The influence of season of lambing and lactation on reproductive activity and plasma LH concentrations in Merino ewes

B. J. Restall and B. G. Starr

*N.S.W. Department of Agriculture, P.O. Box 239, Blacktown,
New South Wales 2148, Australia*

Summary. Groups of Merino ewes which were lactating for 40 days (Group I) or had had their lambs removed at birth (Group II) after lambing in the winter (June) or spring (November) were fed on a high plane of nutrition. Ovarian inspections were carried out at 15 and 30 days after lambing and plasma LH levels were measured at 6-h intervals for 20–30 days.

First ovulation was earlier in ewes lambing in the winter (16.6 days, range 11–26) than in the spring (24.7 days, range 15–30) but there was no difference in the number of ewes ovulating. LH levels were higher in winter-lambing ewes (2.79 ± 3.4 ng/ml) than in those lambing in the spring (1.78 ± 0.23 ng/ml). LH peaks were usually associated with an ovulation in spring lambing ewes but were not consistently so in the others.

More ewes ovulated in Group II (72%) than in Group I (40%) but the mean time of first ovulation was similar. In the winter-lambing ewes the mean daily LH concentration was 2.40 ± 0.32 ng/ml in Group I and 3.18 ± 0.31 ng/ml in Group II but there were no differences between the spring-lambing ewes (I, 1.75 ± 0.20 ng/ml; II, 1.80 ± 0.26 ng/ml). There were more elevations in LH levels in Group II ewes (64%) than in Group I ewes (43.8%).

After lambing the LH levels increased slowly, indicating a gradual recovery of pituitary function.

Introduction

While there is general agreement that season of lambing has a profound influence on the length of the post-partum anoestrus of the ewe (see review by Hunter, 1968), there is controversy concerning the effect of suckling and lactation. On the one hand several studies (Mauléon & Dauzier, 1965; Restall, 1971; Mallampati, Pope & Casida, 1971; Shevah, Black, Carr & Land, 1974) have shown that ewes without lambs resume reproductive activity sooner after lambing than do lactating ewes, while on the other, Fletcher (1973) and Hunter & Van Aarde (1973) could not demonstrate any effect of early weaning. The latter authors concluded that nutritional stress was responsible for the suppressive effect of lactation on reproductive activity. Suckling, as distinct from lactation, has been implicated in the reports of Mauléon & Dauzier (1965), Fletcher (1971) and Kann & Martinet (1975).

Irrespective of how these factors interact and the circumstances in which they do so, they are presumed to be mediated by the endocrine system, but knowledge of circulating hormone levels in the puerperium of sheep is incomplete. This study describes the reproductive activity and circulating gonadotrophin levels in lactating and non-lactating Merino ewes lambing at two times of the year.

Materials and Methods

Animals and management

Oestrus was synchronized in two groups of mature Merino ewes to facilitate mating and to obtain contracted lambings in the spring (November 1970) and the winter (June 1971). Before lambing the ewes were placed in pens inside an animal house and as lambing occurred ewes were

randomly assigned to one of two groups. In Group I, the lambs remained with the ewes, 21 of which lambed in the spring and 14 in the winter. Group II contained ewes whose lambs were weaned at birth; 19 lambed in the spring and 13 in the winter. A third group of 4 nonpregnant ewes was included in the spring study. The groups were kept separate in large pens and had free access to a mixture of hammermilled oats and lucerne hay supplemented with concentrates. A salt mixture containing iron, copper and cobalt, as suggested by Shutt & McDonald (1965), was added to the ration to prevent anaemia due to frequent blood sampling. Eight ewes from each group were randomly selected to provide plasma samples for hormone assay. These ewes were fitted with indwelling catheters in the jugular vein and blood samples were collected at 09.00, 15.00, 21.00 and 03.00 h from the spring-lambing ewes, and at 09.00, 15.00 and 21.00 h from the winter-lambing ewes.

Observations

Vasectomized rams fitted with Sire-Sine harnesses and raddles were run continually with the ewes to detect oestrus. Ovarian inspections of all ewes were performed via midventral laparotomy at 15 and 30 days after lambing, the number, appearance and estimated age (see Restall, 1964) of corpora lutea (CL) being recorded. The experiments were terminated 40 days after lambing.

It was intended that plasma samples be collected for the 30 days following lambing, but the period was less for some ewes because of problems with the indwelling catheters. Blood samples were collected into heparinized tubes, and the plasma stored at -15°C until measurement of the LH content by radioimmunoassay (Goding *et al.*, 1969) using a standard equivalent to NIH-LH-S17. The samples for one or two ewes (selected randomly) were assayed as a batch in random order. Internal standards were included and were used to correct the LH values for assay variations. Coefficients of variation were 6–10% within assays and 13% between assays, and the sensitivity was approximately 0.5 ng/ml.

