THE EFFECT OF LACTATION ON THE RESUMPTION OF REPRODUCTIVE ACTIVITY AND THE PRE-OVULATORY RELEASE OF LH IN FINN×DORSET EWES

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Summary. The resumption of cyclic reproductive activity after lambing was studied in a total of fifty Finn × Dorset ewes which lactated for 1, 24 or 56 days. The ewes lambd in December 1971 and were maintained on a high plane of nutrition throughout pregnancy. Eight of the seventeen ewes which suckled for 1 day showed spontaneous oestrus between 2 and 20 days after lambing compared to only one of the thirty-three lactating ewes. Following treatment with 'progestagen' vaginal sponges for 12 days from 20 to 24 days *post partum*, all the ewes showed oestrus. The mean interval between sponge withdrawal and the onset of oestrus was 35.3 ± 1.5 and 33.2 ± 1.6 hr for ewes which suckled lambs for 1 and 24 days respectively, and 41.0 ± 1.5 hr for the lactating ewes ($P < 0.01$). Thirty-one of thirty-three ewes ovulated. No significant differences were found between the numbers of corpora lutea observed for the three treatments: 3.09 ± 0.3 , 2.4 ± 0.3 and 3.09 ± 0.3 , respectively. A pre-ovulatory LH release was detected in the peripheral plasma of thirty-four of the thirty-five ewes studied. The mean intervals from the onset of oestrus to the start of the LH discharge were 9.1 ± 1.2 and 6.1 ± 1.3 hr for the ewes which suckled lambs for 1 or 24 days and 12.6 ± 1.3 hr for the lactating ewes ($P < 0.01$). The magnitude and duration of the LH peak were not influenced by lactation.

INTRODUCTION

The influence of lactation on the resumption of reproductive activity in the ewe following lambing has been reported as inconsistent (Hunter, 1968; Fletcher, 1973). Recently Hunter & Van Aarde (1973) concluded that lactation had only an indirect effect on post-partum anoestrus, acting through nutritional stress. By contrast, Mauléon & Dauzier (1965) suggested that lactation *per se* has a direct effect during the breeding season, and Restall (1971) has reported a delay in the first post-partum ovulation in ewes which suckled lambs compared with those which were non-lactating.

The resumption of cyclic reproductive activity during different periods of lactation was therefore studied in (F₁) Finnish Landrace × Dorset Horn (Finn ×

Dorset) ewes. Land (1971) has reported that such ewes can show oestrus and may be fertile during lactation.

MATERIALS AND METHODS

Fifty Finn $\times$ Dorset (F_1) ewes from the Edinburgh School of Agriculture's accelerated lambing flock were allocated at random to three groups, two of seventeen and one of sixteen. One of the groups of seventeen ewes had their lambs weaned at 1 day of age, whereas the ewes in the other two groups suckled twin lambs for 24 and 56 days respectively. All ewes lambed during the 3rd week of December 1971, well within their normal breeding season and all were in good body condition.

The ewes were housed in three separate pens and were allowed free access to hay, consisting of 5% digestible crude protein (DCP) and 1.8 Mcal metabolic energy (ME)/kg and concentrate (10% DCP and 2.6 Mcal ME/kg). The lambs had access to creep feed. The nutritional status of the ewes was monitored once a week by weighing; bleeding was undertaken by acute puncture of the jugular vein using heparinized evacuated tubes. The blood samples were immediately centrifuged and the plasma was stored in a deep freeze (-15°C) for the analysis (Patterson, 1963) of non-esterified fatty acids (FFA).

Post-partum oestrus was detected by a daily introduction of vasectomized rams from 2 to 20 days following lambing. Synchronization of oestrus was then effected by the insertion of intravaginal sponges treated with 30 mg Cronolone (G. D. Searle) and 400 mg progesterone, as described by Gordon (1971) for 12 days from Day 20 to 24 after lambing.

The time of onset of oestrus and the discharge of LH were studied in twelve ewes of the 1- and 56-day lactation groups and eleven of the ewes which lactated for 24 days.

