Effect of chronic treatment with a GnRH agonist to suppress pulsatile LH secretion on the ability of exogenous melatonin to advance oestrous cyclicity in ewes

J. J. Robinson¹, J. M. Wallace¹, R. P. Aitken¹ and A. S. McNeilly²

¹The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK; and ²MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK

Thirty-two Scottish Blackface ewes that lambed outdoors in March and were weaned at the end of April and individually penned indoors under the natural photoperiod at 57°N were used to determine whether the ovine ovary that was deprived of gonadotrophic support was capable of early activation by melatonin. From 5 May (day 0), 16 of the ewes received an oral dose of 3 mg melatonin in a 4:1 (v:v) mixture of water and ethanol daily at 15:00 h. The remaining 16 ewes received the vehicle alone. Within each of these groups, eight were implanted s.c. on day 0 with an osmotic minipump which infused 50 µg of the gonadotrophin releasing hormone agonist (GnRH_a), buserelin day⁻¹. On day 25, a second minipump was inserted to ensure continued infusion of the agonist and on day 50 (24 June) both minipumps were removed. Ovarian activity was assessed by laparoscopy at intervals of 3 weeks from day 29 until the experiment was terminated on day 200 (21 November). Blood samples taken by jugular venepuncture three times a week for the first 50 days, daily from days 51–78 and thereafter three times a week were analysed for progesterone, prolactin and LH. Samples taken at intervals of 15 min for 10 h on days -1, 14, 28, 49, 56, 70 and 91 were assayed for LH. Treatment with GnRH_a reduced LH concentrations and abolished pulsatile LH secretion. The onset of ovarian activity (progesterone > 3.8 nmol l⁻¹) was not affected by the 50-day GnRH_a treatment and occurred for the melatonin-treated ewes at mean (±SEM) intervals from 5 May of 66 ± 2.9 (range 51–75) and 71 ± 0.9 (range 68–75) days for non-GnRH_a and GnRH_a ewes, respectively. For the ewes not receiving melatonin the corresponding intervals were 113 ± 11.6 and 125 ± 9.2 days, respectively. The mean numbers of corpora lutea at first oestrus were not affected by GnRH_a treatment and were 1.4 ± 0.13 and 1.5 ± 0.13 for control and melatonin-treated ewes, respectively. First ovulation following GnRH_a treatment resulted in luteal concentrations of progesterone of normal duration and magnitude but, within the melatonin-treated ewes, those that received GnRH_a returned to anoestrus 21 days earlier (*P* < 0.03) than did their non-GnRH_a counterparts. After their initial suppression by melatonin, prolactin concentrations began to increase after 80 days of melatonin treatment in both non-GnRH_a and GnRH_a ewes and approximately three months before the ewes returned to anoestrus. The results demonstrate that a period of isolation of the ovaries of the ewe from the pituitary gonadotrophins does not alter their ability to respond to the melatonin-induced activation of the GnRH pulse generator.

Introduction

The ewe is seasonally polyoestrous and the timing of her oestrous cyclicity by the declining photoperiod in the autumn is controlled by an increase in the activity of the gonadotrophin-releasing hormone pulse generator (Bittman, 1984). Pineal melatonin mediates this photoperiodic effect on the pulse generator (Bittman *et al.*, 1985), thus making the administration of melatonin an effective method for advancing the breeding season by up to four months (Robinson *et al.*, 1992b). A consist-

ent feature for the melatonin-induced advance of oestrous cyclicity is the 60–70 days that is required for its expression. This period represents the time taken for the extended duration of the circadian melatonin signal to increase the frequency of pulsatile LH release from the pituitary to the level that stimulates ovulation (Bittman *et al.*, 1985).

