Seasonal effects on fetal growth in sheep

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ABSTRACT

Previous studies have shown that autumn-born lambs are lighter (by 0.4 to 1.0 kg) at birth than spring-born lambs, an effect which is associated with impaired placental development in ewes mated to lamb in the autumn. As previous studies have focused on near-term fetal weights and birth weights, the objective of this study was to determine the pattern of growth throughout gestation in lambs destined to be born in the autumn or spring, and to examine associated changes in placental development. Mature N.Z. Romney ewes were randomly assigned to groups mated in December (n=23) or March (n=28) and managed on pasture in both seasons for similar live-weight profiles from mating to day 140 of gestation. Ewes were slaughtered at day 56, 84, 112 or 140 of gestation, and measures of fetal and placental weight were determined. Fetal weights (kg) of December- versus March-mated ewes were: d56 (0.040 ± 0.002 vs 0.038 ± 0.002); d84 (0.432 ± 0.020, vs 0.490 ± 0.017); d 112 (2.009 ± 0.101 vs 2.097 ± 0.083); d140 (4.772 ± 0.288 vs 5.616 ± 0 .262). Both the season and season by stage of gestation effects were significant ($P<0.05$), the interaction reflecting the gradual divergence in weight between the two seasonal groups as gestation proceeded. December-mated ewes had lower cotyledon weights (g) (221.47 ± 22.26 vs 333.27 ± 20.59, $P<0.001$) and placentome (385.31 ± 23.12 vs 472.63 ± 21.32, $P<0.05$), but not caruncle (163.81 ± 14.32 vs 139.32 ± 13.21, $P>0.10$) weights, than March-mated ewes (pooled across gestational ages). There were no significant differences in caruncle or cotyledon numbers. These results suggest that the effect of season on fetal development is established early in pregnancy (by day 84 of gestation). This seasonal effect on fetal development was associated with impaired placental development in the December-mated ewes, which is consistent with previous results.

Keywords: fetal sheep; birth weight; placenta; seasonality.

INTRODUCTION

Earlier reports have indicated that autumn-born lambs have substantially (by up to 25%) reduced growth *in utero* and birth weights compared with spring-born lambs (Reid *et al.,* 1988; Peterson, 1992; Morris *et al.,* 1993; Jenkinson *et al.,* 1995). This difference occurs in the absence of marked differences in maternal live-weight change and is associated with impaired placental development in ewes destined to lamb in the autumn (Jenkinson *et al.,* 1995).

Because studies conducted to date have examined only seasonal effects on birth weight or on the weight of fetuses at day 140 of gestation, it is not known at what stage of gestation the difference in weight arises. This experiment was therefore designed to address that question. Specifically, it sought to test the hypothesis that there is a gradual divergence in weight between fetuses destined to be born in the autumn versus spring and to examine further the seasonal differences in placental development first identified by Jenkinson *et al.* (1995).

MATERIALS AND METHODS

Animals and treatments

Sixty-two N.Z. Romney ewes aged 5 to 6 years were used in a 2 x 5 factorial design incorporating two seasons of conception (conceived to lamb in autumn or spring) and five stages at slaughter, days 0 (controls), 56, 84, 112, and 140 of gestation, with 4-6 ewes per cell. Ninety-six ewes in the autumn-lambing group and 66 ewes in the spring-lambing group were mated after being induced to cycle synchronously. Autumn-lambing (December-mated) ewes were synchronised using progesterone-impregnated CIDRs (Eazi-breed CIDR type G, Carter Holt Harvey Plastic Products, Hamilton, New Zealand) plus 400 i.u. of PMSG (PMSG Folligon, Intervet International B.V. Boxmeer, Holland), whereas CIDRs alone were used in the spring-lambing (March-mated) group. Six ewes for the control groups (slaughtered on day 0 of gestation) in each season were randomly chosen following CIDR removal. The same six Suffolk rams were introduced at CIDR removal in December and March, and mating marks produced by the crayon-harnessed rams were recorded daily. Pregnancy status (single versus twin) was determined by ultrasound at day 53 after mating (and again at day 81 in the autumn-lambing group to confirm previous results). This resulted in 23 and 28 single-bearing ewes in the autumn-lambing and spring-lambing groups respectively. Ewes were assigned at random to slaughter dates.

Ewes within each seasonal mating group were grazed on pasture as one mob and monitored (via weekly weighing) so that the two seasonal groups had similar patterns of live-weight change throughout gestation. Controlled grazing was used to minimise seasonal difference in maternal nutrition (as measured by live weight) which could lead to changes in fetal growth.

