe hypoxia (~2% fall, Table 18). In their study of 21 days of hypoxia, Kitakana et al (1989) reduced maternal Pao_2 from ~102 to 57 mm Hg. This resulted in a 12% fall in arterial O_2 content, and as discussed above, uterine blood flow fell during the second day of the experiment. However, the resulting fall in uterine O_2 delivery was only ~25% and was not associated with any change in uterine Vo_2. This study and the current investigation illustrate the limitations of lowering uterine O_2 delivery by decreasing maternal oxygenation. Because the adult operates on the upper, flat portion of the hemoglobin-oxygen dissociation curve, very large reductions in arterial Po_2 must occur to lower O_2 saturation and content, and hence uterine O_2 delivery. However, even when greater reductions of uterine O_2 delivery are achieved, as with experimental reductions in uterine blood flow, there is no evidence for a fall in uterine or utero-placental O_2 consumption. Hooper et al (1995) reduced uterine blood flow by ~50% for 24 h in pregnant sheep at ~120 d gestation. Uterine O_2 delivery was reduced by ~52% with no change in uterine or uteroplacental placental O_2 uptake. Fetal oxygen delivery (umbilical blood flow x umbilical venous O_2 content) was reduced by a lesser extent (~38%), due to an increase in umbilical blood flow, and fetal O_2 consumption was maintained. However, as discussed above, fetal lactate concentration increased markedly

and there was net uptake of lactate by the placenta from the fetal circulation, a reversal of the normal situation. There was also a reduction in uteroplacental glucose consumption. Similar findings were obtained by Gu et al (1985) in studies involving varying degrees of uterine blood flow reduction (10-70%) of 60 min duration. Even when uterine blood flow (and hence O_2 delivery) was 30-50% of normal, total uterine and uteroplacental Vo_2 was maintained. However, fetal O_2 consumption was decreased, associated with marked lactic acidemia and net lactate uptake by the placenta from the fetal circulation. There was also a marked fall in uteroplacental glucose consumption. Fetal O_2 delivery values cannot be determined from the data given in Gu et al (1985), but it is likely that it fell be more than 50% when uterine blood flow was 30-50% of control (Wilkening and Meschia, 1983). This has been a common finding in studies of the fetal tolerance to reduced oxygen delivery, which it is primarily achieved via a reduction in fetal Po_2. A major fetal compensatory response is an increase in the extraction of oxygen by the fetus, which serves to maintain oxygen consumption in the face of a fall in oxygen delivery (Edelstone, 1984; Rurak, 1994). Via this mechanism, the fetal lamb can compensate for acute reduction in oxygen delivery of up to 50%. If oxygen delivery is reduced by more than 50%, then oxygen consumption falls and lactic acidemia develop (see Rurak at al, 1990a), indicative of an inability to compensate for the severe perturbation in the oxygen supply. Thus, in comparison to the placenta, the fetus seems less able to tolerate severe reductions in O_2 delivery, suggesting that the placenta may be protected in this situation, at the expense of the fetus. In the current study, it is unlikely that fetal O_2 consumption was reduced, even with severe hypoxemia, because the reduction in fetal O_2 delivery was likely considerably less than 50%.

