Effect of season on LH secretion in ovariectomized Australian cashmere does

Henniawati¹, B. J. Restall¹ and R. J. Scaramuzzi^{2*}

¹Wollongbar Agricultural Institute, NSW Agriculture, Wollongbar, NSW 2477, Australia; and

²CSIRO, Division of Animal Production, Prospect, NSW 2148, Australia

An experiment was conducted to define the seasonal changes in LH secretion in ovariectomized does with oestrogen implants and the effect of immunization against melatonin. Fifteen mature Australian cashmere goats were ovariectomized and given either no further treatment or one or two implants containing oestradiol; another similar group of 15 does were immunized against melatonin before ovariectomy and oestrogen treatment. LH concentrations and livemasses were recorded every week for 2 years. Livemasses of both groups showed a distinct seasonal pattern with a summer maximum and a winter minimum irrespective of treatment. LH concentrations also showed distinct seasonal patterns with a significant interaction between the number of implants and the time of the year. In the nonimmunized does, the presence of a constant low dose of oestrogen (one implant) resulted in low concentrations of LH except from May to August, the normal period of spontaneous ovulatory activity in intact does. In contrast, nonimmunized does receiving a high dose of oestrogen (two implants) showed a rise in LH concentrations in February, and concentrations remained high until August. Immunization against melatonin abolished this differential LH secretory pattern, and both doses of oestrogen were associated with a short period of high LH concentration between May and September. These results indicate that a negative feedback effect of oestrogen results in low LH secretion for most of the year and that hypothalamic sensitivity to LH decreases for only a short period between May and August. It is suggested that the early increase in LH secretion in does with two oestrogen implants is due to a positive oestrogen feedback mechanism that may depend on a photoperiodic signal for activation.

Introduction

The annual reproductive cycle in Australian goats is characterized by a relatively short period of spontaneous ovulation, preceded by a sensitive anovulatory period in which reproductive activity can be readily induced, and followed by an insensitive, or quiescent, anovulatory period (Restall, 1992). Mating occurs readily in the period preceding spontaneous ovulation in response to a marked male effect (Chemineau, 1983; Restall, 1988). The precise endocrinological control of this annual rhythm in goats has not been described, but is presumed to be similar to that of sheep and to involve control of the gonadotrophic system by an interaction between photoperiod and circulating steroid hormone concentrations (Pelletier and Ortavant, 1975; Legan and Karsch, 1979) and a transduction of photoperiodic information to the hypothalamic–pituitary axis by melatonin (sheep: Karsch *et al.*, 1984; Arendt, 1986; goat: Chemineau *et al.*, 1988a; Maeda *et al.*, 1988). Behavioural, environmental and pheromonal

factors affect the expression of seasonal reproductive activity in sheep and goats (Martin and Thomas, 1990; Walkden-Brown *et al.*, 1993).

This report describes seasonal changes in LH secretion in ovariectomized does with oestrogen implants, the effect of immunization against melatonin on these animals and discusses the control of the seasonal reproductive rhythm in goats.

Materials and Methods

Location and animals

The experiment was carried out at the Pearces Creek substation of the Wollongbar Agricultural Institute (28°48'S, 153°23'E) using the domesticated offspring of cashmere animals bred from captured feral goats. The location, the general characteristics of the experimental animals and their management have been described elsewhere (Restall and Pattie, 1989). Animals were ovariectomized under general anaesthesia using standard surgical procedures (Hecker, 1974).

*Present address: Department of Veterinary Basic Sciences, Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, UK. Revised manuscript received 22 August 1994.

Experimental design

Thirty, 19 month old, Australian cashmere does were randomly assigned to two groups: group 1: 15 does ovariectomized in June 1987, and group 2: 15 does immunized against melatonin in May, June and July 1987, and ovariectomized in November 1987.