Investigations into the nature of the mechanisms involved in the stimulation of LH secretion by melatonin have used ovariectomized ewes with and without oestradiol implants (Karsch *et al.*, 1984). For ovariectomized ewes without an oestradiol implant, the photoperiodic or melatonin-induced drive of the LH pulse generator is expressed in the form of a

progressive increase in LH pulse frequency. In contrast, when ovariectomized ewes are treated with a constant release implant of oestradiol, LH pulses are partially or completely obliterated and remain so until a time that is coincident with the establishment of oestrous cyclicity in ovary-intact ewes. The ability of a constant-release oestradiol implant to align the melatonin-induced stimulation of LH secretion to the beginning of the breeding season provides the basis of current knowledge on the role of ovarian products in timing seasonal breeding.

However, there are endocrine features of the ovary-intact ewe that differ from those seen in the 'ovariectomized/oestradiol implanted' model. Oestradiol concentrations are not constant in ovary-intact ewes, neither is LH pulsatility completely obliterated during anoestrus (Martin, 1984). In the female goat, which has the same breeding season as the ewe, melatonin stimulates a progressive, as opposed to an abrupt increase, in LH pulse frequency (Chemineau *et al.*, 1988). The 'ovariectomized/oestradiol-implanted' model does not provide a test of whether these features are important in timing the response of the ovary to melatonin. Nor does it allow a direct test of whether temporary removal of pituitary gonadotrophin support influences the subsequent ability of the ovary to respond to the melatonin-induced activation of gonadotrophin secretion.

The purpose of the present study was therefore to test whether, in ovary-intact ewes, the temporary isolation of the ovaries from the pituitary gonadotrophins altered the subsequent timing of their response to melatonin. The procedure used to inhibit gonadotrophin production was that described by McNeilly and Fraser (1987) and involved downregulation of the GnRH receptor for the first 50 days of melatonin treatment by the chronic administration of a GnRH agonist (GnRH_a). In addition to identifying the onset of ovarian activity, observations were also made on ovulation rate, the duration of oestrous cyclicity and changes in the concentrations of prolactin in plasma. A brief report of the preliminary observations was presented by Robinson *et al.* (1989).

Materials and Methods

Experimental design

During seasonal anoestrus, thirty-two Scottish Blackface ewes, aged 3–4 years, were removed from their lambs, moved indoors and housed under a natural photoperiod at the Rowett Research Institute located at 57°N 2°W. The ewes were individually penned in the absence of males but could see each other and touch those immediately adjacent to them. Ewes were weighed, ranked and allocated to one of four treatments so that weights were equivalent between treatment groups (mean $\pm$ SEM, 56.7 ± 1.12 kg). All ewes were offered 1.4 kg of a complete diet supplying 12.6 MJ metabolizable energy a day.

Commencing on 5 May (day 0), sixteen ewes received 3 mg melatonin at 15:00 h each day. The melatonin (Sigma Chemical Co., Poole) was dissolved in a mixture of water and alcohol (4:1 v:v) and administered orally by a drenching gun in a final volume of 20 ml. Another 16 ewes were given the vehicle alone and acted as controls. On day 0, eight ewes from each of these groups were implanted with an osmotic minipump (Alzet osmotic pump model 2ML4; Alza Corp., Palo Alto, CA) contain-

ing 2 ml of a synthetic GnRH agonist [D-Ser (Bu)⁶] GnRH (1–9) nonapeptide-ethylamide (Buserelin; Hoechst AG, Frankfurt; infusion rate $50 \mu\text{g day}^{-1}$). The minipumps were preincubated in physiological saline at 37°C for 24 h before insertion.

The pumps were inserted under local anaesthesia, induced by filtration (10 ml Lignovet-plus, C-Vet Ltd, Bury St Edmunds), s.c. into the flank. The insertion site was closed using Michel clips. On day 25 of the study, a second pump was inserted to ensure continued infusion of the GnRH_a. Both minipumps were removed on day 50 of the study (24 June).