Statistical analyses

To analyse the variance in the data concerning the daily basal LH concentration in the ewes, two manipulations were performed. Firstly, peak concentrations of LH in excess of 5 times the mean basal levels were removed and these data treated separately. Secondly, the daily LH concentration (a mean of up to 4 measurements) for each ewe was arbitrarily grouped into 5-day periods. A standard analysis of variance of these data was performed, with the component 'between days within periods' being treated as a main effect. The missing data were treated as suggested by Snedecor (1957) and appropriate error variances were used for testing of significance.

Table 1. The incidence of oestrus and ovulation within 30 days of parturition in lactating (Group I) or non-lactating (Group II) ewes in the spring or winter

Group	Spring-lambing ewes				Winter-lambing ewes			
	No.	No. showing oestrus	No. ovulating	Mean day of first ovulation (range)	No.	No. showing oestrus	No. ovulating	Mean day of first ovulation (range)
I	21	—	8	25.3 (21–29)	14	—	6	18.2 (11–24)
II	19	—	14	24.1 (15–20)	13	2 (days 1 and 2)	9	15.0 (7–26)

Results

Oestrus and ovulation

The incidence of oestrus and ovulation in the treatment groups is shown in Table 1. During the 40-day observation period oestrus was observed on the 1st and 2nd day after lambing in only 2 ewes, both non-lactating and in the winter group. The incidence of ovulation was variable; more ewes ovulated in Group II (72%) than Group I (40%) in both seasons although the differences were not significant ($\chi^2 = 6.96$, $0.05 < P < 0.1$). Ovulations tended to occur earlier in Group II ewes (both seasons) and in ewes lambing in the winter. Ovulation occurred over a longer period in winter (7–26 days) than in the spring (15–30 days) and most ovulations occurred between Days 15 and 25. Three of the 4 non-lambing ewes in the spring group exhibited regular oestrous cycles during the period of observation.

Concentration of LH in peripheral plasma

Some problems were experienced in maintaining patency of the indwelling jugular vein catheters which were replaced when necessary. For the spring study, blood samples were obtained for 30 days from 8 ewes in Group I, 6 ewes in Group II and 3 non-lambing ewes, and 2 of the ewes in Group II were sampled for 28 days. In the winter study, LH results were obtained from 8 Group-I and 6 Group-II ewes for 20–25 days.

In view of this variability the results are presented and analysed for 30 days after spring lambing and 20 days after winter lambing.

Basal LH concentrations. The mean daily basal LH concentrations for the spring and winter lambing ewes are shown in Tables 2 and 3 respectively, together with the analyses of variance.

Table 2. The mean $\pm$ S.E.M. daily basal* LH levels (ng/ml) in peripheral plasma of non-lambing, lactating (Group I), and non-lactating (Group II) ewes in the spring

Group	No. of ewes	Period after lambing (days)						Means
		1–5	6–10	11–15	16–20	21–25	26–30	
I	8	1.29 $\pm$ 0.13	1.49 $\pm$ 0.15	1.52 $\pm$ 0.16	1.86 $\pm$ 0.20	2.23 $\pm$ 0.25	2.10 $\pm$ 0.28	1.75
II	8	1.44 $\pm$ 0.20	1.53 $\pm$ 0.30	1.68 $\pm$ 0.21	2.22 $\pm$ 0.27	2.09 $\pm$ 0.32	1.69 $\pm$ 0.25	1.80
Non-lambing	4	2.11 $\pm$ 0.17	2.39 $\pm$ 0.13	2.36 $\pm$ 0.13	2.27 $\pm$ 0.18	2.57 $\pm$ 0.18	2.54 $\pm$ 0.18	2.38

* Peak LH discharges are omitted (see text).