Within each group, does were given either 0, 1 or 2 oestrogen implants ($n = 5$). Each implant was constructed of Silastic tubing (internal diameter 3.35 mm, external diameter 4.65 mm) containing crystalline oestradiol and sealed with Silastic glue. The internal and external capsule lengths were 20 mm and 25 mm, respectively and although oestrogen concentrations were not subsequently measured in these animals, oestradiol assays of plasma from castrated bucks with two identical implants from the same batch gave values of 14.7 ± 0.95 pg ml⁻¹ (S. W. Walkden-Brown, personal communication).

All animals were run together in small (0.5–1.0 ha) paddocks and isolated from other intact females and males on the station. During the course of the study, one nonimmunized animal with one implant died, another lost both of two oestrogen implants, and data from these animals were not considered.

Immunization

The melatonin antigen used was a melatonin–HSA conjugate made up in Freund's complete adjuvant. Melatonin–HSA conjugate, 2 ml, was injected i.m. on 29 May, 29 June and 29 July, 1987. The melatonin-binding antibody titres, defined as the dilution of antiserum which bound 50% of the radio-labelled hormone available, were measured in August 1987, April 1988 and December 1989 using the method described by Foldes *et al.* (1983). The titres were in the range of 1:220–9000 in August 1987, 1:91 000–1:500 000 in April 1988, and 1:433–18 000 in December 1989. These animals were considered to be 'highly immune' to melatonin and maintained significant antibody concentrations for more than two years. The titres recorded in the experimental animals would be expected to bind the normal nightly physiological concentrations of melatonin (Malpoux *et al.*, 1988) for the duration of the experiment.

Assay procedures

Blood samples were collected once a week in heparinized tubes; the plasma was removed after centrifugation (1700 g for 5 min) and stored at -20°C until assayed.

LH was measured in duplicate plasma samples using a double antibody radioimmunoassay (Campbell *et al.*, 1991) with ovine LH antisera R115 (Scaramuzzi *et al.*, 1970). Standards were prepared using an LH preparation extracted and purified from goat pituitaries obtained from an abattoir by a modification of the method of Papkoff *et al.* (1965). Homogenized pituitaries were acidified twice, the precipitates discarded after centrifugation, the resulting supernatant neutralized and the LH present precipitated by centrifugation (at 1700 g for 30 min). This precipitate was suspended in 0.2 mol K₂HPO₄ l⁻¹, heated to 55–60°C for 2–3 min, and dialysed in distilled water for 4 days. Partially purified caprine LH (170.25 mg) was obtained after lyophilization. A portion

(20 mg) was resuspended in phosphate, centrifuged (at 1700 g for 30 min) and dialysed to yield 9.85 mg of purified caprine LH.

The two purified LH preparations were assayed against an ovine LH standard (NIH-oLH-S20). The caprine LH was diluted with LH-free plasma from oestrogen-treated bucks to provide a stock solution from which standard solutions were serially diluted. The crossreactivity between the ovine LH and the ovine anti-serum was greater than for either of the caprine LH preparations, and both caprine preparations exhibited parallelism with the ovine standard curve. The caprine LH preparation was assayed to detect FSH activity in a radioreceptor assay by the method of Cheng (1975) using calf testicular membrane, PMSG and purified bovine ¹²⁵I-labelled FSH. The ratio of affinity of caprine LH for the bovine FSH receptors was 625:1, indicating that the LH preparation used had low FSH activity.

This LH preparation was used to prepare standards for assays of the plasma samples. The mean assay sensitivity was 1.0 ± 0.16 ng ml⁻¹. Each assay contained five replicates of quality control standards containing 2.5, 5.0, 10.0, 20.0 and 50.0 ng caprine LH ml⁻¹. The mean intra-assay coefficients of variation for these standards were $17.37 \pm 2.56\%$, $9.42 \pm 1.18\%$, $8.30 \pm 0.90\%$, $5.98 \pm 0.67\%$ and $4.89 \pm 0.56\%$, respectively, while the mean interassay coefficients of variation were $13.26 \pm 0.06\%$, $9.59 \pm 0.10\%$, $7.35 \pm 0.15\%$, $7.29 \pm 0.28\%$ and $3.32 \pm 0.32\%$, respectively. The relationship between the amount of hormone added and recovered was highly significant ($P < 0.001$) and described by $y = 0.546 + 1.005x$, $R = 1.00$.