All ewes underwent laparoscopy two days before the start of the experiment to confirm that they were in anoestrus. Thereafter, ovarian activity, as reflected by the presence of a corpus luteum or recent corpus albicans, was recorded by laparoscopy on days 29, 50, 71, 92, 113, 134, 155, 179 and 200 corresponding to 3 June, 24 June, 15 July, 5 Aug, 26 Aug, 16 Sept, 7 Oct, 31 Oct and 21 Nov, respectively. A more comprehensive picture of ovarian activity was provided by collecting blood samples three times a week (Mon, Wed and Fri) during the 50 days that the minipumps were *in situ* and once a day from day 51 to day 78 and thereafter three times a week from day 81 to day 200. These samples were analysed for progesterone, prolactin and LH. Blood samples were collected more frequently to determine pulsatile LH secretion from four ewes per group, via a jugular cannula, at intervals of 15 min from 08:00 to 18:00 h on days –1, 14, 28, 49, 56, 70 and 91 of the study.

Hormone assays

Concentrations of LH and prolactin were measured in duplicate by specific double-antibody radioimmunoassays as described by McNeilly and Andrews (1974) and McNeilly *et al.* (1986). The sensitivities of the assays were $0.05 \text{ ng LH ml}^{-1}$ (NIADDK-oLH-25) and $0.08 \text{ ng prolactin ml}^{-1}$ (NIADDK-oPrL-17). The intra- and interassay coefficients of variation were 4.5 and 9.5% for LH and 3.6 and 6.7% for prolactin, respectively. Progesterone concentrations were measured in 100 μl aliquots of plasma by radioimmunoassay as described by Djahanbakhch *et al.* (1981). The intra- and interassay coefficients of variation were 9.5 and 12.7%, respectively, and the detection limit was 0.9 nmol l^{-1} and the recovery of progesterone from plasma was $65.7 \pm 0.3\%$ (SEM; $n = 32$).

Data analysis and definitions

The onset of ovarian activity was defined as the first rise in progesterone concentrations to exceed 3.8 nmol l^{-1} for two consecutive samples. The validity of this threshold value was confirmed by the presence of a corpus luteum at laparoscopy. An LH rise was considered to be a pulse if the value of two consecutive samples was greater than the mean of the two previous samples (basal value) and the value of at least one of the peak samples exceeded the mean basal value by more than four times the intra-assay coefficient of variation of the assay (based on Backstrom *et al.*, 1982).

Student's *t* test was used to determine the effect of melatonin treatment on the onset of ovarian activity. The LH and prolactin profile data were summarized by linear contrasts defined within

Table 1. Onset of the first luteal phase as determined by peripheral progesterone concentrations in Scottish Blackface ewes treated daily with melatonin or the vehicle alone (control) from 5 May (day 0) in the presence or absence of GnRH_a for the initial 50 days of treatment

Treatment	Onset of first luteal phase	
	Date (range)	Number of days from start of treatment (range)
Control	26 August (7 July–19 October)	113 ± 11.6 ^a (63–167)
Melatonin	10 July (25 June–19 July)	66 ± 2.9 ^b (51–75)
Melatonin plus GnRH _a	15 July (12 July–19 July)	71 ± 0.9 ^b (68–75)
GnRH _a	7 September (1 August–24 October)	125 ± 9.2 ^a (88–172)

Values are means ± SEM, *n* = 8. ^a versus ^b *P* < 0.01.

each animal and these were subjected to analysis of variance for treatment effects. The discrete LH pulse data were analysed by a generalized linear model using GENSTAT (1990).