Summary of the analysis of variance

Treatment of variation	d.f.	Mean square	Variance ratio
Between treatments (A)	2	18.2325	76.41** (error 3)
Between ewes within treatments (B)	17	17.8520	24.23** (error 2)
Between periods (C)	5	7.6022	10.32** (error 2)
Between days within periods (D)	24	0.1120	0.47 (error 3)
Interactions:			
A $\times$ C	10	1.1351	1.54 (error 2)
A $\times$ D (error 3)	48	0.2386	1.01 (error 1)
B $\times$ C (error 2)	85	0.7363	3.13** (error 1)
Remainder (error 1)	408	0.2351	—

** $P < 0.01$, using the appropriate errors as shown.

Table 3. The mean $\pm$ S.E.M. daily basal* LH levels (ng/ml) in peripheral plasma of lactating (Group I), and non-lactating (Group II) ewes in the winter

Group	No. of ewes	Period after lambing (days)					Means
		1-5	6-10	11-15	16-20	21-28	
I	8	1.85 $\pm$ 0.25	2.28 $\pm$ 0.27	2.55 $\pm$ 0.35	2.50 $\pm$ 0.29	3.05 $\pm$ 0.45	2.40
II	6	2.65 $\pm$ 0.27	2.99 $\pm$ 0.27	3.22 $\pm$ 0.35	3.49 $\pm$ 0.29	3.45 $\pm$ 0.35	3.18

* Peak LH discharges are omitted (see text).

Summary of the analysis of variance

Source of variation	d.f.	Mean square	Variance ratio
Between treatments (A)	1	45.6680	108.91** (error 3)
Between ewes within treatments (B)	12	15.0226	21.33** (error 2)
Between periods (C)	3	5.9777	8.48** (error 2)
Between days within periods (D)	16	0.5391	1.29 (error 3)
Interactions:			
A $\times$ C	3	0.5342	0.76 (error 2)
A $\times$ D (error 3)	16	0.4193	0.85 (error 1)
B $\times$ C (error 2)	36	0.7042	1.43 (error 1)
Remainder (error 1)	192	0.4918	—

** $P < 0.01$, using the appropriate errors as shown.

In the spring study there was considerable variation between ewes in basal LH concentration, varying from a mean of 0.57 to 3.52 ng/ml/day with no significant differences between treatment groups. The non-lambing ewes had significantly higher LH concentrations. As shown in Text-fig. 1(b), the LH levels in the non-lambing ewes were relatively constant, while those for ewes in Groups I and II were low immediately after lambing (1.37 ± 0.17 ng/ml) and rose significantly ($P < 0.01$) during the 30-day period (1.90 ± 0.27 ng/ml). The variation between days within periods was not statistically significant (Table 2).

In the winter-lambing ewes, there were significant variations of daily basal LH levels between ewes (1.35–4.67 ng/ml) and between groups (Table 3). LH concentrations increased gradually

Table 4. The incidence of peak discharges of LH in non-lambing, lactating (Group I) and non-lactating (Group II) ewes after lambing in the spring or winter

Season of lambing	Group	No. of ewes	No. of ewes with LH peaks (no. ovulating)	Days after lambing of LH peaks (ng/ml) for each ewe
Spring	I	8	3 (3)	26 (104); 27 (32); 28 (108)
	II	8	5 (5)	20 (36); 20 (60); 30 (54); 15 (23); 27 (61)
	Non-lambing	4	3 (3)	3 (46) and 25 (44); 8 (74) and 26 (69); 10 (75) and 28 (75)
Winter	I	8	4 (3)	11 (54); 19 (25)*; 19 (35); 20 (70)†
	II	6	4 (2)	10 (19)* and 25 (21)*; 11 (78)* and 21 (66); 11 (63); 13 (59)*

* Ovulation did not follow these LH peaks.

† The CL from this ovulation appeared abnormal.

Text-fig. 1. The mean daily basal LH levels in the plasma of non-lambing (Δ , 4 ewes), lactating (Group I, $\bullet$, 8 ewes) and non-lactating (Group II, $\circ$, 8 ewes) ewes in the (a) winter and (b) spring. Peak discharges of LH are omitted (see text).

throughout the 20 days after lambing (Text-fig. 1a) and there were significant differences between periods. The values for the Group II ewes particularly were higher than those for the spring-lambing ewes.

Transient elevations in LH concentrations. Short-term elevations of LH over the basal level were observed intermittently (Table 4). During the spring study, LH peaks were detected in 3 non-lambing ewes, in 5 of the 8 Group-II ewes and in 3 of the 8 Group-I ewes. In all cases these elevations in LH were associated with the subsequent ovulation. In the winter study, peak discharges of LH were detected in 4 of the 8 Group-I ewes and in 4 of the 6 Group-II ewes. Only 5 of the 10 peaks detected were associated with an ovulation, and one of these was presumably abnormal because the CL were small and pale, the follicle having apparently failed to luteinize. The LH peaks seemed to occur earlier in the puerperium in Group-II ewes, and in ewes lambing in the winter.