Statistical analyses

The LH and livemass data for each week were grouped into monthly means for individual animals before being subjected to analyses of variance with repeated measures. Analyses of the combined data for both group 1 (nonimmunized) and group 2 (immunized against melatonin) showed significant third-order interactions between oestrogen treatment, month and group for LH and livemass. Further analyses were carried out within groups as follows. The livemass and LH data from group 1 does were subjected to an initial analysis of variance with two levels of repeated measurements (months and years), with months as replicates. As a significant year by month interaction was evident, the analysis was repeated omitting year as a factor. Similar data from group 2 were subjected to analysis of variance with only months as a repeated measure as data from only 8 months were available for the second year. The LH data from does with one or two implants only, for both groups, were subjected to further analyses of variance using the year subsets separately or combined. Because of the possibility of overestimating the statistical significance of effects where repeated measures are large, appropriate conservative estimates of error were used (Little and Hills, 1978).

The apparent difference between oestrogen implant treatments in the nonimmunized (Group 1) does was evaluated further by examining the weekly LH data in more detail. The date of commencement of a sustained change in LH concentration was determined by inspection of the raw data, and taken as the time when the mean of four subsequent sequential samples was above the mean of the previous four samples at

Fig. 1. Mean monthly changes in livemass of nonimmunized (●) ovariectomized does and does immunized against melatonin (○). Daylength is shown as dashed line.

the commencement of the sustained rise. The end of a putative high LH secretion period was determined as the date of return to the pre-rise value. These dates were determined for the rise and fall of LH for all animals in both years, each animal contributing two complete rise and fall cycles. From these dates the following parameters were calculated: (i) time in days from summer solstice to beginning of LH rise; (ii) duration of elevated LH secretion; and (iii) time in days from winter solstice to end of the period of high LH.

These data were subjected to analysis of variance. All statistical procedures were carried out using Systat 5.1 statistical program (Wilkinson, 1990).

Results

Livemass

The mean monthly livemasses for both nonimmunized and immunized does are shown in Fig. 1, with a summary of the variance analyses in Table 1. There were no significant differences between the treatment groups for mean monthly livemasses of does but a significant seasonal pattern was evident (interaction between month and year, or month main effect, $P < 0.01$) with annual maximum masses recorded in summer and the lowest masses in winter. Although the livemasses of the immunized group were lower than those of the non-immunized does, the magnitude of the seasonal changes was similar. There was significant variation between animals for mean monthly livemass but the does increased in mass overall during the experiment attaining a final mass in excess of 40 kg.

LH concentration

The mean monthly LH concentrations for the immunized and nonimmunized does are shown in Fig. 2 with summaries of the analyses of variance in Table 1. The analyses revealed significant variation due to the number of implants, and an interaction between month and number of implants. Comparison of the immunized and nonimmunized does receiving oestrogen implants (the no implant data omitted) revealed a significant ($P < 0.05$) interaction between number of implants

Table 1. Summary of the analyses of variance of mean monthly livemass and LH for ovariectomized does either immunized against melatonin or not immunized, and with 0, 1 or 2 oestrogen implants

Source of variation	df ^a	Mean square for LH	Mean square for livemass
Nonimmunized does			
Treatment (T)	2	61 308.79*	318.64
Between animals (Error 4)	10	7728.58**	204.10**
Month (M)	11	479.78	190.27
M × T	22	720.31*	6.15
Error (3)	110	254.03	3.62
Year (Y)	1	4.00	5.07**
Y × T	2	12.31	0.21
Error (2)	10	10.72	0.18
M × Y	11	74.98	8.07**
M × T × Y	22	99.86	0.38
Error (1)	110	38.43	0.55
Immunized does			
Treatment (T)	2	21 128.42**	321.59
Between animals (Error 2)	12	424.171**	156.21**
Month (M)	19	619.31**	130.67**
M × T	38	256.31**	1.95
Error (1)	228	15.81	1.77

* $P < 0.05$; ** $P < 0.01$.

