Melatonin secretion in Romney ewes differing in wool growth and reproduction is not aligned to photoperiod during spring and summer

I.C. SCOTT, D. HEATH[1] AND THE LATE H. HAWKER

AgResearch, Invermay Agricultural Centre, P.O. Box 50034, Mosgiel, New Zealand.

ABSTRACT

Romney ewes with consistently high (H, n=5) or low (L, n=5) seasonal changes in wool growth and with differences in ovulation rate and the onset of anoestrus, were blood sampled every 30 min for 24 h at the winter and summer solstices and at the spring and autumn equinoxes. Plasma samples were assayed for melatonin by direct radioimmunoassay.

Mean melatonin levels were higher during the hours of darkness than during daylight at all sampling times. However, the ratio between day- and night-time melatonin levels tended to be smaller in spring (1.8) and summer (1.5) than autumn (2.9) and winter (1988 - 3.8; 1989 - 4.1). There were no significant differences between H and L ewes in mean melatonin concentrations during daylight or darkness at any of the seasons. There was however, considerable variation between individual ewes in the diurnal pattern of melatonin secretion in spring and summer. The high levels of melatonin usually associated with darkness were sometimes found during the day. These disrupted patterns of melatonin secretion occurred in all ewes at one or other of these two seasons. The results suggest that during anoestrus, melatonin profiles may differ from the normal pattern of secretion that is aligned to photoperiod.

Keywords Sheep, photoperiod, melatonin, daylight, darkness, reproduction, wool growth.

INTRODUCTION

Seasonal changes in wool growth and the reproductive status of sheep are entrained by annual changes in photoperiod (Lincoln, 1984). In primitive breeds such as the Soay, photoperiod produces a marked seasonality in these characteristics. Wool growth ceases in winter (Ryder, 1978), and the rut occurs over a six week period in autumn (Hafez, 1952). Conversely, in Merino sheep photoperiod accounts for less than 10% of the within year variation in wool growth (Nagorcka, 1979), and breeding may occur all year round (Pearce and Oldham, 1988). A relationship between the seasonal nature of wool growth and reproduction within breeds has been demonstrated by Montgomery and Hawker (1987), who reported that a low amplitude of change in seasonal wool growth was associated with a higher ovulation rate and a delayed end to the breeding season.

Blood concentrations of melatonin closely reflect transitions from light to dark, with high levels of melatonin secreted from the pineal gland during darkness (Rollag and Niswender, 1976). Melatonin thus mediates the effects of changing photoperiod on reproduction (Bittman *et al.*, 1983) and wool growth (Lincoln and Ebling, 1985). Since differences exist between and within breeds in the extent of the annual wool growth and reproductive cycles despite exposure to the same photoperiodic information, this may reflect differences in sensitivity of the pineal gland to photoperiod, and be reflected in the daily and seasonal profiles of melatonin secretion. Alternatively, these differences may be due to the post-pineal processing of the melatonin signal.

The present experiment was designed to test the hypothesis that ewes of the same breed that differ in the extent of their seasonal wool growth and reproductive responses, also differ in melatonin secretion when exposed to the same ambient photoperiod.

MATERIALS AND METHODS

Animals

Ten animals that had previously been identified as having a consistently high (H, n=5) or a consistently low (L, n=5) seasonal amplitude in wool growth were used in this experiment. When selected, the L ewes grew 54% more wool in winter (5.7 v 3.2 g/d) and 14% less in summer (10.8 v 12.4 g/d) than the H ewes (Hawker and Crosbie, 1985). It was subsequently found that the L ewes also had a higher proportion of multiple ovulations throughout the breeding season, and a breeding season that ended 17 days later than the H ewes (Montgomery and Hawker, 1987)

In June 1988, at the start of the present experiment, these ewes were 7 or 8 years old and had a mean liveweight of 63 kg. They were grazed as one mob on mixed ryegrass/white-clover pasture at the Invermay Agricultural Centre (latitude 45° 51'S) throughout the experiment.