Slaughter procedure

Ewes were slaughtered by captive bolt pistol and exsanguination. Slaughter was conducted between 0800 h

and 1300 h, and the data on each ewe (excluding caruncle and cotyledon weights) collected within 45 minutes of slaughter. The uterus was removed from the ewe and ligated at the utero-cervical junction, and the cervix, vagina, ovaries and excess tissue removed before the gravid uterus was weighed. The outer curvature of the gravid uterus was carefully opened to expose the intact amniotic and allantoic fluid sacs. The weights of amniotic and allantoic fluids were recorded separately. The fetus(es) were gently squeezed by hand to remove amniotic fluid from the wool. The umbilical cord was ligated near the point of attachment to the fetus and approximately 5 cm distal to that point, and the umbilical cord severed between the two ligatures. The fetus was then removed from the uterus and fetal weight, sex, curved crown-rump length (with the fetus lying in a "relaxed" position) and chest girth measurements were recorded. Fetus(es) still alive at this stage (n=19) were euthanased using an overdose of sodium pentobarbitone (Pentobarb 500, Chemstock Animal Health, Christchurch, New Zealand). Uteri were stored in sealed plastic bags until dissection on the afternoon following slaughter, except for the uteri from the December-mated ewes which were slaughtered on day 0, 56 and 112 days of gestation. These uteri were immediately placed into sealed plastic bags following slaughter and frozen for no more than 3 months before dissection. The number and weights of individual caruncles dissected from the non-pregnant uteri, and cotyledons and caruncles dissected from the pregnant uteri, were recorded. The weights of the fetal membranes and the myoendometrium were also recorded. Following dressing out of the ewes, the maternal carcass weight was recorded.

Statistical analyses

All data were analysed using an analysis of variance for a 2 x 5 factorial to determine effects of season, stage of gestation and their interaction. Data are expressed as means and standard errors for ewes and their fetuses in both groups. Statistical analyses were conducted using the computer package 'Minitab'.

RESULTS

The autumn-lambing (n=17) and spring-lambing (n=22) ewes used in this trial each carried a single fetus. In addition to these animals, 6 ewes in each season were slaughtered at day 0 (controls).

Table 1 shows the live weight and carcass weight profiles for autumn- and spring-lambing ewes from day 0 (mating) through to day 140 of gestation. There were no significant effects of season on live weight but spring-lambing ewes had significantly (P< 0.05) heavier carcasses than autumn-lambing ewes.

TABLE 1. Live weight and carcass weight of Autumn (Aut)- vs Spring (Spr)-lambing ewes (mean±S.E.) at 0, 56, 84, 112 and 140 days of gestation, and the significance of Season, Stage of Gestation (Stage) and the Season x Stage interaction.

Season		Stage of gestation (days)					Significance		
		0	56	84	112	140	Season	Stage	Season x Stage
Live weight (kg)	Aut	58.5 ± 2.1	57.7 ± 2.3	57.3 ± 2.3	57.7 ± 1.5	62.0 ± 2.2	NS	*	NS
	Spr	55.4 ± 2.1	57.9 ± 2.1	58.5 ± 2.0	61.6 ± 1.2	64.7 ± 2.0			
Carcass weight(kg)	Aut	26.1 ± 1.5	26.1 ± 1.6	23.8 ± 1.4	24.0 ± 1.9	21.5 ± 1.9	*	NS	NS
	Spr	27.2 ± 1.5	26.8 ± 1.5	27.6 ± 1.2	27.6 ± 1.6	27.4 ± 1.7			

NS, P> 0.10;*, P 0.05.

TABLE 2. Total gravid uterus weight (TGUW), and weights of placental components of Autumn (Aut)- vs Spring (Spr)-lambing ewes (mean ± S.E.), at days 56, 84, 112 and 140 of gestation and the significance of Season, Stage of Gestation (Stage) and the Season x Stage interaction

