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## The effects of season on placental development and fetal growth in sheep

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### ABSTRACT

Autumn-born lambs have been reported to have lower birth weights than spring-born lambs by 0.4 to 1.0 kg, but the physiological basis of this difference is unknown. This study examined the effects of season on fetal growth and placental development independent of the confounding effects of maternal nutrition. Romney ewes (aged 5 and 6 years) were randomly allocated to groups mated in December (n=13) or March (n=13) with a similar live weight at mating ( $55.6 \pm 1.8$  vs  $55.7 \pm 1.9$  kg, mean  $\pm$  SE,  $P > 0.10$ ) and managed for a similar live weight at day 140 of gestation ( $62.5 \pm 1.8$  vs  $62.9 \pm 1.9$  kg,  $P > 0.10$ ). At day 140 of gestation, measures of fetal growth and placental development, adjusted for litter size, were (December- vs March-mated): individual fetal weight ( $4.15 \pm 0.16$  vs  $5.07 \pm 0.16$  kg,  $P < 0.001$ ); total fetal weight per ewe ( $6.42 \pm 0.18$  vs  $7.14 \pm 0.22$  kg,  $P < 0.01$ ); caruncle number ( $114.5 \pm 4.1$  vs  $121.0 \pm 4.2$ ,  $P > 0.10$ ); placentome number ( $89.4 \pm 4.2$  vs  $106.9 \pm 4.3$ ,  $P < 0.01$ ); caruncle occupancy, ie number of placentomes/number of caruncles ( $0.79 \pm 0.03$  vs  $0.88 \pm 0.03$ ,  $P < 0.05$ ); and total placentome weight ( $564.7 \pm 34.0$  vs  $679.0 \pm 34.9$  g,  $P < 0.05$ ). These results suggest that seasonal differences in birth weight of lambs are mediated not by differences in maternal nutrition but rather by a direct seasonal effect on placental size (specifically the formation of placentomes and hence total placental weight).

**Keywords:** Season; birth weight; placental development; fetal growth; sheep.

### INTRODUCTION

There is good reason to believe that New Zealand sheep farmers will increasingly be required to provide overseas markets with lambs on a year-round basis. To achieve this, ewes will have to be mated out-of-season to lamb in the autumn or winter, in addition to the normal time in spring (Taylor, 1982). Recent studies have shown that lambs born in the autumn or winter have substantially lower birth weights (by up to 25-30%) than those born in spring (Reid *et al.*, 1988; Morris, 1992; Peterson, 1992). Given that lamb birth weight has an important effect both on the neonatal survival of lambs (Dalton *et al.*, 1980; McCutcheon *et al.*, 1981), and on their subsequent growth to weaning (Schinckel and Short, 1961), this is an important finding in the context of sheep production. However, it is not known whether the seasonal effect on birth weight is a consequence of differences in maternal nutrition, photoperiod, or other seasonally-related factors.

The objective of this study was to determine whether seasonal differences in fetal growth, and hence birth weight (as observed previously), are associated with differences in placental development, independent of differences in maternal nutrition.

### MATERIALS AND METHODS

#### Animals and treatment

The study involved 26 Romney ewes aged 5 and 6 years which were pregnant to December- (n=13) or March- (n=13) mating. All ewes had lambed in the previous spring and were randomly allocated to treatment groups after weaning in mid-November. Both groups of ewes were shorn one month prior to mating.

Mating in December (rams joined 21 December) was accomplished using a combination of progesterone-impregnated controlled internal drug releasers (Eazi-breed CIDR Type G, Carter Holt Harvey Plastic Products, Hamilton, New Zealand) and pregnant mare's serum gonadotrophin (PMSG Folligon, Intervet International B.V. Boxmeer, Holland), to induce ovulation outside the normal breeding season, and high ram to ewe ratios (1:10). The CIDRs were inserted for 14 days. PMSG (400 iu/ewe) was injected intramuscularly the day before CIDR removal. March mating of ewes (rams joined 22 March) was as above, but PMSG was not used. Four 1988-born, entire Border Leicester x Poll Dorset rams were introduced at CIDR withdrawal, the same four rams being used in December and March.

Rams were harnessed and crayon marks were recorded on a daily basis (between 1600 and 1800 h). Single or multiple pregnancy was confirmed at day 58 of pregnancy (and again at day 70 to verify previous results) using real-time ultrasound scanning (Carter, 1987). All ewes in the trial were pregnant to matings in the first 72 hours after CIDR removal.

Ewes in each group were managed as two separate mobs on pasture, mainly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), to achieve the same patterns of liveweight change from mating to slaughter. Ewes were weighed on electronic scales (Tru-Test Distributors, Auckland) straight off pasture (between 0900 and 1100 h) on a two-weekly basis to monitor changes in live weight. Electric fences were used from late December until early February to maintain ewes to be mated in March at the mean mating weight of 55.6 kg recorded in December-mated ewes. Both groups were flushed four weeks prior to mating on the best quality pasture available.