Discussion

The Merino ewes in this study generally show maximal reproductive activity in March–April and have an extended breeding season, as is evident from the observation that 3 of the 4 non-lambing

ewes showed regular cyclic activity during November. Despite this extended breeding season, there was a difference in the mean time of appearance of the first ovulation after lambing, but not in its incidence, with the winter-lambing ewes ovulating earlier (16.6 days) than the spring lambing ewes (24.7 days). This seasonal effect is in accord with the observations of other workers (reviewed by Hunter, 1968).

Oestrus generally does not occur until 5–8 weeks after lambing even during the breeding season (Hunter, 1968), and one or more ovulatory cycles usually precede the first normal oestrus (Mauléon & Dauzier, 1965). The almost complete absence of oestrous activity in the present 40-day study is therefore not surprising. However, evidence from highly fertile crossbred ewes indicates that oestrus can occur in a significant proportion of ewes in the first 3 weeks after lambing (Restall, 1971; Shevah *et al.*, 1974).

Removal of lambs at birth, a treatment that confounds the effects of suckling and lactation, resulted in more ewes in this Group (II) ovulating than those whose lambs were not weaned (Group I). Several workers have shown that early weaning advances the time of first ovulation and oestrus (Mauléon & Dauzier, 1965; Mallampatti *et al.*, 1971; Restall, 1971; Shevah *et al.*, 1974). In addition there is evidence that suckling *per se* delays the resumption of reproductive activity (Mauléon & Dauzier, 1965), although this study has been criticized (Hunter, 1968) on the grounds of a possible milk-yield difference, and hence of metabolic demand, between the suckling and lactation treatments. Fletcher (1971) reported a small but significant correlation between the frequency of suckling in the first 2 weeks after lambing and time of first oestrus. The most convincing evidence, however, for a suckling effect is shown by Kann & Martinet (1975), who found that removal of the suckling stimulus by denervation of the udder markedly advanced the time of first ovulation and oestrus, without affecting lamb growth.

Hunter & Van Aarde (1973) have suggested that lactation exercises its effect through nutritional stress, and they found no differences in the duration of post-partum anoestrus when ewes were fed according to their nutritional requirements. In contrast, Shevah *et al.* (1974) found such differences in spite of there being no evidence of nutritional stress. The two studies are difficult to compare because of the differences in breed and location; it would be surprising if the highly fertile Finn $\times$ Dorset ewes (Shevah's study) were not different from the mutton Merinos (Hunter's study) even under similar conditions. It is possible, however, that small differences in dietary quality, while not being evident in gross measures of nutritional stress, may be important in the high demand period of early lactation.

Jaume, cited by Chamley *et al.* (1973), showed that the pituitary LH and FSH content in lambing ewes increased 15-fold and 4-fold, respectively, from parturition to a few weeks later. Chamley *et al.* (1974) recorded low circulating levels of FSH and LH at parturition and much higher levels 3 weeks later. Similar observations have been reported by O'Reilly & Dziuk (1973). Pelletier & Thimonier (1973) presented evidence that LH synthesis and release are reduced during lactation, and others have suggested that the pituitary may be unresponsive to releasing hormone during lactation (Chamley *et al.*, 1974). Altogether this suggests that recovery of pituitary function is gradual.

The frequent sampling procedure employed in the present study has provided a detailed description of circulating LH levels. There was a gradual increase after parturition and a distinct seasonal difference in basal peripheral concentrations of LH (Text-fig. 1). In the ewes lambing in the winter, a time that may be considered to be a transitional or declining phase of annual reproductive activity, there were significant differences in circulating LH levels between the lactating and non-lactating ewes. This difference was not apparent in the spring-lambing ewes, in which circulating LH levels were lower. These endocrine changes are compatible with a gradual recovery of pituitary function after lambing, and in the absence of a seasonal suppression lactation and/or suckling appears to inhibit this recovery. However, the endocrine changes observed are at variance with those of Foster & Crighton (1973), who found no differences between circulating LH in lactating, non-lactating and cyclic ewes.

The observed variations in peak discharges of LH also provide support for the above conclusion; in the winter-lambing ewes major discharges of LH tended to occur earlier than in the spring-lambing ewes, although those in the former were not all associated with ovulation. The significance of this

observation is not clear, but it does indicate another fundamental endocrine difference between ewes lambing in the two seasons.

Prolactin, LH and ovarian steroids are known to be involved in the control of luteal function in early pregnancy (see Kann & Denamur, 1974) and in lactation (see Hunter & Van Aarde, 1974; Restall, 1971), and the endocrine observations of the present study need to be extended to include other gonadotrophic and steroid hormones.

We thank Mr S. Collins, Mr R. Coyte and Mr N. Bradbury for care of the experimental animals. The study was supported by a grant from the Australian Wool Corporation.