Season		Stage of gestation (days)				Significance		
		56	84	112	140	Season	Stage	Season x Stage
TGUW (g)	Aut	623.7 ± 62.2	2083.0 ± 151.6	3610.0 ± 277.2	7593.2 ± 533.9	**	***	**
	Spr	683.8 ± 62.2	2347.0 ± 135.6	4260.0 ± 226.3	9504.7 ± 487.4			
Amniotic fluid wt(g)	Aut	145.2 ± 8.1	538.5 ± 61.1	521.5 ± 156.2	632.8 ± 137.7	**	***	*
	Spr	147.8 ± 7.3	581.8 ± 49.9	735.2 ± 127.6	1250.3 ± 125.7			
Allantoic fluid wt (g)	Aut	31.9 ± 13.9	75.3 ± 14.4	196.8 ± 36.7	908.4 ± 199.3	NS	***	NS
	Spr	41.6 ± 12.5	77.8 ± 11.7	234.0 ± 29.9	1033.2 ± 182.0			
Myoendometrium wt (g)	Aut	123.2 ± 9.5	233.3 ± 21.45	337.1 ± 25.1	584.3 ± 41.3	**	***	NS
	Spr	146.1 ± 8.5	269.1 ± 17.5	398.9 ± 20.5	668.9 ± 37.7			
Fetal membranes wt (g)	Aut	42.1 ± 9.4	97.5 ± 22.3	142.2 ± 12.1	199.8 ± 13.8	***	***	NS
	Spr	68.3 ± 8.4	119.5 ± 18.2	176.0 ± 9.8	274.5 ± 12.6			

NS, P> 0.10;*, P< 0.05;**, P< 0.01; ***, P< 0.001

TABLE 3: Caruncle, cotyledon and placentome weights and numbers, of Autumn (Aut)- vs Spring (Spr)-lambing ewes (mean ± S.E.), at days 56, 84, 112 and 140 of gestation, and the significance of Season, Stage of Gestation (Stage) and the Season x Stage interaction.

	Season	Stage of gestation (day)				Significance		
		56	84	112	140	Season	Stage	Season x Stage
Placentome wt (g)	Aut	166.1 ± 19.8	561.9 ± 72.6	378.6 ± 34.9	366.7 ± 40.7	**	**	NS
	Spr	171.4 ± 17.7	691.4 ± 64.9	501.4 ± 28.5	512.2 ± 37.2			
Cotyledon wt(g)	Aut	70.3 ± 14.0	348.6 ± 80.2	150.7 ± 20.7	253.4 ± 31.3	***	***	NS
	Spr	96.0 ± 12.5	463.0 ± 71.8	356.2 ± 16.9	401.4 ± 28.6			
Caruncle wt (g)	Aut	95.8 ± 10.9	213.4 ± 57.8	227.9 ± 17.6	113.3 ± 20.0	NS	**	NS
	Spr	75.4 ± 9.8	228.4 ± 51.7	145.2 ± 14.3	110.8 ± 18.3			
Cotyledon no.	Aut	83.8 ± 7.4	97.0 ± 8.0	87.2 ± 8.9	97.4 ± 9.9	NS	NS	NS
	Spr	87.6 ± 6.6	95.5 ± 6.5	87.0 ± 7.3	87.8 ± 9.1			
Caruncle no.	Aut	126.2 ± 6.4	132.0 ± 11.3	87.0 ± 23.5	136.6 ± 12.4	NS	NS	NS
	Spr	129.6 ± 5.8	130.0 ± 9.2	118.8 ± 19.2	115.3 ± 11.3			

NS, P> 0.10;**, P< 0.01;***, P< 0.001

TABLE 4: Fetal weight, crown-rump length (CRL) and girth measurements of fetuses from Autumn (Aut)- vs Spring (Spr)- lambing ewes (mean ± S.E.), at days 56, 84, 112 and 140 of gestation, and the significance of Season, Stage of Gestation (Stage) and the Season x Stage interaction

	Season	Stage of gestation (day)				Significance		
		56	84	112	140	Season	Stage	Season x Stage
Fetal weight(g)	Aut	39.7 ± 2.3	431.6 ± 19.1	2009.0 ± 101.3	4772.0 ± 288.0	*	***	*
	Spr	38.4 ± 2.1	490.8 ± 17.1	2097.0 ± 82.7	5616.0 ± 262.9			
CRL (mm)	Aut	129.5 ± 1.9	282.5 ± 6.2	436.2 ± 5.8	605.0 ± 15.4	NS	***	NS
	Spr	126.3 ± 1.8	287.0 ± 5.5	449.2 ± 7.8	585.0 ± 14.1			
Girth (mm)	Aut	76.3 ± 1.9	166.3 ± 3.3	266.3 ± 5.3	362.0 ± 8.4	**	***	*
	Spr	71.4 ± 1.7	168.0 ± 2.9	282.5 ± 4.4	389.2 ± 7.6			

NS, P> 0.10; *, P< 0.05; **, P< 0.01; ***, P< 0.001.

