Effects of maternal bromocriptine and melatonin treatments during early gestation on fetal lamb growth

SW Peterson, S Wigati and CMC Jenkinson

School of Agriculture and Environment, Massey University, Private Bag 11 222, Palmerston North, New Zealand.

Corresponding author: Email: s.peterson@massey.ac.nz

Abstract

Lower birth weights of autumn-born than of spring-born lambs may be due to a seasonal impairment of placental development, established by d84 of gestation, and perhaps mediated by high summer prolactin concentrations. This study attempted to determine whether reducing circulating summer prolactin concentrations in ewes during early gestation, using bromocriptine and melatonin, would improve placental and fetal growth and birth weights of autumn-born lambs. Pregnant mixed-age Romney ewes were randomly allocated to three treatment groups, balanced for age, live weight (LW) and source flocks, on d15 after mating. Ewes in the Bromocriptine group (n=20, LW 58.3±6.7 kg) were injected with 50 mg Parlodel®LA, the Melatonin group (n=20, LW 58.6±6.6 kg) each received one 18-mg Regulin® implant, while the Control group (n=21, LW 58.5±5.8 kg) was injected with saline solution (0.9% NaCl). At d139 of gestation, ewes were randomly allocated to a 'Slaughter group' sacrificed at d140-2 (n=30 LW 61.2±5.6 kg) and a 'Live-birth group' (n=31, LW 60.0±7.1 kg). Maternal plasma prolactin concentrations were significantly reduced by both bromocriptine (Control vs. Bromocriptine): 48.3±9.2 vs. 0.7±9.9 ng/ml ($P<0.001$) at d20 of gestation, and 82.6±8.5 vs. 4.5±9.1 ng/ml ($P<0.001$) at d40 of gestation, and melatonin treatments (Control vs. Melatonin): 82.6±8.5 vs. 18.4±9.5 ng/ml ($P<0.001$) at d40 of gestation, and 16.8±3.7 vs. 2.9±4.2 ng/ml ($P<0.05$) at d60 of gestation. Fetal plasma prolactin concentrations were not affected by treatments (Control vs. Bromocriptine vs. Melatonin): 32.4±18.3 vs. 32.4±19.1 vs. 59.8±16.4 ng/ml. At d140 of gestation, there were no treatment effects (adjusted for sex and litter size) on fetal weight, crown-rump length, placental parameters, and maternal and fetal organ weights, except thymus reduced 20% ($P<0.05$) by melatonin treatment. Birth weights and subsequent growth rates were similar among groups. Despite treatments reducing plasma prolactin concentrations there was no affect on placental or fetal growth, indicating that high prolactin concentrations early in pregnancy are not responsible for low birth weight in ewes lambing in May.

Keywords: bromocriptine; melatonin; prolactin; lamb birth weight; placental development; fetal growth; autumn-lambing ewes

Introduction

Autumn lambs are up to 30% lighter at birth than those born in spring (Reid et al. 1988; Peterson et al. 1990; Morris et al. 1993), and at d140 of gestation (Jenkinson et al. 1994; 1995; McCoard et al. 1996). Low birth weight reduces lamb survival (Dalton et al. 1980; McCutcheon et al. 1981) and growth rate (Schinckel & Short 1961), so ability to control fetal growth may improve birth weight and reduce lamb mortality.

Jenkinson et al. (1994; 1995) proposed that low birth weights of autumn lambs were due to a direct seasonal effect on placental development that reduced fetal growth, rather than to effects of maternal nutrition. Autumn-lambing ewes had the same number of caruncles, fewer placentomes and lower total placental weight, than did spring-lambing ewes (Jenkinson et al. 1994; 1995). McCoard et al. (1996) reported that seasonal difference in placentome weight was due to cotyledonary rather than caruncular weight and that the effect is established by d84. Jenkinson et al. (1995) speculated that the differences may be mediated by seasonal differences in prolactin concentrations.

Plasma prolactin concentrations are high in autumn-lambing ewes during early to mid-gestation (December-February) (Jenkinson et al. 1994; 1995; Munro 1980; Peterson et al. 1990) primarily due to photoperiod (Bassett 1992; Clarke et al. 1993; Munro 1980; Pearson et al. 1996). Although there is no evidence that prolactin regulates seasonal placental and/or fetal development, there is a negative relationship between birth weights of autumn-born lambs and plasma prolactin concentrations during early to mid-gestation (Jenkinson et al. 1994). Hence, changes in seasonal prolactin concentrations may regulate conceptus growth, particularly during the early gestation period. Alexander (1964a,b) reported that the number of placentomes associated with each fetus is fixed at placentation and birth weight is closely correlated with placental size. Thus, if seasonal prolactin concentration regulates placental development, reducing prolactin concentrations early in pregnancy may improve placental and fetal growth.