Results

Ovarian activity

Melatonin treatment significantly advanced the date of the first luteal phase relative to controls by an average of 51 days (*P* < 0.001) (Table 1). This advance in the onset of ovarian activity occurred irrespective of whether ewes were infused with GnRH_a during the first 50 days of melatonin treatment. Similarly there was no significant difference in the date of onset of the first luteal phase in control compared with animals treated with GnRH_a only. For ewes in the melatonin and melatonin plus GnRH_a groups, the early onset of ovarian activity occurred over a total of 24 days, contrasting with 109 days in the control and 'GnRH_a only' groups (*P* < 0.001). This close synchrony in the onset of ovarian activity in both of the melatonin-treated groups is further emphasized (Fig. 1). A single control ewe that commenced ovarian activity early formed a persistent corpus luteum following her fifth ovulation. All other ewes, irrespective of treatment, had luteal phases of normal duration and magnitude. Daily melatonin treatment did not prolong the mating period. All eight ewes in the melatonin plus GnRH_a group and seven of eight ewes in the 'melatonin only' group had ceased oestrous cyclicity, as determined by peripheral progesterone concentrations, by the time the last blood sample was collected on 16 November. This was confirmed by laparoscopy 5 days later, by which time the remaining melatonin-treated ewe had also ceased ovulating. In contrast, all control and 'GnRH_a only' ewes were still cycling at this time as determined by a combination of progesterone analysis and laparoscopy. Although both groups of ewes on continuous melatonin treatment had between four and seven ovarian cycles, melatonin plus GnRH_a

treated ewes returned to anoestrus on average 21 days earlier (*P* < 0.03) than did 'melatonin only' ewes.

Ovulation rate at the first and second cycle was calculated on the basis of laparoscopic observations made at intervals of approximately 21 days throughout the study. Mean ovulation rate (± SEM) at the first and second cycle for control versus melatonin-treated ewes was not significantly different, did not change within groups between the first and second observations and was completely independent of GnRH_a treatment (first cycle, control: 1.4 ± 0.13 versus melatonin: 1.5 ± 0.13; second cycle, control: 1.3 ± 0.12 versus melatonin: 1.3 ± 0.12).

LH concentrations

In both melatonin plus GnRH_a and 'GnRH_a only' groups, plasma LH concentrations fell to baseline values by day 11 of the GnRH_a infusion (Fig. 2). Thereafter mean LH concentrations remained at basal values and were significantly lower (*P* < 0.001) than those of control and melatonin-treated ewes for the duration of the GnRH_a infusion. Concentrations of LH in plasma began to return to normal in both groups of GnRH_a-treated ewes 3–4 days after the removal of the minipumps and subsequently were similar to that of control and 'melatonin only' ewes throughout the remainder of the study.

The effectiveness of GnRH_a in abolishing pulsatile LH secretion was confirmed by the frequent measurements of LH secretion on days 14, 28 and 49 of GnRH_a infusion (see Table 2 and Fig. 3). On all 3 days, pulses could not be detected in any of the ewes infused with GnRH_a, whereas there were one or two pulses in 10 h in the controls. Six days after GnRH_a removal (i.e. day 56 of the study) the mean number of LH pulses in the GnRH_a-treated ewes had increased to about five or six (*P* < 0.001 for comparison with those that had not received GnRH_a) and this was accompanied by a corresponding decrease in pulse amplitude. These alterations in the characteristics of LH secretion were similar in both control and melatonin-treated

Fig. 1. Individual luteal phases (plasma progesterone > 3.8 nmol l⁻¹, □) for (a) control, (b) melatonin-treated, (c) melatonin plus GnRH_a-treated and (d) GnRH_a-treated ewes kept under the natural photoperiod at 57°N. The melatonin and GnRH_a treatments were imposed from 5 May (day 0) and continued for the duration of the experiment (melatonin) or for 50 days (GnRH_a).

Fig. 2. Plasma LH concentrations (means and SEM, *n* = 8 per treatment) for (a) control, (b) melatonin-treated, (c) melatonin plus GnRH_a-treated and (d) GnRH_a-treated ewes kept under the natural photoperiod at 57°N. The melatonin and GnRH_a treatments were imposed from 5 May (day 0) and continued for the duration of the experiment (melatonin) or for 50 days (GnRH_a). ⊥ denotes mean ± SEM for the onset of ovarian activity (plasma progesterone > 3.8 nmol l⁻¹).