## Slaughter procedures

Ewes were slaughtered immediately off pasture, commencing at 0830 h on day 140 of gestation, by stunning with a captive bolt pistol and exsanguination. The abdominal cavity was opened and the uterus removed. A ligature was tied at the junction of the cervix and uterus, and the cervix, vagina and associated tissue removed. The allantoic and amniotic fluids were removed separately through an incision made along the greater curvature of the pregnant horn and weight of each fluid compartment recorded. The fetuses were removed from the uterus and the umbilical cord ligated at the abdomen before being cut. Fetal number, weight and sex were recorded. Placentomes were dissected from the uterus, separated into their maternal (caruncle) and fetal (cotyledon) components, counted and their individual weights recorded. The myoendometrium was then weighed.

The liver, spleen, heart, kidneys, lungs and thyroid gland of the ewes were removed, blotted dry and their fresh weights recorded (combined weights of bilateral organs). Carcasses were weighed.

Fetal crown-rump length was measured using a length of string and a tape measure (Mellor and Matheson, 1979). Fetal liver, spleen, heart, kidneys, lungs, brain, thyroid and thymus glands were removed, blotted dry and weighed (combined weights of bilateral organs).

## Statistical methods

Analysis of covariance was used to test effects of season on weights of maternal organs and uterine components (adjusted to a common maternal live weight and pregnancy rank (single vs multiple)) and on fetal weight and fetal organ weights (adjusted for fetal weight, rank and sex). Data are expressed as least square means and standard errors for the two groups of ewes and their fetuses. Statistical analyses were conducted using the computer package 'REG' (Gilmour, 1990).

## RESULTS

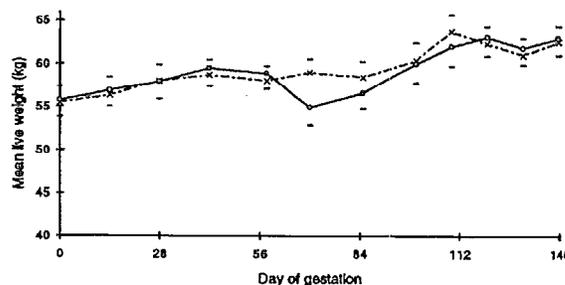
Figure 1 shows the changes in ewe live weight from day 0 (mating) through to day 140 of gestation (slaughter). Ewes mated in December or March had a similar live weight at mating ( $P>0.10$ ) and at slaughter ( $P>0.10$ ). The maximum difference in live weight between the two groups (4.07 kg or 6.9%) occurred at day 70 of gestation.

The thirteen ewes pregnant to the December mating carried nine singles, two sets of twins and two sets of triplets, while the 13 ewes pregnant to the March mating carried eight singles and five sets of twins.

Live weights, carcass weights and organ weights of the December- and March-mated ewes are presented in Table 1. None of these parameters was significantly affected by season.

Total fetal weight was higher ( $P<0.01$ ) in the March-mated ewes (Table 2), although there were no significant differences between seasons in myoendometrium or fetal fluid weights. March-mated ewes had a significantly ( $P<0.01$ ) greater number of placentomes formed and, since there was no seasonal difference in mean weight of individual placentomes, a greater total placentome weight ( $P<0.05$ ). The numbers of caruncles in December- and March-mated

**FIGURE 1:** Live weights (mean±SE) of December-mated (---x---) and March-mated (---o---) ewes from mating (day 0) to slaughter (day 140 of gestation).



**TABLE 1:** Final liveweight, carcass weight and organ weights of pregnant ewes, mated in December or March, at day 140 of gestation (mean ± SE).

	December-mated	March-mated
n	13	13
Final live weight (kg)	62.5±1.8	62.9±1.9
Carcass weight (kg)	23.3±0.7	23.5±0.7
<b>Organ weight (g)</b>		
Liver	887.5±24.4	856.1±24.6
Lungs	508.5±11.5	502.0±12.0
Heart	255.9±6.4	254.0±4.6
Spleen	82.0±8.1	96.5±8.4
Kidneys	137.4±3.4	147.5±3.6
Thyroid gland	5.6±0.5	5.7±0.5

ewes were similar but caruncle occupancy (ie. number of placentomes/number of caruncles) was greater ( $P<0.05$ ) in the March-mated ewes (Table 2).

**TABLE 2:** Uterine components of pregnant ewes, mated in December or March, at day 140 of gestation (mean ± SE).

	December-mated	March-mated
n	13	13
<b>Weight (g)</b>		
Total fetal weight	6417.3±183.8	7139.3±215.1**
Amniotic fluid	1490.3±95.6	1319.7±98.2
Allantoic fluid	1051.9±84.6	885.0±86.9
Myoendometrium	632.3±21.8	672.2±22.4
Placentome(individual)	6.31±0.41	6.28±0.43
Placentomes (total)	564.7±34.0	679.0±34.9*
No. of caruncles	114.5±4.1	121.0±4.2
No. of placentomes	89.4±4.2	106.9±4.3**
Caruncle occupancy <sup>a</sup>	0.79±0.03	0.88±0.03*

<sup>a</sup> Number of placentomes/number of caruncles

\*\*  $P<0.01$

\*  $P<0.05$

Table 3 shows that fetuses from the March-mated ewes had higher ( $P<0.001$ ) mean body weights ( $5.07 \pm 0.16$  kg) than fetuses from the December-mated ewes ( $4.15 \pm 0.16$  kg). Thy-mus weights were significantly ( $P<0.001$ ) higher in fetuses from December-mated ewes but thyroid weights were higher ( $P<0.05$ ) in fetuses from March-mated ewes. Crown-rump length and weights of other fetal organs were not significantly different between lambs conceived in the two seasons.

