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Effect of Hypoxia on the Initiation of Secondary Wool Follicles in the Fetus

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Abstract

The development of secondary wool follicles in single fetal sheep subjected to hypobaric hypoxaemia was studied. One group of pregnant ewes were exposed to 57·1 kPa from 30 to 135 days gestation. Fetal weights (mean ± s.d.) for the hypoxaemic group (3·35 ± 0·53 kg; $n = 4$) were significantly lower than for the controls (4·19 ± 0·31 kg; $n = 3$, $P < 0·05$). At 110 days gestation, a second group had arterial and venous catheters surgically implanted into the ewe and fetus and skin samples were taken from the fetus. At 120 days gestation (10 days after surgery) these animals were subjected to hypoxia for 20 days, at a level to maintain fetal carotid pO_2 between 1·47 and 1·87 kPa (mean carotid pO_2 for the control fetuses was 2·84 ± 0·28 kPa). Fetal weight at 140 days was not significantly different in the hypoxaemic and control groups. Morphometric analysis revealed that the secondary to primary follicle ratio (S : P) was less in both groups of hypoxaemic fetuses than in their respective controls. Although hypoxia for 20 days did not significantly alter fetal weight, it produced a low S : P ratio similar to the longer-term hypoxaemic animals. It is concluded that hypoxia has a marked effect in reducing the initiation of secondary follicles in the last third of gestation.

Introduction

Most secondary wool follicles are initiated during the last third of gestation, but maturity may not occur till several weeks after birth (Fraser 1954). Primary follicles first appear in mid-gestation (Hardy and Lyne 1956; Fraser and Short 1960) and the formation of follicles is complete prior to the appearance of secondary follicles.

The measurement of secondary to primary (S : P) follicle ratio has been used to express changes in secondary development (Ryder 1957; Hutchinson and Mellor 1983), since it has been shown that primary follicles alter little in response to maternal nutrition and heat stress (Cartwright 1971; Corbett 1979). The use of S : P ratio overcomes the many problems associated with estimating primary and secondary follicle densities (Kleiber 1961).

Although several studies have been made on the effects of nutrition on follicle initiation and development in the prenatal and postnatal sheep (Schinkel and Short 1961; Williams and Henderson 1971; Hutchinson and Mellor 1983), the influence of low oxygen availability to the fetus has not been examined. Fetal hypoxaemia commonly accompanies spontaneous and induced fetal growth retardation (Robinson 1979; Robinson *et al.* 1979). Oxygen may also be a limiting factor in instances of multiple pregnancy; where placental blood flow is reduced or growth of the placenta

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restricted. The consequences of hypoxia for wool follicle initiation is little understood and we therefore investigated the changes in S : P ratios associated with fetal hypobaric hypoxaemia in early and late gestation.

Materials and Methods

Animals

Merino × Border Leicester ewes were mated to a Border Leicester ram and tupping dates were accurately recorded. At 30 days gestation pregnancy was confirmed using an ultrasound scanner and the ewes randomly allocated into experimental and control groups. The control animals were placed in metabolism cages in an animal house, whilst the experimental group were housed in metabolism cages in a hypobaric chamber.

Another group of ewes from the same flock were mated but were not housed until 90 days gestation. At 110 days gestation, catheters were inserted unilaterally into the jugular vein and carotid artery of the ewe (o.d. 2·0 mm, i.d. 1·5 mm) and fetus (o.d. 1·5 mm, i.d. 1·0 mm) under conditions of strict asepsis and antisepsis, essentially as described by Robinson *et al.* (1979). A skin sample was taken from the lateral surface of the fetal neck, approximately midway along its length. This site was chosen for S : P ratio analysis as the added trauma and risk to the fetus in obtaining a midside sample (the more usual site) from the catheterized group was considered unnecessary for purposes of the present study.