Melatonin regulates seasonal reproduction in the ewe (Yellon & Longo 1987) and prolactin responds to changes in photoperiod mediated by melatonin. Increasing plasma melatonin concentrations to mimic those in winter will reduce circulating prolactin concentrations; prolactin can also be reduced to minimal concentrations using bromocriptine (Peterson et al. 1990) a dopaminergic agonist at D2 and serotonin receptors.

Our objective was to determine whether reducing prolactin concentrations in autumn-lambing ewes during early pregnancy using bromocriptine or melatonin would improve placental and fetal growth and increase birth and weaning weights of lambs.

Materials and methods

Animals and treatments

All procedures were approved by the Massey University Animal Ethics Committee. This study involved Romney ewes aged 3-6 years that had lambed in the spring, were shorn in November, weaned in early December, and were pregnant to December mating. CIDRs (EAZI-breed CIDR Type G, Carter Holt Harvey Plastic Products, Hamilton) were inserted intravaginally for 10 days. PMSG (Folligon, Intervet, Chemavet Division Pharmaco (NZ) Ltd, Auckland) (400 IU/ewe) was injected intramuscularly on the day before CIDR removal. Border Leicester rams, at a ratio of 1:10 ewes (Knight et al. 1989), were introduced 24 h after CIDR removal.

Rams were harnessed with mating crayons (Radford et al. 1960) and marks on ewes were recorded daily. The day after the rams were joined was the mean mating date (18 December).

At d15 after mating, ewes were randomly allocated to three treatment groups balanced for age and pre-mating LW: 'Bromocriptine group' (LW 58.3±6.7 kg), 'Melatonin group' (LW 58.6±6.6 kg), and 'Control group' (LW 58.5±5.8 kg).

Bromocriptine-group ewes were injected intramuscularly with Parlodel LA® (Sandoz Pharma Ltd, Basle, Switzerland), a long-acting inhibitor of prolactin secretion, containing 50 mg bromocriptine mesylate in 2 ml vehicle (Dextran 70 in 0.9% NaCl). Each ewe in the Melatonin group was implanted subcutaneously at the base of the ear with Regulin® LA (Regulin Limited, South Melbourne, Vic., Australia), an implant containing 18 mg melatonin designed to release melatonin at a constant rate for 30-40 days (Handbook, Regulin Limited). Control-group ewes were injected in the neck muscle with 2 ml physiological saline solution (0.9% NaCl).

To avoid affecting the maternal recognition of pregnancy (13 days after conception) (Smith 1982; Flint 1995), treatments commenced at d15 of gestation (about 18 days after ovulation or about 13 days after the embryo enters the uterus)(Wimsatt 1975).

Pregnancy was diagnosed by ultrasound at d80 and ewes grazed together until d139 of gestation when 61 ewes were randomly allocated to two subgroups: a 'Slaughter group' (n = 30) and a 'Live-birth group' (n = 31) balanced for treatment group, LW, age and litter size. The 'Slaughter group' was divided into three groups (balanced for treatment and litter size) slaughtered on d140-142 of gestation, whereas the 'Live-birth group' was monitored for changes in LW until lambs were weaned (at eight weeks of age).

Ewes were weighed at twenty-day intervals; lambs from the 'Live-birth group' were weighed at birth and thereafter on the same day as their dams. Blood samples were collected by jugular venepuncture between 1000-1600 h at intervals of 20 days.

Slaughter and sampling procedures

Ten ewes were slaughtered on each of d140, d141 and d142 by stunning with a captive bolt pistol and exsanguination. The gravid uterus was removed and weighed. Fetuses were removed, their umbilical cord ligated and cut before blood sample by cardiac puncture. Fetal number, weight and sex were recorded. Allantoic and amniotic fluids were removed without being weighed, and placentomes dissected from the uterus, separated into maternal (caruncular) and fetal (chorionic) components, counted, and individual weights recorded. The myoendometrium was weighed. Fetal crown-rump lengths (CRL) and girths were measured (Mellor & Matheson 1979). Major organs of the dams and fetuses were weighed.