^aTwo years data for non-immunized does (months as replicates), 20 months data for immunized does.

and the immunization treatment. Subsequent separate analyses of variation of the data for the implanted immunized and nonimmunized does are given in Table 2 and results for each group were as follows:

Nonimmunized does. In ovariectomized does without oestrogen implants, LH concentrations initially fell in the first month before increasing steadily for the next 7 months (Fig. 2). The LH concentrations then appeared to reach a plateau and were relatively constant for the remainder of the experiment in three of the five does. In the other two does, LH concentrations rose sharply in August 1988 and remained high until February 1989, then dropped back to the plateau levels.

In both years during the normal anoestrous period (September–December) the mean monthly LH concentration was low in does with oestrogen implants. The LH concentration appeared to rise in January or February in the does with two oestrogen implants, but not until May in those with one oestrogen implant. Irrespective of the number of oestrogen implants, LH concentrations fell at the beginning of the anoestrous period in September (Fig. 2).

Considerable individual variation was evident (Table 2) and both combined or separate analyses of the data for each year revealed significant variation due to the month of observation ($P < 0.01$) and a significant interaction between the month of observation and the number of implants ($P < 0.052$). This interaction was examined in more detail by analysis of the weekly LH concentrations.

The mean weekly LH concentrations for the four animals in each implant group from January to September of each year are

Fig. 2. Seasonal changes in mean monthly LH concentration in ovariectomized does. Figures on the left are for nonimmunized does with (a) no implant or (b) one (●) or two (○) oestrogen implants. Corresponding data for does immunized against melatonin are shown on the right (c, d). Note that the y axes have different scales.

shown in Fig. 3; as the data showed heterogeneous variation it has been transformed to logarithms. For the does receiving two oestrogen implants, the mean weekly LH concentration was more variable than for those receiving only one implant, but the rise in LH concentration was clearly in advance of that of does receiving one implant. The mean interval from the summer solstice to the commencement of the rise, the duration of the rise and the interval from winter solstice to the end of the rise are shown in Table 3. The analysis of variance of these data showed a significant effect of the number of implants on the commencement of the rise, with those does receiving two implants commencing, on average, 58 days earlier than those with one implant (Table 3). The average date of commencement of the LH rise was 28 April for does with one implant, and 29 February for those with two implants.

There was considerable variation between animals in the group that received two implants particularly in the first year (Fig. 3); two animals in one or other year showed either a very early rise (1 January) or a late rise (22 April) in weekly LH concentration. However, of the eight secretory periods observed for the two implant does, six began at the earlier date.

The duration of the high LH secretory period was significantly ($P < 0.05$) longer in does with two implants than in those with one, with an average extension of 51 days (Table 3).

There were no significant differences between does with one or two implants in the time after the winter solstice when the high LH secretory pattern ended. However, the high LH

secretory periods ended significantly ($P < 0.05$) earlier in the second year; the mean dates of cessation were 6 September (year 1) and 18 August (year 2), a difference of about 3 weeks.

Does immunized against melatonin. The mean monthly LH concentrations for the animals immunized against melatonin are shown in Fig. 2, with the summary of the analyses of variance in Tables 1 and 2. Ovariectomized immunized does without oestrogen implants showed a steady increase in LH concentration during the 20 months of observation without a discernible plateau. When only animals with implants were considered, both separate and combined analyses of the data for each year (Table 2) showed significant variation due to the month of the year ($P < 0.01$) and the month by treatment interaction ($P < 0.052$). Immunized does with oestrogen implants showed a much smaller seasonal rise in LH concentration than did the nonimmunized animals. In contrast to the nonimmunized does, those animals with two implants of oestrogen showed significantly ($P < 0.01$) lower LH concentrations than those with one implant. There was no evidence of an early rise in LH concentration due to the number of implants.