Blood Samples

The ewes were housed indoors under natural lighting conditions for five days before, and during each blood sampling period. Over the period from winter 1988 to winter 1989, blood samples (3ml) were collected via in-dwelling jugular catheters every 30 min for 24 h at the winter and summer solstices, and also at the spring and autumn equinoxes. Sampling for each 24 h melatonin profile began at 9:00 am GMT. During the hours of darkness, samples were taken with the aid of a dim green light. Blood samples were centrifuged at 4° C within 30 min of collection and the plasma removed and stored at -20° C until assayed.

Melatonin Assay

Melatonin concentrations were measured by the direct radioimmunoassay procedure of Fraser *et al.*, (1983) as modified by English *et al.*, (1986). Within this study the minimum level of

[1] AgResearch, Wallaceville Research Centre, P.O. Box 40063, Upper Hutt, New Zealand.

detection for the assay was 10 pg/ml. A standard serum pool with a melatonin concentration of approximately 100 pg/ml was included after every 50 duplicates. Intra-and inter-assay coefficients of variation were 8.2% and 18.0% respectively.

Statistical Analysis

Two of the group L ewes died during the course of the experiment, one prior to the autumn equinox and one prior to the 1989 winter solstice. Data available from these ewes were included in the analyses. All data were log-transformed to allow for heterogeneity of variances between daylight and darkness sampling. All analyses compare the mean melatonin concentrations for daylight and darkness periods, where sunrise and sunset (as published in the New Zealand Almanac) delineated the changes between light and dark. Two-way analysis of variance (Anova) was used to test for any interaction between treatment (H, L) and time (light, dark), treatment and season, or time and season. One-way Anova was used to test for any seasonal effects on melatonin concentrations during daylight or darkness, and also to test for any time effects within each season.

RESULTS

There were no significant differences between the H and L ewes in mean plasma melatonin concentrations during daylight or darkness at any of the seasons. Therefore, data from the two treatments were combined for all further analyses.

The mean 24 h plasma melatonin concentrations of ewes at each season are shown in Figure 1. At each season, the mean melatonin concentration was aligned to photoperiod such that during the hours of darkness the mean melatonin levels were significantly greater ($P<0.01$) than during daylight (Table 1). However, there was a significant interaction ($P<0.01$) between mean light and dark levels of melatonin and season. The mean daylight levels of plasma melatonin were higher in summer (31 ± 4 pg/ml) and autumn (22 ± 2 pg/ml) than at the other seasons (11 ± 0.3, 11 ± 0.5, and 15 ± 2 pg/ml for winter '88, spring and winter '89 respectively), and during darkness the mean melatonin levels were lower in spring (23 ± 3 pg/ml) than at the other seasons (49 ± 10, 47 ± 6, 72 ± 16 and 64 ± 11 pg/ml for winter '88, summer, autumn and winter '89 respectively). Also, the ratio between mean light and dark levels in plasma melatonin tended to be smaller in spring (1.8) and summer (1.5) than both autumn (2.9) and winter (1988 - 3.8; 1989 - 4.1).

TABLE 1 Mean (± SEM) plasma melatonin concentrations (pg/ml) during daylight and darkness at the winter and summer solstices and at the spring and autumn equinoxes in Romney ewes. N = number of ewes sampled.

	Season	N	Mean Melatonin Concentration (pg/ml) Day	Night
1988	Winter	10	11 (0.3)	49 (10)
	Spring	10	11 (0.5)	23 (3)
	Summer	10	31 (4)	47 (6)
1989	Autumn	9	22 (2)	72 (16)
	Winter	8	15 (2)	64 (11)

At each season, high fluctuating levels of melatonin were found during the hours of darkness. During spring and summer however, there was considerable variation between individual animals in the diurnal pattern of melatonin secretion. Five ewes (3L, 2H) had high (>20 pg/ml) levels of melatonin occurring during daylight and low (10 pg/ml) levels during darkness in spring, while in summer, the other five ewes (2L, 3H) had high (>20 pg/ml) melatonin levels during both daylight and darkness. Examples of disrupted 24 h melatonin profiles in spring and in summer are shown in Figure 2.

