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## The effect of peripartum administration of ovine prolactin on lactogenesis in autumn-lambing ewes

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### ABSTRACT

Two routes of oPRL supplementation were used to elevate peripheral or local concentrations of PRL in autumn-lambing ewes which, based on previous results, were expected to have low plasma PRL concentrations and milk yields relative to spring-lambing ewes. Administration of 10 mg oPRL directly into the gland or subcutaneous injection of 0.5 mg/kg oPRL did not increase the milk yields, or change the composition of milk, compared to controls. These results suggest that the circulating and intramammary concentrations of PRL in autumn-lambing ewes are not limiting lactogenesis. However, because the plasma prolactin concentration in the ewes was unexpectedly high, it was not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations. Nevertheless, the results indicate that raising the intramammary concentration of PRL around the time of parturition, in ewes with circulating PRL concentrations characteristic of normal spring-lambing ewes, does not enhance lactogenesis.

**Keywords:** Ovine prolactin; intramammary; lactation; autumn-lambing ewes.

### INTRODUCTION

Although prolactin (PRL) is known from *in vitro* studies to act directly on the mammary gland tissue to stimulate the synthesis of all the major milk solids (Kelly *et al.*, 1984), no studies have established whether PRL is active when introduced directly into the mammary gland of ruminants, and attempts to increase milk yields by peripartum oPRL supplementation of ewes (either with artificially reduced, or with normal, circulating PRL concentrations) have not been reported.

PRL has been administered to ruminants by continuous intravenous (i.v.) infusion (Hooley *et al.*, 1978; Akers *et al.*, 1981) and by daily injections (Plaut *et al.*, 1987). However, if oPRL were effective when administered directly into the mammary gland of the ewe then problems associated with systemic administration could be avoided. Direct administration of oPRL into the mammary gland of pseudopregnant rabbits (via the teat duct) resulted in the secretion of milk and increased activity of lipoprotein lipase in the mammary gland (Falconer & Fiddler, 1970) suggesting that oPRL acts directly on the mammary gland to initiate lactogenesis (at least in the rabbit).

If intramammary injection of oPRL were effective in increasing the milk yield of sheep, this route would have several major advantages. First, the amount of hormone required might be much lower than that needed for administration via other routes, since there would be no need to raise circulating concentrations of PRL. Second, the secretions within the gland might act as a reservoir for PRL, presenting it to the epithelial cells in high concentrations over an extended period (a natural slow release mechanism). Third, if the dose was correct, oPRL release into the circulation might be so slow that plasma concentrations would not rise significantly. Thus any response obtained could be attributed directly to an action of PRL within the gland, not requiring the

presence of a hypothetical intermediate hormone or systemic actions of PRL.

Reduced milk yields and lamb growth rates are associated with lower circulating PRL concentrations in autumn-lambing ewes versus spring-lambing ewes (Peterson *et al.*, 1990). Although these seasonal differences were confounded with corresponding differences in live weight (LWT) the results are in agreement with reports (Bocquier *et al.*, 1986; Perier *et al.*, 1986) indicating that longer photoperiod increases milk yields of ewes. If the peripartum plasma PRL concentration is responsible for determining the potential milk production of the gland, then administration of supplementary oPRL into the glands of autumn-lambing ewes (with naturally low plasma PRL concentrations) would be expected to result in increased milk yields.

Therefore the objective of this study was to determine whether supplementary oPRL, administered to autumn-lambing ewes during the peripartum period by subcutaneous or intraductal injection, affects subsequent milk yield and composition.

### MATERIALS AND METHODS

#### Animals and Treatments

Two trials were carried out to determine the effects of intramammary and subcutaneous oPRL administration in autumn-lambing ewes. Trial 1 involved intramammary injection of oPRL while trial 2 compared the milk production of ewes treated with subcutaneous injections of oPRL with that of untreated ewes.

For both trials, multiparous ewes (aged from 4-7 years) synchronised with progesterone-impregnated CIDRs and induced to ovulate using PMSG, were mated during November to lamb in April. Ewes were grazed at pasture (mainly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)) through-

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out both trials. Following ultrasound pregnancy diagnosis, ewes were selected on the basis of mating date and number of foetuses, and allocated to the 2 trials at random, except that groups were balanced as much as possible for age, live weight and pregnancy rank.