Twenty-two hours after the sponges were removed, vasectomized rams were introduced to the ewes for 15 min and then again at 2-hr intervals for further 15-min periods until oestrus was detected. Ewes in oestrus were removed to individual pens and blood samples were then taken from each ewe at 2-hr intervals from 2 to 30 hr after the onset of oestrus. The blood samples were treated as above before estimation of the concentration of LH. A horse anti-bovine LH antiserum was used in a solid-phase radioimmunoassay to measure immunoreactive plasma LH concentrations, using NIH-LH S17 (supplied by the National Institutes of Health, U.S.A.) as the standard reference preparation. Polystyrene tubes coated with antibody by the method of Catt & Tregear (1967) were used in the assay, which gave somewhat higher results than a double antibody assay on the same material, particularly in samples of low activity. The antiserum cross-reacted only very slightly with FSH but appreciably with TSH.

The number of corpora lutea in each ovary was counted at laparoscopy in a sample of fifteen ewes 2 weeks after parturition, and in all the thirty-five ewes on the 8th day of the synchronized oestrous cycle.

RESULTS

Energy intake and ewe performance

The nutritional status of the ewes was assessed by measuring the FFA in the

Table 1. Voluntary energy and protein feed intakes, body weight gain and non-esterified free fatty acids in the peripheral blood during the first 5 weeks after lambing of ewes completing 24 or 56 days of lactation

<i>Duration of lactation (days)</i>	<i>No. of ewes</i>	<i>Daily feed intake</i>		<i>Mean body wt after lambing (kg $\pm$ S.E.)</i>	<i>Mean body wt gain (kg/ewe $\pm$ S.E.) (range in parentheses)</i>	<i>Mean plasma FFA (μequiv./litre $\pm$ S.E.) (range in parentheses)</i>
		<i>Energy (Mcal ME/ewe)</i>	<i>Protein (g DCP/ewe)</i>			
1	17	5.2	200	60.2 $\pm$ 1.5	6.4 $\pm$ 1.3	181 $\pm$ 13
24	16	6.4	240	62.0 $\pm$ 2.3	3.2 $\pm$ 0.8	403 $\pm$ 27
56	17	6.9	260	62.7 $\pm$ 2.6	5.2 $\pm$ 0.9	446 $\pm$ 46

ME, metabolic energy; DCP, digestible crude protein; FFA, free fatty acids.

peripheral plasma and the body weight gain during the period of the experiment. The results represented in Table 1 indicate that the body weight gain was similar for ewes which lactated for either 1 day or 56 days. (The smaller increase in body weight of the 24-day lactating group could have arisen from feed restriction at the time of weaning.) All the ewes lactating for 56 days either

Table 2. Mean body weight gain and mean plasma FFA levels in the peripheral blood during the first 3 weeks after lambing for ewes which showed oestrus during this period compared with those which did not

Group	Sub-group	No. of ewes	Mean body wt gain (kg/ewe $\pm$ S.E.) (range in parentheses)	Mean plasma FFA (μ equiv./litre $\pm$ S.E.) (range in parentheses)
Dry ewes*	Ewes in oestrus	8	2.1 $\pm$ 1.2 (-1 to + 8)	234 $\pm$ 43 (105 to 510)
	Ewes not in oestrus	9	4.2 $\pm$ 1.1 (0 to + 11)	196 $\pm$ 23 (115 to 337)
	Total	17	3.2 $\pm$ 0.8 (-1 to + 11)	214 $\pm$ 23 (105 to 510)
Lactating†	Ewes in oestrus	1	0	427
	Ewes not in oestrus	32	3.0 $\pm$ 0.6 (-3 to + 12)	365 $\pm$ 28 (88 to 631)
	Total	33	2.9 $\pm$ 0.6 (-3 to + 12)	364 $\pm$ 26 (88 to 631)

FFA, free fatty acid.

* Lambs weaned 1 day after birth.

† Lambs weaned on the 24th or the 56th day of age.