Assays

The prolactin assay was a double-antibody competitive-binding radioimmunoassay based upon the method of van Landeghem and van de Weil (1978). Details have been described previously (Peterson et al. 1994). Assay binding was typically 50-65% and mean assay sensitivity 0.4 ng/ml. Serially diluted ovine plasma samples exhibited parallelism with the standard curve. Plasma samples were assayed in triplicate. The mean intra-assay coefficient of variation (CV) calculated over six assays was 8.3% and the mean inter-assay CV was 12.8% for five reference plasma samples the concentrations of which were on the linear portion of the standard curve.

Statistical analyses

Analysis of variance for a 3x2x2 factorial design was used to compare the effects of the treatments, litter size and sex on maternal and fetal parameters. All data were analysed using ProcGLM. Uterine components, fetal weights and CRLs, fetal plasma prolactin concentrations and birth weights were analysed using analysis of covariance or variance. Significant differences among treatments were tested using the LSM-test. Sequential weights of lambs and ewes, and maternal plasma prolactin concentrations were analysed using multivariate (repeated-measures) analysis of covariance or variance to determine treatment effects (adjusted to a common litter size and sex for lamb weights). Data are expressed as least-square means and standard errors of the mean (LSM±SEM).

Results

Mean LW of ewes treated with bromocriptine and melatonin did not differ from those of control ewes (Table 1). Bromocriptine treatment significantly lowered prolactin concentrations from day 15-50 ($P<0.001$), whereas melatonin treatment reduced prolactin concentrations from day 30-60 of gestation (Figure 1). The maximum difference in prolactin concentrations between control and treatment groups occurred at day 40 of gestation ($P<0.001$).

There were no significant differences in the mean weights of the uterus and placental components among groups (Table 2) nor weights of organs of dams or fetuses, except that the thymus was lighter ($P<0.05$) in fetuses from

Figure 1 Plasma prolactin concentrations in synchronised mixed-age Romney ewes treated with bromocriptine (---■---) (n=20) melatonin (– –◆– –) (n=20) and saline solution (control) (solid line) (n=21), of both the 'slaughter' (slaughtered on days 140-142) and the 'live birth' groups from three days before mating to d140 of gestation, and until 60 days postpartum in the 'live-birth' ewes (n=11, 10 and 10 for control, bromocriptine and melatonin groups, respectively). Vertical bars represent the standard error of the mean. Day 0 (mating) was 18 December.