Following recovery from surgery, all animals were placed in metabolism cages. Blood samples were taken from mother and fetus every second day to determine blood gases (Corning Blood Gas Analyzer 168) and packed cell volumes (PCV, micro-haematocrit method). At 120 days gestation (10 days post-operative) the experimental animals were moved to the hypobaric chamber and exposed to simulated high altitude.

In both studies all animals were fed identical diets of lucerne chaff and pellets at a maintenance level for their weight and stage of pregnancy (McDonald *et al.* 1981).

Hypobaric Hypoxia

Hypobaria was achieved in a 12 157 litre chamber with a suction pump which maintained the required pressure and allowed a complete change of air approximately every 10 min. This ensured that the relative gas mixture, temperature and humidity of the chamber was similar to both ambient and the environmental conditions of the control group. A timed light switch simulated normal photoperiod.

For the unoperated group (animals exposed to hypoxia from 30 to 135 days gestation), the chamber simulated 4572 m altitude (57·1 kPa). The pressure used for the catheterized animals was dependent on the fetal pO_2. The chamber pressure was set to establish a fetal carotid pO_2 of between 1·47 and 1·87 kPa within 2 h of the initial exposure to hypoxia. As a consequence the simulated altitude was dependent on the individual fetal response, but was in the range of 3048–4572 m (69·7–57·1 kPa).

The chamber pressure was returned to ground pressure every second day (immediately following blood sampling) for 20–30 min in order for the animals to be fed, watered and their waste removed. Return to the required level of hypobaria took 15–20 min.

Skin Processing

The uncatheterized animals were killed at 135 days and the catheterized animals at 140 days gestation. At post-mortem, fetuses were dried, weighed and a skin sample taken from the right lateral surface of the fetal neck, away from the previous sample site.

All skin samples were fixed in 10% (v/v) buffered formalin. The wool was trimmed prior to embedding. Dehydration of the tissue followed the method of Carter and Clarke (1957). The skin was embedded in wax and horizontal sections were cut at a thickness of 8 μm. The sections were stained with haematoxylin, eosin and picric acid.

The method for counting follicles was similar to that described by Hutchinson and Mellor (1983). The tissue slides were examined under a microscope at ×40 magnification. Primary follicles were identified by accompanying sweat glands, whilst secondary follicles were identified by the absence of associated sweat glands. One hundred primary follicles were counted along with associated secondary follicles, irrespective of the stage of development of the follicle. This was repeated 10 times for each tissue sample, using different fields and between three and four sections per sample.

Data Analysis

Student *t*-test was used to compare fetal body weights, S : P ratio and PCV between control and experimental animals for each study. Multiple pregnant animals were excluded from the investigations.

Results

Hypoxia produced a significant polycythaemia in the experimental group in both studies (Table 1). There was no difference in PCV between fetuses and between dams exposed to hypobaric hypoxia for 105 or 20 days.

Table 1. Effects of exposure to hypobaric hypoxaemia from 120 to 140 days and 30 to 135 days gestation on maternal and fetal packed cell volume, fetal body weight and S : P wool follicle ratio

Values represent means ± s.d. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$, when compared with controls

Maternal PCV		Fetal PCV		Fetal body weight (kg)		S : P ratio[A]	
Control	Hypoxic	Control	Hypoxic	Control	Hypoxic	Control	Hypoxic
Exposure to hypoxia from 120 to 140 days gestation							
35·1	56·6***	37·8	51·5***	3·95	3·41	3·43[B]	1·34[C]
±2·3	±2·5	±2·6	±1·7	±0·42	±0·88	±0·34	±0·54
Exposure to hypoxia from 30 to 135 days gestation							
33·7	57·8***	41·0	54·0**	4·19	3·35*	2·84	1·00***
±1·6	±3·1	±1·0	±3·0	±0·31	±0·53	±0·23	±0·49

[A] Pre-treatment values (110 days gestation) 1·30 ± 0·29 for fetuses exposed to hypoxia 120–140 days gestation.

[B] Significantly less than pre-treatment values (110 days gestation); $P < 0{\cdot}001$.