Spring-lambing ewes had a greater total gravid uterus weight (TGUW) than autumn-lambing ewes (P<0.01, Table 2). This difference in TGUW between seasons was associated with a difference in amniotic fluid (P<0.01), myoendometrium (P<0.01) and fetal membrane (P< 0.001) weights but there was no effect of season on allantoic fluid weight. Spring-lambing ewes had significantly heavier placentomes (P<0.01, Table 3), due to a significant difference in cotyledon weight (P<0.001) rather than an effect of season on caruncle weight. Despite differences in placentome weight, there was no effect of season on cotyledon or caruncle numbers (Table 3).

Table 4 shows the weights of fetuses from autumn- and spring-lambing ewes, and their crown-rump length (CRL) and girth measurements. There was a significant overall effect of season on fetal weight (P<0.05) and a significant season by stage of gestation interaction (P< 0.001), reflecting the gradual divergence in weight (in favour of fetuses from spring-lambing ewes) as gestation advanced. There was no effect of season on CRL, although fetuses of spring-lambing ewes had significantly larger girth measurements (P< 0.01) than those of autumn-lambing ewes.

DISCUSSION

Studies conducted to date have examined seasonal effects only on fetal weight at day 140 of gestation (Jenkinson *et al.,* 1995) or on birth weight (Reid *et al.,* 1988; Morris *et al.*, 1993; Peterson, 1992). However, it is not known when during gestation the seasonal effect on fetal weight begins. The objective of this study therefore, was to determine the pattern of fetal growth throughout pregnancy in fetuses from the two seasonal groups and to examine further the difference in placental development first identified by Jenkinson *et al.* (1995).

The magnitude of the seasonal difference in fetal weight at day 140 of gestation (and by inference in birth weight) is consistent with that observed in previous studies. Our results suggest that this seasonal effect is associated with a gradual divergence in fetal weight from about day 84 of gestation through to day 140 of gestation in favour of fetuses destined to be born in the spring. However, the bulk of the fetal weight difference arises in the last month of gestation. This effect on fetal weight appears not to be due to maternal live-weight differences, as the live-weight profiles for both the spring- and autumn-lambing ewes were similar throughout gestation, with the greatest difference (3.9 kg) being observed at day 112 of gestation. However, there was a significant overall effect

of season on carcass weight. This difference in carcass weight (up to 5.9 kg) is unlikely to be the main cause of the fetal weight difference as live-weight differences of up to 10-12 kg have been observed to day 100 of pregnancy with no obvious adverse effect on birth weight (Parr *et al.*, 1986; Rattray *et al.*, 1987).

The reduced weights of fetuses from December-mated as compared to March-mated ewes were associated with impaired placental development in the December-mated ewes, which is consistent with the findings of Jenkinson *et al.*, (1995). However, this impairment of placental development, as measured by a reduction in total placentome weight in December-mated ewes, was not associated with a reduction in placentome number as observed by Jenkinson *et al.*, (1995). The difference in placentome weight was due to a difference in cotyledon weight rather than a seasonal effect on caruncle weight. Thus, cotyledon weight was depressed in the group exhibiting low fetal weights, which is consistent with the observation of Alexander (1964) that birth weight is more closely correlated to cotyledon weight than to cotyledon number. Since the placentome is the site of nutrient transfer between the dam and the fetus, a reduction in the amount of placentome tissue may restrict nutrient supply to the fetus and hence, could retard fetal growth. Thus, it is likely that the early restriction of placentome weight in ewes due to lamb in the autumn is associated with their retarded fetal growth, although the fact that the reduced placentome weight is mainly a reflection of reduced cotyledonary growth suggests that the seasonal effect is mediated via the fetus rather than via the dam.

In conclusion, these results show that there is a gradual divergence in the weight of fetuses destined to be born in the autumn versus the spring. The weights of the fetuses from autumn-lambing ewes were substantially reduced as compared to fetuses from spring-lambing ewes, an effect which was established early in pregnancy (by day 84 of gestation). This effect of season on fetal growth was associated with reduced cotyledon weights in the autumn-lambing ewes. These result suggest that further studies on the cause of this seasonal effect on fetal weight should target stages earlier than 84 days of gestation as the seasonal effect on fetal and placental growth appears to be established by this stage of gestation.

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