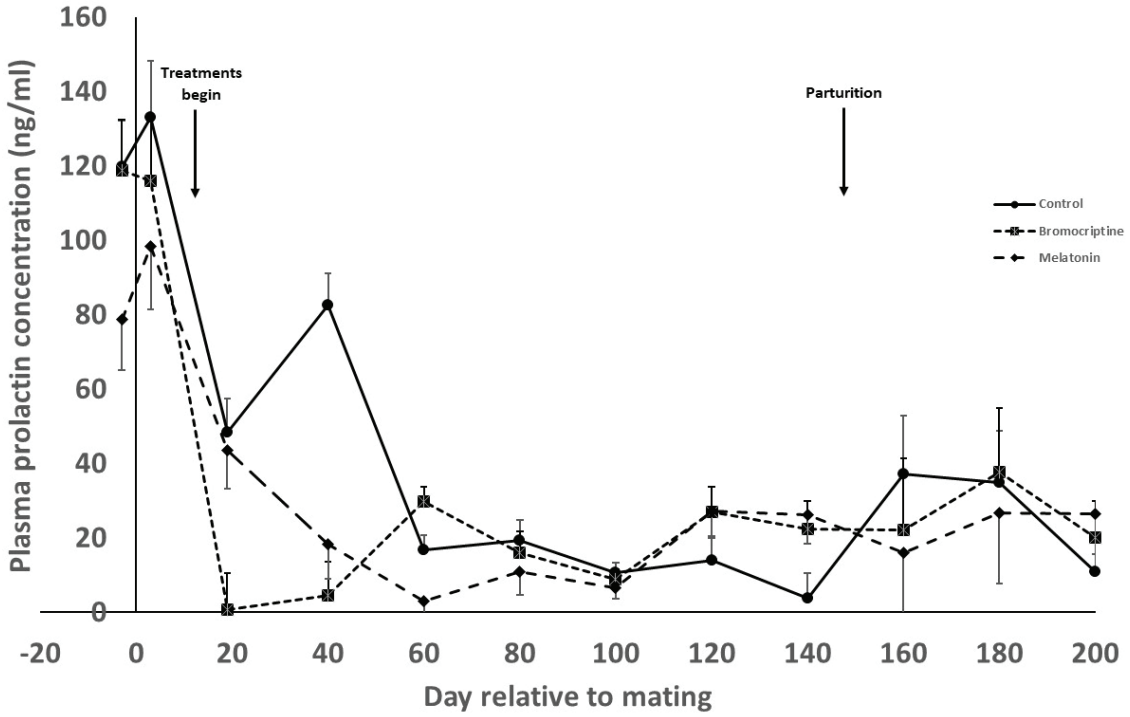

Table l Live weights (LSM±SEM) of Romney ewes treated with bromocriptine, melatonin or saline solution (control) on d15 of gestation. Day 0 (mating) was 18 December. There were no significant differences due to treatment.

Day of gestation	Bromocriptine (n=20)	Melatonin (n=20)	Control (n=21)
-3	60.00 ± 1.32	56.39 ± 1.39	59.18 ± 1.26
12	59.18 ± 1.35	56.66 ± 1.38	58.48 ± 1.28
19	56.31 ± 0.56	55.97 ± 0.56	55.86 ± 0.51
40	55.84 ± 0.59	55.81 ± 0.59	55.81 ± 0.59
60	55.18 ± 0.76	54.86 ± 0.76	55.68 ± 0.69
80	55.81 ± 0.65	55.59 ± 0.65	56.26 ± 0.60
100	58.83 ± 1.12	56.34 ± 1.13	59.26 ± 1.03
120	60.41 ± 0.81 (n=10)	60.56 ± 0.81 (n=10)	61.44 ± 0.74 (n=11)
140	62.68 ± 0.83	63.37 ± 0.84	64.00 ± 0.76
160	58.19 ± 2.21	60.18 ± 2.17	56.55 ± 1.74
180	61.28 ± 2.24	62.14 ± 2.20	61.48 ± 1.76
200	55.90 ± 2.05	59.16 ± 2.02	56.36 ± 1.61

melatonin-treated ewes (data not shown).

Fetuses from bromocriptine- and melatonin-treated ewes had similar circulating concentrations of prolactin to those of fetuses from control ewes (32.4±19.1 *vs*. 59.8±16.4 *vs*. 32.4±18.3 ng/ml) and had a similar CRL, girth and body weight to those of control ewes (Table 3).

There was no significant difference in birth weights of lambs (4.3±0.2 *vs*. 4.4±0.2 *vs*. 4.2±0.2 kg for control, bromocriptine and melatonin groups respectively) and no treatment-by-sex interactions. Lambs from all groups grew at similar rates until weaning (data not shown).

Discussion

Bromocriptine and melatonin significantly reduced maternal plasma prolactin concentrations for about 30 days, but bromocriptine reduced maternal plasma prolactin concentrations to almost zero by d20, and concentrations remained very low until d50, whereas melatonin reduced maternal plasma prolactin concentrations more gradually, reaching minimal levels at d40-60 of gestation. Although the beginning of the treatments coincided with the natural decline in plasma prolactin in December, treatments did eliminate the January peak.

Data from control ewes indicate that the profile of prolactin concentration was consistent with the very few published New Zealand studies (Craven et al. 1994; Pearson et al. 1996), i.e., plasma prolactin concentrations rise during spring until early to mid-December, decline during late December, then increase reaching a summer peak in late January, then decline to spring values. This summer profile of prolactin secretion differs from the winter profile (Peterson et al. 1990; Bassett, 1992) when plasma prolactin concentrations in pregnant ewes are low during winter and increase during spring. Hence, there is an inverse profile of maternal plasma prolactin concentrations

Table 2 Intrauterine parameters (LSM±SEM) at day 140 of gestation of ewes treated with bromocriptine, melatonin, or saline solution on d15 of gestation. There were no significant differences due to treatment.

Treatment group	Bromocriptine	Melatonin	Control
n	10	10	10
Weight (kg) of:			
Gravid uterus	10.8±0.4	10.9±0.4	11.4±0.4
Individual fetus	5.0±0.2	4.9±0.2	5.1±0.2
Weight (g) of:			
Myoendometrium	674.8±21.9	672.0±22.1	704.6±21.9
Fetal membranes	276.7±11.6	277.6±11.7	275.0±11.6
Occupied caruncle (individual)	2.5±0.3	2.0±0.3	2.6±0.3
Cotyledon (chorionic) (individual)	3.1±0.4	3.0±0.4	3.3±0.4
Placentome (individual)	5.8±0.5	4.6±0.5	5.9±0.5
Total placentome	473.6±25	434.0±27.2	447.7±28.9
Number of:			
Placentomes	92.0±7.1	99.2±7.1	92.4±7.1
Unoccupied caruncles	19.1±5.3	23.9±5.3	27.6±5.3
Total caruncles	114.1±7.7	123.1±7.7	121.0±7.7
Caruncle occupancy (%)	80.4±4.1	81.3±4.1	76.9±4.1