Discussion

The seasonal pattern of live mass changes observed in the study reported here is similar to other studies of Australian

Table 2. Summary of the analyses of variance (mean squares) for mean monthly LH in nonimmunized and immunized ovariectomized does with 1 or 2 oestrogen implants

Source of variation	df	Year 1	Year 2	Combined year 1 and year 2		
Nonimmunized does ^a						
Number of implants (T)	1	1348.47	4.19	751.52		
Between animals (Error 1)	6	1329.73	221.01	1202.5		
Month (M)	11	268.21**	179.18**	410.41**		
M × T	11	156.28*	88.94*	275.98*		
Error (2)	66	89.32	46.32	147.69		
Year (Y)	1			16.75		
Y × T	1			2.72		
Error (3)	6			9.94		
M × Y	11			71.5**		
M × Y × T	11			23.6		
Error (4)	66			18.6		
Immunized does ^b						
		df	df			
Number of implants (T)	1	133.04**	1	666.92**	1	638.44**
Between animals (Error 2)	8	25.64**	8	9.23	8	21.86**
Month (M)	11	117.27**	7	108.46*	19	116.00**
M × T	11	4.14	7	15.00	19	16.42*
Error (1)	88	7.23	56	7.71	152	7.71

* $P < 0.05$; ** $P < 0.01$.^aData for two years with months as replicates in the combined analysis.^bData for 20 months. **Fig. 3.** Mean weekly LH ($\log_{10}$ transform) for ovariectomized does with (a) one or (b) two oestrogen implants between January and October for two consecutive years.

Table 3. Commencement, duration and end of the seasonal rise in LH secretion in nonimmunized ovariectomized does treated with either 1 or 2 implants of oestradiol (data are means and standard deviations) together with a summary of the analyses of variance

Parameter	Treatment			
	Ovariectomized + 1 oestrogen implant		Ovariectomized + 2 oestrogen implants	
	Year 1	Year 2	Year 1	Year 2
Period from summer solstice to start of LH rise (days)	132.5 (4.04)	124.5 (4.04)	73.3 (46.3)	66.8 (29.9)
Duration of LH rise (days)	126 (20.6)	123.3 (23.8)	183.5 (54.8)	167.5 (36.8)
Period from winter solstice to end of LH rise (days)	76.5 (17.6)	65.8 (21.6)	78.3 (14.4)	51.8 (12.0)

Summary of the analyses of variance				
Source of variation	df	Days from summer solstice to LH rise	Duration of LH rise	Days from winter solstice to end of LH rise
Number of implants (T)	1	13 689**	10 353.07**	150.07
Year (Y)	1	210.25	351.57	1387.57*
T × Y	1	2.25	175.55	248.06
Error	12	768.46	1337.23	282.77

* $P < 0.05$; ** $P < 0.01$.

goats (Ash, 1986; Klören, 1991; Walkden-Brown *et al.*, 1994), and to ruminants in general (Forbes, 1982; Kay, 1985). This pattern of growth is considered to be due to a circannual cycle in voluntary feed intake that is photoresponsive and may be secondary to a primary growth cycle. The magnitude of the observed loss of mass in autumn (14.3% for nonimmunized does, 12.5% for immunized does) is similar to that observed by Walkden-Brown (1991) for grazing bucks at the same location (14.8–19.5% loss in mass). Walkden-Brown (1991) also provided evidence for an influence of age; the magnitude of the livemass effect changed from a growth stasis in the first year of life to an increasing loss of mass as animals grew older. The exact nature of the effect is unknown.