FIG 1 Mean (±sem) plasma melatonin concentrations (pg/ml) over 24 h in Romney ewes at the winter (a) and summer (c) solstices and spring (b) and autumn (d) equinoxes. Darkness is indicated by the dark shading, and dawn and dusk (30 min either side of sunrise and sunset) by the light shading. Blood samples were taken at 30 minute intervals.

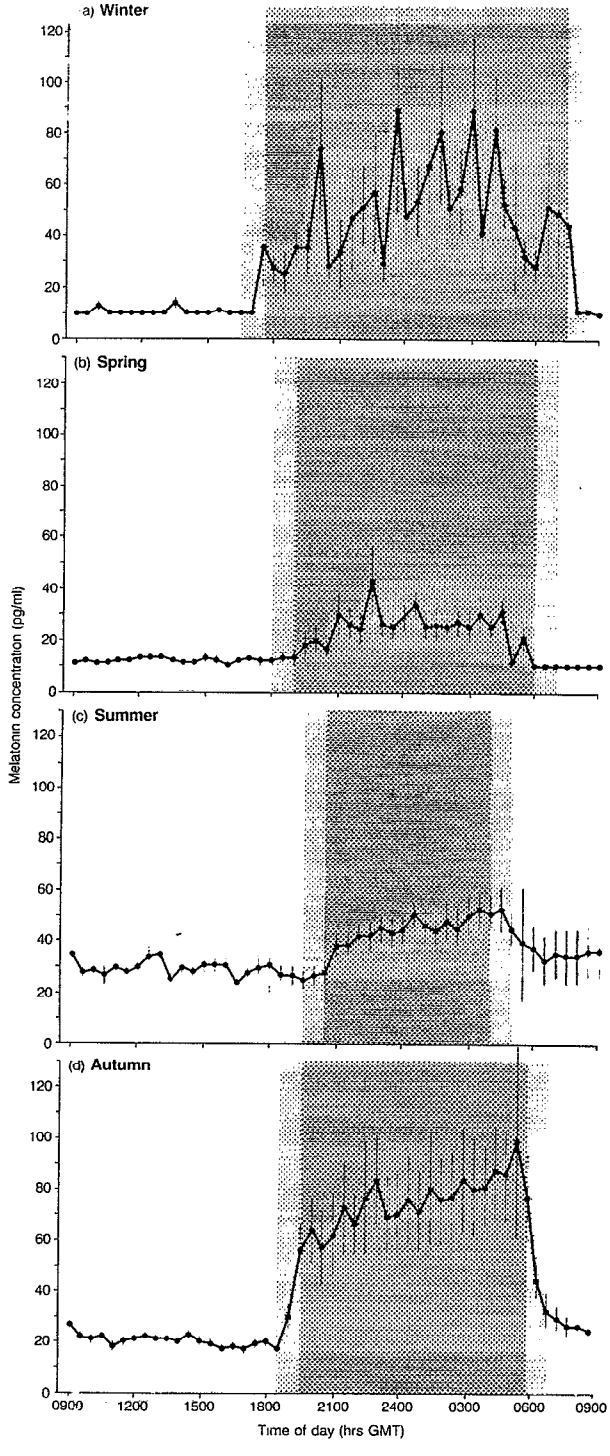
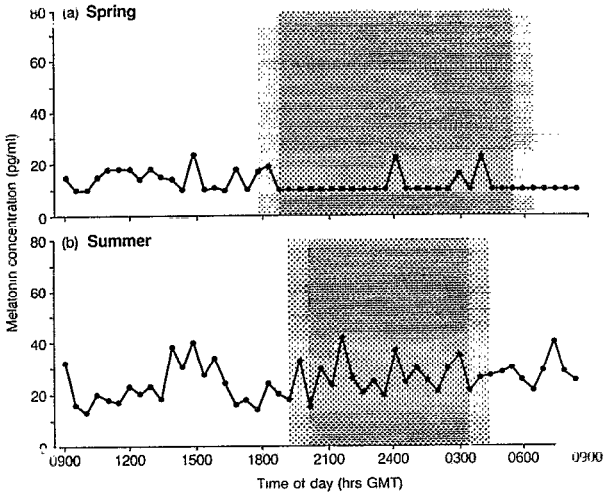