Table 3 Crown-rump length (CRL), girth, and body weights at d140 of gestation (LSM±SEM) of sheep fetuses from ewes treated with bromocriptine, melatonin or saline solution (control) on d15 of gestation. There were no significant differences due to treatment.

Treatment group	Bromocriptine	Melatonin	Control
n	11	15	13
CRL (cm)	57.8±0.8	57.4±0.7	59.0±0.7
Girth (cm)	37.3±0.6	36.2±0.5	36.8±0.5
Body weight (kg)	5.0±0.2	4.9±0.2	5.1±0.2

in summer and winter periods of gestation, but at about d140 of gestation, both spring- and autumn-lambing ewes experience the same photoperiod (Jenkinson et al. 1994) though one is increasing whilst the other is decreasing.

Neither treatment affected fetal plasma prolactin concentrations. Since prolactin does not cross the ovine placenta (Alexander et al. 1973) neither treatment altered fetal prolactin secretion. However, since melatonin does cross the ovine placenta (Zemdegs et al. 1988) the fetus still presumably maintained its seasonal clock signal in the bromocriptine-treated ewes. In retrospect, we should have also measured daytime melatonin in the ewes and fetuses.

There were no differences in the mean LW of groups during the trial (December-July). Individual fetal weights, fetal CRL, girths and birth weights were not affected by either treatment.

Neither treatment affected weights of placental components. Furthermore, there was no direct effect of melatonin on placental or fetal growth. We know of no published data showing results of melatonin administration in early pregnancy, although Eifert et al. (2014) reported that maternal melatonin supplementation during mid to late gestation increased umbilical blood flow by 20%. Furthermore, Sales et al. (2019) reported that melatonin implants (both 18 and 36 mg) increased weight of twin male fetuses (but not singletons or female fetuses) at d140 of gestation. We did not find such differences, but our numbers were too small and their treatment regimen commenced at d100 whilst our treatment commenced at d15. Nevertheless, in agreement with our results, their comparable treatment (18 mg) did not alter the placental parameters that we measured. However, they found 36-mg of melatonin increased the proportion of type-C placentomes (we did not type our placentomes) and increased the partial pressure of oxygen in cord blood, concluding that melatonin increased fetal growth by improving oxygen supply to the fetus. Although these are interesting findings, they do not directly relate to our hypothesis since their treatments occurred in the last third rather than the first third of pregnancy.

Hormones other than prolactin and melatonin may be involved in the seasonal differences in fetal growth. Stelwagen et al. (1991) reported that treatment of pregnant ewes with rbGH increased placentome number but Jenkinson et al. (1999) concluded that bGH applied to pregnant ewes can stimulate fetal growth only after d100 of gestation, probably reflecting changes in maternal nutrient partitioning or placental function, rather than placental size.

Although reduced maternal plasma prolactin concentrations caused by bromocriptine and melatonin in this study did not affect placental and fetal growth or lamb birth weight, it is not possible to conclude that seasonal prolactin change does not have any role in fetal growth regulation. Perhaps the period of reduced plasma prolactin concentration produced by treatments was inadequate to mimic the low winter plasma prolactin concentrations of spring-lambing ewes. It is also possible that since the treatments commenced when the prolactin concentrations were naturally declining, that the reduced prolactin concentrations, might not have affected parameters in the treated ewes. Further studies may determine whether suitable timing and greater length of treatments can mimic the period of low winter plasma prolactin concentrations. In the New Zealand situation, it seems that the treatments need to be carried out earlier to remove the natural first peak of prolactin secretion, which means that the ewes need to be mated earlier than mid December. However, the results of Sales et al. (2019) suggest that seasonal differences in birth weight may be due to direct effects of melatonin rather than of prolactin.

Acknowledgments

Biological and iodination grade oPRL were donated by National Institute of Diabetes and Digestive and Kidney Diseases supplied through the National Hormone and Pituitary Program, University of Maryland School of Medicine. The Massey University Agricultural Research Foundation and The Riverside Research Fund provided financial support.

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