Trial 1 involved 8 ewes (7 single-bearing, 1 twin-bearing) each of which received 10 mg of oPRL (NIADDK-oPRL-18, AFP 8277E, 30 i.u./mg protein) injected into one gland (4 ewes injected in the left gland and 4 in the right gland). The oPRL (10 mg/ml) was dissolved in 0.15M saline and 0.03M sodium bicarbonate at pH 10.8 and adjusted to pH 9.0 before injection. The contralateral gland was treated with 1 ml of the excipient (BIC) as a control to the oPRL treatment.

In trial 2, 9 ewes (6 single-bearing, 3 twin-bearing) received subcutaneous oPRL treatment (PRLsc group), while 8 ewes (6 single, 2 twin) were in the control group. The PRLsc group received subcutaneous injections (in the shoulder region) of 30 mg oPRL dissolved in 3 ml of BIC. Control group ewes received no injections.

All of the ewes from both trials were run together as one flock and, apart from the treatment differences described above, were subjected to the same management and sampling regimen. Because of the inability to accurately predict the lambing date of ewes, the oPRL was administered on two consecutive days immediately prior to the expected mean date of parturition in an attempt to elevate intramammary or plasma PRL concentrations at a time close to parturition. Injections were administered at about 1700 h. To minimise infection, teats were cleaned before treatment with a solution of Hibitane (Chlorhexidine gluconate 5% w/v, ICI PLC, Macclesfield, Cheshire, England) in water and then with 70% ethanol. The needle used for intramammary injections was immersed in 70% ethanol. All ewes were given 5 ml Streptopen s.c. on each day of oPRL treatment. In addition, on the first day of milking, each ewe was treated with 2.5 million i.u. of Leocillin (penethamate hydriodide, Leo Pharmaceutical Products, Ballerup, Denmark) which is actively taken up by the mammary gland (Edwards, 1966). Three ewes in which small traces of blood subsequently appeared in the milk were given 10 ml Streptopen s.c. and no further signs of mastitis were detected.

Following lambing, ewes were milked on days 1-6, 8, 10 and 12 of lactation. Milk samples were refrigerated at 4°C until analysed within 1-2 days. Ewes were returned to pasture between morning (ca 0900 h) and afternoon (ca 1500 h) milkings, while lambs were held indoors and bottle-fed.

On each of the 2 treatment days, blood samples were collected by jugular venipuncture from all ewes before oPRL treatment. Thereafter 2 further blood samples were taken at 5-d intervals. Samples (7 ml) were taken using evacuated tubes containing 100 µl of 7 g/l sodium EDTA as the anticoagulant and placed on ice immediately after collection. They were centrifuged at 1800g for 20 minutes at 4°C and plasma was stored at -20°C until analysed for oPRL. Milk samples were analysed for milk fat, protein and lactose content, and plasma samples were analysed for PRL.

### Assays

The prolactin assay was a homologous double-antibody competitive binding radioimmunoassay (RIA) based upon the method of van Landeghem and van de Wiel (1978). The protocol

utilised was derived from Kirkwood *et al.*, (1984). Iodination grade ovine PRL (NIADDK-oPRL-I-2, AFP-7150B, 35 i.u./mg) was iodinated according to the procedure of Greenwood *et al.*, (1963) using borate instead of phosphate buffers. Iodination time was 5 seconds. Separation of bound and free iodine was carried out using a Sephadex G50 gel column (Lot No. 0870, Pharmacia Fine Chemicals, Uppsala, Sweden). Standards were prepared using biological grade ovine PRL (NIADDK-oPRL-18, AFP-82277E, 30 i.u./mg). The concentration of standards ranged from 1-1200 µg/l oPRL. The linear range was approximately 10-800 µg/l. Optimal dilutions of antibodies were determined by factorial experiments covering a wide range of dilutions of both antibodies. The first antibody, rabbit anti-oPRL antiserum (rabbit 9, 7/5/76, donated by Dr D.F.M. van de Wiel, "Schoonoord" Research Institute for Animal Husbandry, Zeist, The Netherlands), was used at a working dilution of 1:50,000 (final dilution 1:550,000). The second antibody, goat anti-rabbit IgG (Lot No. 8103, Immuno-Chemical Products Limited, Auckland, New Zealand) was used at a working dilution of 1:40. Assay binding was typically 45-55% and assay sensitivity about 1 µg/l. Three ovine plasma samples, assayed neat or at stepwise serial dilutions up to final dilutions of 1:128, 1:256 and 1:512, exhibited parallelism with the standards. Plasma samples were assayed in triplicate. The mean intra-assay coefficient of variation (CV) calculated over 12 assays was 9.7% and the mean inter-assay CV was 12.6% for 3 reference plasma samples corresponding with the linear portion of the standard curve.