TEXT-FIG. 1. The relationship between body weight change and plasma free fatty acid (FFA) concentration for dry ewes which did (●) or did not (x) show oestrus during the first 3 weeks after lambing.

maintained or gained weight, three of the early weaned (1-day) group lost weight as did three of the 24-day group. In terms of body weight changes, therefore, there was no indication of nutritional stress in the lactating ewes relative to the dry ewes. This was confirmed by the observation that the mean FFA concentrations in the peripheral plasma of all three groups were below the

Table 3. Exhibition of oestrus and the incidence of ovulation after progestagen treatment between 22 and 34 days post partum for ewes with 1, 24 or 56 days of lactation, together with a summary of the analysis of variance

Duration of lactation (days)	No. of ewes observed	No. in oestrus	Time between end of treatment and the onset of oestrus (hr)						No. of ewes observed	No. ovulating	No. of corpora lutea					
			Extract from analysis of variance								Extract from analysis of variance					
			Mean	S.E.	d.f.	M.S. between	M.S. within	F			Mean	S.E.	d.f.	M.S. between	M.S. within	F
1	12	12	35.33	1.48	34	189.5	26.8	7.07**	11	11	3.09	0.28	30	1.34	0.86	1.56
24	11	11	33.18	1.57					9	9	2.44	0.30				
56	12	12	41.00	1.48					11	11	3.09	0.28				

M.S., mean square; F, variance ratio.

** $P < 0.01$.

range of 500 to 1200 μ equiv./litre considered to indicate undernourishment in the ewe (Patterson, 1963; Russel, Doney & Reid, 1967).

Post-partum interval to first oestrus

Eight of the seventeen ewes whose lambs were weaned at 1 day of age showed oestrus during the first 3 weeks after lambing compared to only one of the thirty-three lactating ewes, showing that the incidence of oestrus is greater in the dry ewes. The results of laparoscopy during the 3rd week after lambing indicated that 'silent' ovulations did not occur during this period, and all the ewes which had shown oestrus did ovulate. The summary of body weight gain during this period (Table 2) indicates that there was no difference between the dry and lactating ewes. Furthermore, the sub-division of the ewes into those which showed

TEXT-FIG. 2. The time interval between end of progestagen treatment (vaginal sponge withdrawal) and the onset of oestrus in ewes with 1 (-----), 24 (— —) or 56 (—) days of lactation.

oestrus and those which did not, indicates that the gain was smaller in those ewes showing oestrus. The individual weight changes and FFA concentrations are presented in Text-fig. 1, where it can be seen that the display of oestrus was not associated either with body weight change or with FFA concentration.

Synchronization of oestrus 32 to 36 days post partum

The timing of onset of oestrus following progestagen treatment is shown in Text-fig. 2. All thirty-five ewes showed oestrus, and laparoscopy results (Table 3) indicated that thirty-one of thirty-three ewes had ovulated; the ovaries were not examined in two of the ewes. Although eleven of the twelve ewes in the lactating group showed oestrus and ovulated following progestagen treatment, the interval from sponge removal to the onset of oestrus was 41 hr in these ewes compared with 34 hr in the dry ones ($P < 0.01$).

Table 4. The characteristics of the preovulatory release of LH associated with synchronized oestrus 35 days *post partum* for ewes with 1, 24 or 56 days of lactation

Character	Duration of lactation (days)	No. of ewes	Extract from analysis of variance				
			Mean	S.E.	d.f.	M.S. between	M.S. within
Time interval between onset of oestrus and the start of LH release (hr)	1	12	9.08	1.21	33	119.0	17.6
	24	11	6.09	1.27			
	56	11	12.64	1.27			
Maximum concentration of LH released (ng NIH-LH/ml)	1	12	102.3	12.5	33	1517	1872
	24	11	125.3	13.0			
	56	11	112.0	13.0			
Duration of the LH release (hr)	1	12	12.00	0.58	33	11.5	4.1
	24	11	10.09	0.60			
	56	11	10.55	0.60			
Integral of the area under the LH peaks (ng/ml/hr)	1	12	429	55	33	49170	35646
	24	11	560	57			
	56	11	493	57			

M.S., mean square; F, variance ratio.