Annual growth rhythms have been shown to be photo-inducible in a number of domestic species (sheep: Forbes *et al.*, 1979; Kay and Suttie, 1980; Soay rams: Suttie *et al.*, 1984; cattle: Peters *et al.*, 1980) including Australian goats (Ash, 1986). However, our results do not show any significant difference between the immunized and nonimmunized does in the seasonal livemass pattern. The immunized does were unlikely to have been able to transduce photoperiodic signals as two other studies at this centre have shown that similar immunization treatment interferes with the expression of the photoperiodically controlled fleece growth rhythm (Sutherland *et al.*, 1990; Foldes *et al.*, 1992). The immunized does would be expected to behave as pinealectomized animals (Martenz, 1980), unable to transduce photoperiodic changes, and it is known that photoinducible rhythms can persist for up to three years following pinealectomy or superior cervical ganglionectomy (Bittman, 1985; Argo, 1985 cited by Kay, 1985). This persistence is considered evidence of an endogenous rhythm (Bittman, 1985) but the existence of an endogenous rhythm is by no means established. As all goats were fed supplements in the study reported here, it is concluded that the observed seasonal changes in livemass in the does immunized against

melatonin are due to persistence of a previously entrained rhythm, rather than merely reflecting seasonal pasture changes.

LH concentrations showed a distinct seasonal pattern in the presence of circulating oestrogen with significant differences due to the number of implants. It was not possible to measure the concentration of circulating oestrogen resulting from the implants, but two similar sized implants in castrated bucks at the same location resulted in concentrations of 14.7 ± 0.95 pg ml⁻¹ (S. W. Walkden-Brown, personal communication). Slightly higher concentrations would be expected in the does studied because of sex differences in livemass, and does with one implant would be expected to have lower circulating concentrations of oestrogen. These estimates are comparable to the results of Chemineau *et al.* (1982) who found oestradiol concentrations of 10 pg ml⁻¹ in seasonally anoestrous goats. Karsch *et al.* (1980, 1983) showed that sheep with two implants of similar size had circulating oestrogen concentrations three times higher than those with one implant, and we consider that the two treatments imposed on the does in the present study would have resulted in a similar difference in circulating oestrogen.

The seasonal pattern of LH concentration varied with the number of implants, and by inference, with the concentration of circulating oestrogen; the period of high LH concentration in does with one implant corresponds to the period of spontaneous ovulatory activity (Restall, 1992), but it began much earlier and lasted much longer in does with two implants. This earlier rise in LH concentrations in does with two implants corresponds to the responsive period of the seasonal rhythm in which anovulatory animals can be induced to ovulate (Chemineau, 1983; Restall, 1992). The LH pattern in does with one implant is similar to that described for sheep and attributed to changes in hypothalamic sensitivity to negative feedback of oestrogen (Legan and Karsch, 1979; Karsch *et al.*, 1984). However, the mechanisms underlying the anovulatory

responsive period and the following relatively short period of spontaneous ovulation in our goats are not known.

Sutherland (1987) studied seasonal changes in negative oestrogen feedback in Angora $\times$ Australian goats, and concluded that the control was the same as in sheep, that is primarily a differential negative feedback effect of oestradiol. His experiment was conducted in April during the responsive anovulatory period, and used a single low dose of oestrogen analogous to the one implant treatment in the present study, and gave a similar result. This putative seasonal change in sensitivity to negative oestrogen feedback does not explain the earlier rise in LH observed in does with two implants, as the higher oestrogen concentration would be expected to suppress, not release, LH.

A possible explanation for the observed differential LH pattern in does with one or two implants is that a positive oestrogen feedback system is present all year round but with varying sensitivity. In the experiment reported here LH was low in does with one implant in the anovulatory responsive period (Feb–May) due to sensitivity of the negative oestrogen feedback. The higher LH concentrations in does with two implants cannot be due to tonic LH release, as this is suppressed during the anovulatory responsive period, but may be a result of a weak positive oestrogen feedback stimulus. In the following period of spontaneous ovulation (May–July), a positive feedback effect may be masked by the increased tonic LH secretion associated with the change in negative oestrogen feedback. Such a system of differential change in positive and negative oestrogen feedback could control the observed periods of spontaneous ovulation, and the responsive and nonresponsive periods of anovulation in this species (Restall, 1992).