4.3.6 Maternal plasma progesterone concentration and uteroplacental progesterone output

The hypothesis to be tested in this project is that short term maternal hypoxemia decreases placental progesterone production via a reduction in placental oxygen supply from the mother and/or fetus. The data obtained do not support this hypothesis; no decrease in uteroplacental progesterone output was observed during the hypoxia period. In contrast there was a tendency for progesterone output to increase with moderate hypoxia, a change that was observed in 4 of the 5 experiments. The increase in progesterone output was due to a rise in uterine venous progesterone concentration. A similar finding was obtained by Keller-Wood and Wood (1991). They measured progesterone concentration in maternal arterial and uterine venous blood before and during a 30 min hypoxic period (achieved by lowering maternal inspired oxygen concentration). There was no significant effect of hypoxemia on the arterial or venous concentrations of progesterone, although uterine venous progesterone concentrations increased in 7 of 10 experiments. This was associated with a fall in umbilical venous progesterone levels, so that the umbilical veno-arterial progesterone gradient decreased. Thus there was evidence for increased placental progesterone secretion into the maternal circulation and decreased secretion into the fetal compartment. However, veno-arterial differences in progesterone concentration are much greater on the maternal side of the placenta compared to the fetal side (Keller-Wood and Wood, 1991), so that it seems unlikely that the apparent decrement in fetal progesterone uptake could match the apparent rise on the maternal side. Moreover, in the absence of measurements of uterine and umbilical blood flow in the study of Keller-Wood and Wood (1991), data on actual progesterone secretion rates in the ewe and fetus are lacking. However, taken together, the data from both studies suggest that moderate hypoxia increases placental progesterone output into the maternal circulation. However, this must be verified by further studies.

If there is in fact an increase in placental progesterone production with moderate hypoxia, one possible mechanism for this effect would be the rise in fetal PGE_2 concentrations that occurs with reduced oxygenation (Hooper et al, 1990; Sue-Tang et al, 1992; Murotsuki et al, 1995). As was discussed in the Introduction it is likely that the placenta is the source of this PGE_2. Wango et al (1992) demonstrated that progesterone synthesis in ovine binucleate cell preparations is increased by PGE_2 and reduced by indomethacin. They also showed that sheep binucleate cells produce PGE_2 from arachidonic acid. Thus an increase in PGE_2 synthesis by binucleate cells during hypoxia could increase progesterone synthesis in these same cells via an autocrine mechanism. The PGE_2 released into the fetal circulation appears to contribute to the fetal tolerance to hypoxia by minimizing the fetal hyperglycemia and lactic acidemia that occur (Hooper et al, 1992; Thorburn, 1992). PGE_2-induced stimulation of placental progesterone production could also be of benefit to the fetus. Valenzuela et al (1992) reported that acute fetal hypoxia (achieved via uterine artery occlusion) increases amniotic fluid prostaglandin F metabolites in pregnant sheep. They speculated that this was due to increased placental secretion of prostaglandin $F_{2\alpha}$, and this could involve an hypoxia-induced increase in cytokine production, as has been demonstrated in other tissues. They also suggested that this could be a mechanism for premature delivery in pregnancies associated with intrauterine growth restriction and associated fetal hypoxemia (Valenzuela et al, 1993). An increase in placental progesterone secretion could interfere with this mechanism via the inhibitory effects that the hormone has on myometrial gap junctions, oxytocin receptors and perhaps other elements involved in the initiation of effective uterine contractions (Zhang et al, 1992; Lye et al, 1993; Neuland and Breckwoldt, 1994). That such progesterone effects would be desirable with modest, non-acidemic fetal hypoxemia is suggested by the fact that in healthy fetal lambs modest ($\leq$ ~5 mm Hg fall in Pao_2), transient decreases in blood oxygen levels occur frequently as a result of prelabor uterine

activity (contractures) and fetal skeletal muscle activity in the form of breathing and body movements (Harding et al, 1983; Rurak and Gruber, 1983; Rurak, 1994). Contractures differ markedly in character from labor contractions in that they are of much longer duration (~5 min) and lower amplitude (≤5 mm Hg), and occur once every ~20-40 min. They result in transient fetal hypoxemia via a reduction in uterine blood flow (Sunderji et al, 1984). Maternal bolus i.v. injection of a small amount of oxytocin can elicit a contracture. This is associated with transient fetal hypoxemia and a rise in ACTH and cortisol concentration, and the ACTH rise is abolished by maintaining fetal normoxia (Lye et al, 1985; Woudstra et al, 1991; Sadowsky et al, 1992). It is not known whether fetal prostaglandin levels increase as well. Thus with contractures, and possibly with vigorous fetal activity, there is transient activation of the elements which are involved in the initiation of parturition in sheep. A concomitant increase in placental progesterone release could counteract any ACTH and cortisol influences on the uterine contractility, thereby preventing premature labor. However, in situations where there is more severe fetal hypoxia associated with acidemia that puts the fetus at risk, initiation of labor and delivery, even if premature, might increase the survival odds for the fetus. This could explain why, in the current study, severe hypoxia was not associated with any evidence of increased uteroplacental progesterone secretion. However the mechanism for such an effect is unclear at present.