** $P < 0.01$.

The preovulatory release of LH

The LH release was characterized by a sharp increase in plasma LH concentration to more than 100 ng/ml in some ewes over a period of 2 to 4 hr with a rapid return to basal levels by 10 hr after the initial rise. The characteristics of the discharge in each of the three groups are given in Table 4, and illustrated in Text-fig. 3 by representative animals from each treatment group. Only one of the thirty-five ewes failed to register a discharge, this being in the lactating group.

TEXT-FIG. 3. The preovulatory LH release in three ewes representing 1(-----), 24 (— — —) or 56 (——) days of lactation.

The interval between the onset of oestrus and the start of the LH discharge differed markedly between treatments (Table 4). This interval was only 9.1 and 6.1 hr in the non-lactating groups compared with 12.6 hr in the lactating groups ($P < 0.01$). But the baseline levels of LH in the three treatments were similar, ranging between 2.5 and 10.0 ng/ml plasma, as were the mean maximum values and the mean duration of the discharge (Table 4).

DISCUSSION

The observation that only one of thirty-three lactating ewes, and eight of seventeen dry post-partum ewes showed oestrus during the first 3 weeks after lambing indicates that the reproductive activity of the present flock of lactating sheep was almost completely suppressed following lambing in the middle of the breeding season (December), when they would normally be expected to show regular cyclical oestrus. This is in agreement with the report of Land (1971) that in 2 consecutive years, lactating Finn-Dorset ewes did not show oestrus until

approximately 45 days after winter lambing. As in the cow (Short, Bellows, Moody & Howland, 1972), silent ovulations were not detected by laparoscopy in any group during this study period.

The reduced incidence of oestrus observed during lactation in well-fed ewes was in accord with the conclusion of Restall (1971) that the presence of a lamb appeared to block the expression of a regular pattern of oestrus and to cause delay in the mean time of first ovulation, and also with that of Mauléon & Dautier (1965) who reported that whereas over 35% of Île de France ewes with lambs removed had their first heat before the 34th day after lambing, this occurred in only 10% of ewes that were suckling. By contrast, Hunter & Van Aarde (1973) concluded that if lactating and non-lactating ewes were fed to meet their respective nutritional requirements, the duration of their post-partum anoestrous periods did not differ. The present results support the hypothesis that suckling delays the resumption of reproductive activity following lambing in adequately fed ewes. The same conclusion has been reached for the cow (Short *et al.*, 1972). The display of oestrus and the observation of ovulation in most of the ewes following treatment with a progestagen during lactation, however, indicates that such treatment may facilitate the resumption of ovarian activity.

A typical preovulatory LH release similar to that found in cyclic ewes, and previously reported by several workers (e.g. Pelletier, Kann, Dolais & Rosselin, 1968; Mauer, Revenal, Johnson, Moyer, Hirata & White, 1972) was detected in the peripheral blood plasma of thirty-four out of thirty-five ewes. The time interval between the onset of oestrus and the release of LH in the lactating ewes however, was longer than that previously reported.

The observation of a delay in both the onset of oestrus and the discharge of LH in the lactating ewes compared with dry ewes indicates that lactation does have an effect on LH release, as postulated in pigs (Crighton & Lamming, 1969), sheep (Land, 1971) and cows (England, Hauser & Casida, 1973) and as demonstrated in the rat (Hammons, Velasco & Rothchild, 1973) and in sheep during anoestrus (Pelletier & Thimonier, 1973). The difference in the display of oestrus and LH secretion for ewes which suckled for 24 and 56 days is compatible with the findings of Hammons *et al.* (1973). They reported a very rapid increase in the serum level of LH following removal of the litter, which indicates that the suckling stimulus must be applied almost continuously in order to suppress LH secretion.

The effect of lactation on the timing of the onset of oestrus following sponge withdrawal and the release of LH is a factor which should possibly be considered in the choice of mating or insemination times for lactating sheep. The present failure to detect differences in the nutritional status of the lactating and dry ewes indicates that the differences in the incidence of oestrus before progestagen treatment, and the timing of events after treatment, are not mediated by way of nutritional differences but are a direct effect of lactation.

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