FIG 2 24 h plasma melatonin profiles of two ewes with patterns of melatonin secretion not aligned with photoperiod. Figure 2a shows the profile of Ewe 9 in spring, and Figure 2b shows the profile of Ewe 5 in summer. Darkness is indicated by the dark shading, and dawn and dusk (30 min either side of sunrise and sunset) by the light shading.Blood samples were taken at 30 minute intervals.



DISCUSSION

The rationale for the present experiment was that if the same photoperiodic information is able to elicit differing seasonal patterns of wool growth and reproduction between and within breeds of sheep, then the photic message must be either delivered, or interpreted differently. Since melatonin mediates the effects of changing photoperiod on wool growth and reproduction (Lincoln and Ebling, 1985), then either the melatonin signal, or the post-pineal processing of the melatonin signal, is different. We investigated the hypothesis that ewes of the same breed that differ in the extent of their seasonal wool growth and in reproductive responses to photoperiod, may also differ in an aspect of pineal function, namely melatonin secretion.

We were unable to detect any differences between H and L ewes in mean melatonin levels during daylight or darkness at any of the seasons studied. This suggests that differences in response to photoperiodic information are not mediated by differences in melatonin secretion, but rather, are due to differences in the post-pineal processing of the melatonin signal. However, it must be remembered that these ewes were of the same breed, and differences in seasonal wool growth and reproduction were small compared to those recorded between Soay and Merino sheep. Also, the sample size and frequency of blood sampling may not have been large enough to detect any subtle difference in melatonin levels between the two groups.

In the present experiment, we have found that melatonin levels in the blood were higher during darkness than during daylight at all seasons, confirming other studies (eg Kennaway *et al.*, 1983). However, in the present study, the ratio between light and dark levels of melatonin was higher in winter and autumn than in spring and summer. When the individual melatonin profiles of ewes were examined, it was discovered that there was great variation between ewes in the pattern of melatonin secretion in spring and summer, and this contributed to the reduced ratio between light and dark melatonin levels at these seasons. All ewes appeared to have a disrupted profile at one or other of these two seasons, such that high levels of melatonin usually associated with darkness were found during daylight and vice-versa. Almeida and Lincoln (1984) found evidence of similarly disrupted melatonin profiles. When Soay rams were exposed to a long (16 hours light: 8 hours dark) photoperiod for longer than 25 weeks, there were times when the pattern of melatonin secretion was not synchronised with photoperiod. They suggested that when animals are unresponsive to photoperiodic cues, the circadian control of melatonin is disturbed.

The loss of alignment between photoperiod and melatonin secretion observed during spring and summer suggests that, in order to synchronise the circannual cycle of wool growth and reproduction, melatonin profiles that accurately code for light and dark need not be present throughout the year. In agreement with this, Woodfill *et al.*, (1991) have reported that in pinealectomised ewes, the circannual cycle of breeding activity can be synchronised by just one 70-day block of circadian melatonin infusion. However, in their experiment, ewes were exposed to 70-days of long photoperiodic information, whereas in the present experiment, melatonin secretion was disrupted during long photoperiod. Woodfill *et al.*, (1991) also reported that ewes that exhibited synchronous cycles following one 70-day block of long photoperiodic information, tended to have a shorter breeding season than that of control animals kept outdoors. Furthermore, Wayne *et al.*, (1990) found that when ewes were pinealectomised at the summer solstice, effectively blocking the signal of decreasing photoperiod, the following breeding season was shorter than normal. Therefore, perhaps only one block of photoperiodic information per year is required to synchronise the circannual breeding cycle, but this information must be received during decreasing photoperiod to ensure the full duration of the breeding season.