As was discussed in the Introduction, there are several *in vitro* studies which indicate that steroidogenesis is oxygen dependent in various tissue, including the placenta. However, as noted above, no evidence for oxygen dependence of *in vivo* placental progesterone synthesis was obtained in the present study. This may be because the reduction in maternal and/or fetal oxygenation was not severe enough for placental O_2 supply to become a limiting factor. For the reasons discussed above, acute maternal

hypoxia may not be the best experimental paradigm for this purpose. Other methods such as reduced uterine blood flow or maternal hemorrhage/anemia (Paulone et al, 1987) might be more appropriate. In this regard, Challis et al (1989) examined the maternal and fetal endocrine responses to a 48 h reduction in uterine blood flow which reduced fetal Pao_2 from 22.6 to ~14.5 mm Hg. There was also fetal metabolic acidemia that lasted ~8 h. A transient increase in fetal arterial progesterone concentration was observed in the first 1-2 h of reduced uterine blood flow, but the change was not statistically significant. Maternal arterial progesterone concentration was not altered, and uterine venous levels were not measured. Further work on the effects of hypoxia on placental progesterone production seem warranted, as do investigations of other aspects of placental endocrine and metabolic functions during reduced oxygenation.

5. Summary and Conclusions

To examine the effects of hypoxia on placental progesterone production, maternal and fetal hypoxemia was experimentally induced by reducing maternal inspired O_2 for 2 h in chronically instrumented pregnant sheep at 125-136 days gestation. The hypoxemia period was preceded and followed by 2 h pre-hypoxia and recovery periods, respectively. Control experiments, involving 6 h periods of normoxia were also carried out. Samples were taken simultaneously at predetermined time periods from maternal femoral arterial and uterine venous catheters for measurement of progesterone concentration. Blood flow to the uterine horn containing the operated fetus was measured continuously, and utero-placental progesterone output was calculated as the uterine venous - arterial difference in progesterone concentration times uterine blood flow. Blood samples were also collected from the fetal femoral artery and umbilical vein, and in these samples, as well as in the maternal samples, the following variables were measured: Po_2, Pco_2 and pH, hemoglobin concentration, blood O_2 saturation and content, glucose and lactate concentrations and fetal plasma cortisol level. The following variables were calculated from these data: utero-placental oxygen delivery and consumption and glucose uptake and lactate flux. Maternal and fetal arterial pressure and heart rate were continuously monitored. The following results were obtained.

1. Arterial and uterine venous progesterone concentrations were higher and associated with higher uterine blood flow and progesterone output (from the operated horn) in sheep carrying twin fetuses compared to those with a single fetus.

2. Maternal hypoxia resulted in fetal hypoxemia, lactic acidemia and increased cortisol concentration.

3. Fetal arterial O_2 content was reduced by ~ 40% during severe hypoxia, which may have reduced O_2 delivery from the fetus to the placenta.

4. Although there was no significant changes in maternal progesterone levels or utero-placental progesterone output, there was a trend for an increase in uterine venous progesterone concentration and progesterone output with moderate hypoxia. This is similar to published reports.

5. If an increase in utero-placental progesterone output does in fact occur, it may be due to the increase in placental PGE_2 production that occurs with hypoxia. This effect may have been abolished by severe hypoxia. A rise in placental progesterone production during fetal hypoxia could act to inhibit the onset of preterm labor.

5. Placental endocrine function appears to be more resistant to hypoxia, compared to physiologic and metabolic functions in the fetus.

6. The effect on placental progesterone output of other methods of inducing fetal hypoxemia, which have a greater impact on uterine O_2 delivery (e.g. maternal anemia, reduced uterine blood flow), should also be examined.

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