[C] Not significantly different from pre-treatment values, but different from controls; $P < 0{\cdot}001$.

Table 1 details the mean body weight and S : P ratio for fetuses in both experiments. The body weights for the animals exposed to hypoxia from 30 to 135 days gestation were significantly lower than their controls. There was no difference in the weights from fetuses subjected to hypoxia for 20 days in late gestation.

The S : P ratios were significantly lower in the fetuses subjected to hypoxaemia than in controls in both studies. As well, the control animals had similar S : P ratios and likewise the hypoxaemic fetuses showed little difference in secondary follicle development between 20 and 105 days of exposure.

There was no significant correlation between body weight and S : P ratio.

Discussion

Maternal and fetal PCV were significantly higher in both experimental groups than in their controls, indicating a level of adaptation to hypoxaemia. A comparison of PCV data between the long- and short-term hypoxaemic animals suggests that both groups experienced similar levels of hypoxaemia.

Previous studies on the effect of hypoxia on fetal growth have shown that body weight is reduced independently of maternal nutrition (Van Geijn *et al.* 1981; Chang *et al.* 1984). The present results support this finding when the fetus is exposed to hypoxia for most of gestation, but the effect on birthweight is small compared with undernutrition. This suggests that the supply of nutrients may limit fetal growth to a greater degree than availability of oxygen.

Secondary follicle initiation begins in many sheep breeds between 90 and 99 days (Ryder and Stephenson 1968). In the catheterized fetuses, secondary follicles were present at 110 days (Table 1), but increased rapidly by 140 days. Hypoxia abruptly halted secondary follicle initiation at this late stage of fetal development and there was no significant S : P ratio difference between these fetuses and those exposed to low oxygen levels for almost 80% of their gestation.

Underfeeding of ewes during the last third of gestation reduces S : P ratios (Schinkel and Short 1961; Williams and Henderson 1971). Hutchinson and Mellor (1983) have shown that pregnant ewes underfed between 95 and 116 days gestation and refed from 117 to 142 days gestation showed fetal S : P ratios similar to animals that had been well fed throughout gestation. Our work supports their findings that the later phase of secondary follicle initiation (from 120 days onwards) may have the greatest influence on the secondary follicle population of the neonate.

Several mechanisms by which hypoxia may be acting are possible. It has been shown that hypoxia leads to a decrease in uterine blood flow (Dilts *et al.* 1969; Makowski *et al.* 1973). This phenomena would non-specifically alter delivery of metabolites and oxygen to the fetus. It is possible that this mechanism also acts during maternal hyperthermia (Oakes *et al.* 1976) to reduce fetal S : P ratio (Cartwright 1971). Hypoxia may hinder normal fetal skin metabolism by either reducing fetal glucose consumption (Jones *et al.* 1983), redistributing fetal cardiac output, and/or peripheral vasoconstriction, to the disadvantage of the skin (Dawes *et al.* 1968; Creasy *et al.* 1973; Cohn *et al.* 1974; Reuss *et al.* 1982; Court *et al.* 1984).

In addition, recent work has shown that experimentally induced fetal growth retardation in which the fetus is both hypoxaemic and hypoglycaemic, is associated with a reduction in the circulating levels of thyroid hormones (Harding 1982). Investigations by Chapman *et al.* (1974) and Thorburn *et al.* (1981) have provided evidence that a fall in thyroxine and reverse triiodothyronine (rT_3, 3′,5′,3-T_3) is associated with inhibition of secondary follicle development. Chronic hypoxaemia may elicit changes in thyroid functions (either alone or in association with growth retardation) which inhibit normal initiation and development of wool follicles in the fetus.

The way in which oxygen is linked to wool follicle development is as yet unclear, but there is evidence that hypoxia in late gestation is capable of halting secondary follicle initiation. Measurement of S : P ratio in the newborn lamb would appear to be a sensitive non-specific marker of fetal distress in late gestation.

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