The finding of disrupted patterns of melatonin secretion in spring and summer in the present experiment was unexpected, and raises further important questions. What causes the disruption to melatonin secretion in spring, and how does it become realigned with photoperiod between summer and autumn? And for how long, and at what time of the year must accurate photoperiodic information be provided to elicit a synchronous breeding season of normal intensity and duration?

ACKNOWLEDGEMENTS

We thank staff of the Animal Production Unit for assistance with the blood sampling, Seow-Ling Seah and Ken Dodds for the statistical analyses, and Jim Webster and Mark Fisher for helpful comments on the paper.

REFERENCES

Almeida, O.F.X.; Lincoln, G.A. 1984. Reproductive photofractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. *Biology of Reproduction* **30:** 143-158.

Bittman, E.L.; Dempsey, R.J.; Karsch, F.J.; 1983. Pineal melatonin secretion drives the reproductive response to daylength in the ewe. *Endocrinology* **113:** 2276-2283.

English, J.; Poulton, A.L.; Arendt, J.; Symons, A.M. 1986. A comparison of the efficiency of melatonin treatments in advancing oestrus in ewes. *Journal of Reproduction and Fertility* **77:** 321-327.

Fraser, S.; Cowen, P.; Franklin, M.; Franey, C.; Arendt, J. 1983. Direct radioimmunoassay for melatonin in plasma. *Clinical Chemistry* **29** (2): 396-397.

Hafez, E.S.E. 1952. Studies on the breeding season and reproduction of the ewe. *Journal of Agricultural Science* **42:** 189-231.

Hawker, H.; Crosbie, S.F. 1985. Effects of level of nutrition in winter and summer on the wool growth of Romney and Perendale ewes with a history of high or low winter wool growth. *Proceedings of the New Zealand Society of Animal Production* **45:** 225-228.

Kennaway, D.J.; Sanford, L.M.; Godfrey, B.; Friesen, H.G. 1983. Patterns of progesterone, melatonin and prolactin secretion in ewes maintained in four different photoperiods. *Journal of Endocrinology* **97:** 229-242.

Lincoln, G.A. 1984. Central effects of photoperiod on reproduction in the ram revealed by the use of a testosterone clamp. *Journal of Endocrinology* **103:** 233-241.

Lincoln, G.A.; Ebling, F.J.P.; 1985. Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. *Journal of Reproduction and Fertility* **73:** 241-253.

Montgomery, G.W.; Hawker, H. 1987. Seasonal reproduction in ewes selected on seasonal changes in wool growth. *Journal of Reproduction and Fertility* **79:** 207- 213

Nagorcka, B.N. 1979. The effect of photoperiod on wool growth. In *Physiological and environmental limitations to wool growth.* Eds. J.L. Black and P.J. Reis. University of New England Publishing Unit, Armidale. pp. 127-137.

Pearce, D.T.; Oldham, C.M. 1988. Ovulation in the Merino ewe in the breeding and anoestrous seasons. *Australian Journal of Biological Sciences* **41:** 23-26.

Rollag, M.D.; Niswender, G.D. 1976. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. *Endocrinology* **98:** 482- 489.

Ryder, M.L. 1978. Growth cycles in the coat of ruminants. *International Journal of Chronobiology* **5:** 369-394.

Wayne, N.L.; Malpaux, B.; Karsch, F.J. 1990. Photoperiodic requirements for timing onset and duration of the breeding season of the ewe: Synchronisation of an endogenous rhythm of reproduction. *Journal of Comparative Physiology A* **166:** 835-842

Woodfill, C.J.I.; Robinson, J.E.; Malpaux, B.; Karsch, F.J. 1991. Synchronization of the circannual reproductive rhythm of the ewe by discrete photoperiodic signals. *Biology of Reproduction* **45:** 110-121.