Such apparent 'positive' oestrogen feedback effects may be a general phenomenon. A chronic positive feedback effect of oestradiol in ovariectomized heifers in all seasons has been reported (Critser *et al.*, 1983) as well as a seasonal variation in LH concentrations. The treatment of ovariectomized cows with oestradiol increases both mean concentration and amplitude of LH pulses throughout the year (Day *et al.*, 1986; Stumpf *et al.*, 1988); the magnitude of the response depends on the season. There is some evidence that low concentrations of oestradiol administered to ovariectomized cows decrease pulse frequency while enhancing amplitude (Kinder *et al.*, 1991). Similarly, oestradiol increases mean LH concentrations in ovariectomized mares, with a greater enhancement in the breeding season (Garcia and Ginther, 1978). Reports of a chronic positive feedback effect of oestradiol on LH resulting in increases in pulse amplitude but not frequency, for both a seasonal and nonseasonal breeding species, contrast with the dramatic seasonally dependent inhibitory effect of oestradiol in ovariectomized ewes (Legan *et al.*, 1977), in which frequency but not amplitude, of pulses is suppressed (Goodman and Karsch, 1980). Chemineau *et al.* (1988b) found that LH pulse frequency increases gradually by 65% in French Saanen goats as the breeding season approaches which was interpreted as an easing of the negative oestrogen feedback inhibition. Mori *et al.* (1987) report that in ovariectomized Shiba goats the hypothalamic–pituitary axis becomes hypersensitive to both the positive and negative feedback effect of oestradiol under long day conditions, indicative of independent mechanisms.

These reports, together with the results of the study reported here, suggest that there is a general positive oestrogen feedback effect that is expressed differently in different species under similar circumstances. An enhancement of the positive effect during the sensitive anovulatory phase in our goats may underpin the marked responses to environmental stimuli, such as the introduction of males, at that time of the year (Walkden-Brown *et al.*, 1993).

Does immunized against melatonin showed a very different pattern of LH secretion from that of the nonimmunized animals. While the seasonal change in LH secretion corresponding to the period of spontaneous ovulations was still evident, there was no effect of the number of implants, and by inference the dose of oestrogen. In fact the higher dose appeared to be more suppressive of LH secretion than did the lower dose, as might be expected if only the negative feedback mechanism was operative. While the effect of immunization on the biological activity of circulating melatonin has not been demonstrated, studies involving immunization against melatonin are in agreement that immunized animals are similar to pinealectomized animals and this is consistent with the interpretation that immunization has blocked the biological activity of melatonin (Martenz, 1980). If the immunization of our animals effectively removed their ability to transduce the normal light signal, then the absence of the putative positive feedback mechanism evident in the nonimmunized does receiving the higher oestrogen dose implies that it is under photoperiodic control, but is not entrained. The persistence of the negative feedback rhythm without photoperiodic change suggests that it is either an endogenous or entrained rhythm. In sheep that have been pinealectomized or have undergone superior ganglionectomy, negative feedback and behavioural rhythms may persist for long periods, although synchrony and seasonal phasing may be lost quickly (Karsch *et al.*, 1984; Bittman, 1985; Karsch and Woodfill, 1990).

The results reported here are consistent with other studies of goats (Sutherland, 1987; Chemineau *et al.*, 1988a, b) and suggest possible mechanisms underlying seasonal reproductive rhythms. We propose that a negative oestrogen feedback system prevents the expression of ovulatory activity for most of the year, and that a positive oestrogen feedback mechanism, indicated by a rise in circulating LH concentrations, renders the animal responsive to other stimuli for a varying period before the resumption of spontaneous ovulatory activity. This putative positive feedback mechanism may be dependent on a photoperiodic signal for activation, but further research is warranted as it may underlie the ability of goats to respond opportunistically to short-term changes in their physical environment.

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