References

- CHAMLEY, W.A., BROWN, J.M., CERINI, M.E., CUMMING, I.A., GODING, J.R., OBST, J.M., WILLIAMS, A. & WINFIELD, C. (1973) An explanation for the absence of post-parturient ovulation in the ewes. *J. Reprod. Fert.* **32**, 334–335, Abstr.
- CHAMLEY, W.A., FINDLAY, J.K., JONAS, H., CUMMING, I.A. & GODING, J.R. (1974) Effect of pregnancy on the FSH response to synthetic gonadotrophin-releasing hormone in ewes. *J. Reprod. Fert.* **37**, 109–112.
- CHAMLEY, W.A., FINDLAY, J.K., CUMMING, I.A., BUCKMASTER, J. & GODING, J.R. (1974) Effect of pregnancy on LH response to synthetic gonadotrophin-releasing hormone in the ewe. *Endocrinology* **94**, 291–293.
- FLETCHER, I.C. (1971) Relationships between frequency of suckling, lamb growth and post-partum oestrous behaviour in ewes. *Anim. Behav.* **19**, 108–111.
- FLETCHER, I.C. (1973) Effect of lactation, suckling and oxytocin on post-partum ovulation and oestrus in ewes. *J. Reprod. Fert.* **33**, 293–298.
- FOSTER, J.P. & CRIGHTON, D.B. (1973) Comparison of LH levels in post-partum anoestrous and cycling ewes and the effects of synthetic gonadotrophin-releasing factor. *J. Reprod. Fert.* **35**, 599–560, Abstr.
- GODING, J.R., KATT, K.J., BROWN, J.M., KALTENBACH, C.C., CUMMING, I.A. & MOLE, B.J. (1969) Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. *Endocrinology* **85**, 133–142.
- HUNTER, G.L. (1968) Increasing the frequency of pregnancy in sheep. 1. Some factors affecting re-breeding during the post-partum period. *Anim. Breed. Abstr.* **36**, 347–378.
- HUNTER, G.L. & VAN AARDE, I.M.R. (1973) Influence of season of lambing on post-partum intervals to ovulation and oestrus in lactating and dry ewes at different nutritional levels. *J. Reprod. Fert.* **32**, 1–8.
- KANN, G. & DENAMUR, R. (1974) Possible role of prolactin during the oestrous cycle and gestation in the ewe. *J. Reprod. Fert.* **39**, 473–483.
- KANN, G. & MARTINET, J. (1975) Prolactin levels and duration of post-partum anestrus in lactating ewes. *Nature, Lond.* **257**, 63–64.
- MALLAMPATI, R.S., POPE, A.L. & CASIDA, L.E. (1971) Effect of suckling on post partum anestrus in ewes lambing in different seasons of the year. *J. Anim. Sci.* **32**, 673–677.
- MAULÉON, P. & DAUZIER, L. (1965) Variations de durée de l'anoestrus de lactation chez les brebis de race Ile-de-France. *Annls Biol. anim. Biochim. Biophys.* **5**, 131.
- O'REILLY, P.L. & DZIUK, P.J. (1973) Change in level of LH in the sera and pituitaries of ovariectomized ewes at parturition. *Endocrinology* **92**, 1575–1579.
- PELLETIER, J. & THIMONIER, J. (1973) Comparison of the induced preovulatory LH discharge in lactating and dry sheep during seasonal anoestrus. *J. Reprod. Fert.* **33**, 310–313.
- RESTALL, B.J. (1964) The growth and retrogression of the corpus luteum in the ewe. *Aust. J. exp. Agric. Anim. Husb.* **4**, 274–277.
- RESTALL, B.J. (1971) The effect of lamb removal on reproductive activity in Dorset-Horn × Merino ewes after lambing. *J. Reprod. Fert.* **24**, 145–146, Abstr.
- SHEVAH, Y., BLACK, W.J.M., CARR, W.R. & LAND, R.B. (1974) The effect of lactation on the resumption of reproductive activity and the preovulatory release of LH in Finn × Dorset ewes. *J. Reprod. Fert.* **38**, 369–378.
- SHUTT, D.A. & McDONALD, I.W. (1965) Rate of haemoglobin synthesis after blood loss in sheep and the influence of dietary protein. *Aust. J. exp. Biol. med. Sci.* **43**, 475–488.
- SNEDECOR, G.W. (1957) *Statistical Methods applied to Experiments in Agriculture and Biology*, 5th edn. Iowa State College Press, Ames, Iowa.

Received 3 August 1976