Milk samples were analysed for fat, protein and lactose content using a Milkoscan 104 A/B (A/S N. Foss Electric, Denmark). The instrument was calibrated according to the manufacturers recommendations for normal bovine milk using samples provided by the Dairy Research Institute, Palmerston North, New Zealand. Since the response of the machine is linear over a restricted range of protein and fat concentrations, it was necessary to dilute the ewes milk with an equal volume of water so that the concentration of fat and protein fell within the range of calibration. Since the ewes were suckled on the day of parturition and milk samples were not collected until the afternoon of the day after parturition, the first secretions analysed would have contained little colostrum. Thus, although the Milkoscan was not calibrated for ewes milk, the values obtained are considered satisfactory for comparison of groups within these trials.

### Statistical Analyses

Multivariate (repeated measures) analysis of variance was used to analyse all time-series data. PRL data were log transformed and all milk composition data were arc sine transformed for statistical analyses. In trial 1, since all ewes received the same treatment, there was no statistical analysis of PRL data and glands, rather than ewes, were classified as either PRL-treated or BIC-treated for milk yield and composition analyses. Milk yield and composition data from PRL- and BIC-treated glands were analysed with glands nested within ewes. In trial 2, ewes were grouped according to treatment for data analyses. PRLsc-group ewes were those ewes treated with s.c. oPRL injections and the Control group were untreated ewes. In both trials the test for a delay in the onset of lactogenesis was the interaction of treatment-group contrasts with time. Data were analysed using the computer statistical package REG (Gilmour, 1990).

## RESULTS

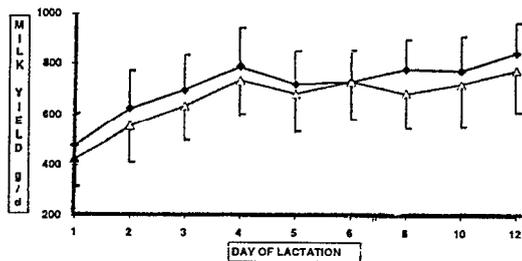
### Trial 1

All ewes lambed within a 4-d period, 16 of them lambing on one or other of the 2 days when oPRL treatments were administered. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures are the mean  $\pm$  (S.E.M.) 0.2 d of the actual sampling days. The mean daily air temperature, on days when blood samples were collected, was 15.2°C.

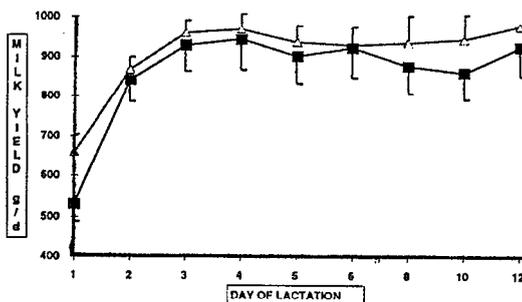
Plasma prolactin concentrations (mean  $\pm$  S.E.M.) in the autumn-lambing ewes treated via the teat duct on 2 days peripartum with 10 mg oPRL in one gland and with 1 ml bicarbonate in the contralateral gland were 324  $\pm$  110  $\mu$ g/l on the day of parturition (day 0), 376  $\pm$  127  $\mu$ g/l (day 1), 182  $\pm$  71  $\mu$ g/l (day 5), 72  $\pm$  32  $\mu$ g/l (day 10).

Milk yields did not differ significantly between the PRL- and BIC-treated glands (Figure 1). Milk fat, protein and lactose percentages likewise did not differ between the PRL- and BIC-treated glands (data not shown).

**FIGURE 1:** Milk yields (Mean  $\pm$  S.E.M. g/d) of individual glands of autumn-lambing ewes (trial 1) treated via the teat duct on 2 days peripartum with 10 mg oPRL in one gland (PRL group, n=8,  $\Delta$ — $\Delta$ ) and with 1 ml bicarbonate (BIC group, n=8,  $\blacklozenge$ — $\blacklozenge$ ) in the contralateral gland.