**TABLE 3:** Crown-rump length, body weight and organ weights of fetuses from December- and March-mated ewes at day 140 of gestation (least square means  $\pm$  SEM).

	December-mated	March-mated
n	19	18
Crown-rump length (cm)	56.0 $\pm$ 0.4	56.8 $\pm$ 0.4
<b>Weight (g)</b>		
Body	4154.5 $\pm$ 156.6	5074.3 $\pm$ 161.3***
Brain	47.33 $\pm$ 2.01	51.33 $\pm$ 2.07
Liver	92.07 $\pm$ 2.62	97.37 $\pm$ 2.70
Lungs	117.90 $\pm$ 3.14	122.96 $\pm$ 3.24
Heart	31.84 $\pm$ 0.69	32.13 $\pm$ 0.71
Spleen	5.27 $\pm$ 0.18	5.15 $\pm$ 0.17
Kidneys	22.36 $\pm$ 0.71	22.73 $\pm$ 0.73
Thyroid gland	1.85 $\pm$ 0.18	2.56 $\pm$ 0.18*
Thymus gland	19.05 $\pm$ 0.92	13.87 $\pm$ 0.95***

\*\*\* P&lt;0.001

\* P&lt;0.05

## DISCUSSION

The objective of this study was to determine whether seasonal differences in fetal growth and hence in birth weight (as observed previously) are associated with differences in placental development. The effect of season on fetal size at a mean day 140 (range 139-141) of gestation (and by inference on birth weight) is consistent with the findings of Reid *et al.* (1988), Morris (1992), and Peterson (1992). In sheep, the plane of maternal nutrition and the size of the placenta are well recognised as major determinants of fetal growth rate (Mellor and Matheson, 1979). The seasonal difference in fetal weight found in this study is most unlikely to have been a consequence of differences in maternal nutrition between the December- and March-mated ewes. Both groups of ewes followed the same pattern of liveweight change as pregnancy progressed, except at day 70 of gestation when a difference in live weight of 4.07 kg (6.9%) occurred between the two groups. However, much larger differences (10-12 kg) have been generated in 50 kg grazing ewes up to day 100 of gestation without any significant carry-over effects on lamb birth weight, provided that the level of feeding is adequate in late pregnancy (Parr *et al.*, 1986; Rattray *et al.*, 1987). Our ewes were not different in final live weight, carcass weight or organ weights, suggesting that nutritional differences between the two seasons were minimal.

The reduced total and average fetal weight (adjusted for litter size) in December-mated compared with March-mated ewes was associated with, and presumably caused by, a reduced number of placentomes and total placental weight. This was in turn due to a lower occupancy of maternal caruncles by fetal cotyledons, despite the total number of caruncles being similar in December- and March-mated ewes. Experimental reduction in placental size (eg. by carunclectomy or placental ablation) has been shown to reduce fetal weight and dimensions with birth weight tending to decline as the number of caruncles removed increases (Alexander, 1964b; Mellor *et al.*, 1977; Owens *et al.*, 1987). As the placenta is the key organ controlling the supply of nutrients to the growing fetus it would appear that any reduction in placental size could restrict the passage of nutrients to the fetus, and hence cause a reduction in birth weight.

The number of placentomes associated with each fetus is supposedly fixed at implantation, but the total weight of placentomes increases until about 90 days of gestation after which there is little change (Alexander, 1964a). This would suggest that the seasonal effect on placentome number observed here arose at the time of implantation in each group of ewes. However, there is increasing evidence that endocrine changes in mid to late pregnancy can influence placentome number. Recent investigations (Min *et al.*, 1994) have shown that treatment of pregnant ewes with bovine growth hormone for a period of only 10 days beginning at day 100 of gestation significantly increases the number of placentomes. This supports the observation of Stelwagen *et al.* (1991) that the treatment of ewes with growth hormone for a period of 28 days increases the placentome number. Thus the possibility exists that the seasonal difference in placentome number/weight, and hence in fetal growth, observed in the present study, occurred as a consequence of differences in the endocrine milieu experienced by the ewes in later gestation rather than at implantation.

It is possible that the seasonal effect on fetal and placental development could have resulted from differences in the time between previous lambing or weaning and mating for this trial. However, this is unlikely given that Morris *et al.* (1993) found similar seasonal differences in lamb birth weight when ewes were managed in different seasonal policies over three years and hence had similar lambing/weaning to mating intervals.

Further studies are required to determine the stage of pregnancy at which this difference in placental size occurs and whether these differences in placental development can be explained in terms of seasonal differences in circulating hormone concentrations in the dam.

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