**FIGURE 2:** Milk yields (Mean  $\pm$  S.E.M. g/d) of individual\* glands of autumn-lambing ewes (trial 2) treated on 2 days peripartum with 30 mg oPRL by subcutaneous injection (PRLsc group, n=18,  $\Delta$ — $\Delta$ ) and of untreated (Control group, n=16,  $\blacksquare$ — $\blacksquare$ ) ewes



\*Milk yields are for individual glands to permit comparison with Figure 1

### Trial 2

Plasma prolactin concentrations in 17 autumn-lambing ewes treated on 2 days peripartum with 30 mg oPRL via subcutaneous injection (PRLsc) did not differ significantly from those in untreated (Control) ewes on the days on which they were sampled. Plasma prolactin concentrations

(mean  $\pm$  S.E.M.) averaged across both groups were 332  $\pm$  89  $\mu$ g/l on the day of parturition (day 0), 231  $\pm$  48  $\mu$ g/l (day 1), 102  $\pm$  28  $\mu$ g/l (day 5), 57  $\pm$  18  $\mu$ g/l (day 10).

Milk yields in PRLsc-group ewes did not differ from those in Control-group ewes (Figure 2). Milk fat, protein and lactose percentages did not differ between the 2 treatment groups (PRLsc or Control) (data not shown).

Milk yields in PRLsc-group ewes (trial 2) were significantly higher ( $P < 0.05$ ) than those in the PRL-treated (trial 1) glands (compare Figures 1 & 2).

## DISCUSSION

These studies were carried out to determine if supplementary oPRL administered to normal (specifically not bromocriptine-treated) ewes could increase milk yields. Since low circulating PRL concentrations in autumn-lambing ewes are associated with low milk yields (compared to those of spring-lambing ewes) (Peterson *et al.*, 1990) it was logical to test the effect of supplementary oPRL in autumn-lambing ewes. Thus, in trial 1, the comparison was between intramammary treatment with oPRL in one gland and with excipient in the contralateral gland of each ewe while, in trial 2, responses in ewes supplemented systemically with oPRL were compared with those in untreated (Control) ewes.

Administration of supplementary oPRL directly into the gland during trial 1 did not increase the milk yields, or change the fat, protein or lactose composition of milk, compared to glands treated with BIC. Neither did subcutaneous injection of oPRL (trial 2) increase milk yields, or alter the fat, protein and lactose percentages of milk compared to Control group ewes. These results suggest that the circulating concentration of PRL, and the intramammary concentration of PRL, in autumn-lambing ewes are not limiting lactogenesis. However, the circulating concentrations of PRL recorded in these trials were much higher than those recorded in previous autumn-lambing trials (Peterson *et al.*, 1990; Peterson, 1992) in which the ewes were housed under artificial lighting and fed different diets compared to the ewes grazed outdoors in the present trials. Thus, it is not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations.

The difference between plasma PRL concentrations in trials 1 and 2 and those recorded in previous autumn trials (Peterson *et al.*, 1990; Peterson, 1992) may be because the sampling times in trials 1 and 2 corresponded much more closely with the mean date of parturition than they did in the previous autumn trials, or because the values were determined in different assays. However, pooled samples present in all assays indicate that differences between assays probably cannot explain the difference between the previous autumn trials and those reported here. Peripartum peak PRL concentrations in the autumn-lambing ewes (trials 1 and 2) were similar in magnitude to those previously recorded (Peterson, 1992) in corresponding samples from spring-lambing ewes, despite the finding (Peterson *et al.*, 1990) that PRL levels in samples collected from autumn-lambing ewes between 2 weeks prepartum and 1 week postpartum (but not coinciding with the mean date of parturition) were significantly lower than those in spring-lambing ewes. This raises

the question of whether circulating PRL concentrations are, in fact, consistently lower in autumn- than in spring-lambing ewes. Since circulating PRL concentrations in goats have recently been shown to respond to seasonal changes in ambient temperature independently of photoperiod (Forsyth, 1992), it is possible that high ambient temperatures during trials 1 and 2 prevented the expected decrease in plasma PRL levels. However, this is considered unlikely since the mean daily temperature during the previous autumn trial (Peterson, 1992), was only 0.8°C lower than that recorded during the trials described here. Further studies involving frequent peripartum blood sampling under conditions of controlled temperature will be required to establish conclusively the effects of ambient temperature on plasma PRL concentrations in sheep.

It is interesting to note that milk yields in PRLsc-group ewes (trial 2) were higher than those in the PRL- and BIC-treated glands (trial 1). Since both groups of ewes were managed as one group, it is possible that the intramammary injections had a detrimental effect on milk secretion. Further study is needed to examine this possibility.

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