Table 2. Treatment mean values ($\pm$ SEM, $n = 4$) for LH pulse frequency and amplitude in Scottish Blackface ewes treated daily with melatonin or the vehicle alone from 5 May (day 0) in the presence or absence of GnRH_a for the first 50 days of treatment

Day	LH pulse frequency (per 10 h)				LH pulse amplitude ($\mu\text{g l}^{-1}$)			
	Control	Melatonin	Melatonin + GnRH _a	GnRH _a	Control	Melatonin	Melatonin + GnRH _a	GnRH _a
1	1.0 $\pm$ 0.41	0.8 $\pm$ 0.25	1.3 $\pm$ 0.48	1.5 $\pm$ 0.50	3.5 $\pm$ 1.52	4.9 $\pm$ 2.53	3.1 $\pm$ 0.66	3.6 $\pm$ 1.63
14	1.0 $\pm$ 0.41	1.0 $\pm$ 0.0	0	0	4.1 $\pm$ 0.92	2.9 $\pm$ 1.28	—	—
28	1.0 $\pm$ 0.41	0.8 $\pm$ 0.25	0	0	3.3 $\pm$ 0.63	2.1 $\pm$ 0.85	—	—
49	1.5 $\pm$ 0.50	2.0 $\pm$ 1.00	0	0	2.5 $\pm$ 0.52	1.8 $\pm$ 0.46	—	—
56	2.3 $\pm$ 0.25	1.3 $\pm$ 0.25	5.3 $\pm$ 0.85	6.0 $\pm$ 0.57	2.4 $\pm$ 0.56	1.9 $\pm$ 0.18	0.8 $\pm$ 0.10	0.7 $\pm$ 0.18
70	0.8 $\pm$ 0.25	2.5 $\pm$ 1.19	3.7 $\pm$ 0.67	1.7 $\pm$ 0.33	2.7 $\pm$ 0.45	1.0 $\pm$ 0.15	0.9 $\pm$ 0.13	1.9 $\pm$ 0.20
91	1.3 $\pm$ 0.66	3.5 $\pm$ 1.32	3.5 $\pm$ 0.86	2.8 $\pm$ 0.25	4.3 $\pm$ 0.38	0.8 $\pm$ 0.12	1.1 $\pm$ 0.33	1.5 $\pm$ 0.22

Fig. 3. Representative examples of pulsatile LH secretion in (a) a control ewe, (b) a melatonin-treated ewe, (c) a melatonin plus GnRH_a-treated ewe and (d) a GnRH_a-treated ewe, on days -1, 14, 28, 49, 56, 70 and 91 of the experiment. Treatment with melatonin started on 5 May (day 0) and continued for the duration of the experiment, whereas treatment with the GnRH_a started on 5 May and terminated on 24 June (day 50). Each arrowhead denotes a significant LH pulse. Onset of ovarian activity (plasma progesterone $> 3.8 \text{ nmol l}^{-1}$) for each ewe was: control, 12 September; melatonin, 15 July; melatonin plus GnRH_a, 12 July and GnRH_a, 7 September.

Fig. 4. Plasma prolactin concentrations (means and SEM, $n = 8$ per treatment) for (a) control, (b) melatonin-treated, (c) melatonin plus GnRH_a-treated and (d) GnRH_a-treated ewes kept under the natural photoperiod at 57°N. The melatonin and GnRH_a treatments were imposed from 5 May (day 0) and continued for the duration of the experiment (melatonin) or for 50 days (GnRH_a). $\vdash$ denotes mean $\pm$ SEM for the onset of ovarian activity (plasma progesterone > 3.8 nmol l⁻¹).

ewes. At the next sampling time (day 70), the nature of the LH secretion in melatonin-treated ewes reflected the early onset of ovarian activity of these ewes in that pulse frequency tended to be higher and amplitude lower ($P < 0.05$) than for ewes not receiving melatonin. Similar effects of melatonin on LH were observed on day 91. In the absence of the GnRH_a, melatonin treatment resulted in a progressive decrease in LH pulse amplitude in the period preceding first ovulation (i.e. up to day 56, see Table 2).

Prolactin concentrations

Control ewes and those infused with the GnRH_a had similar high mean values for concentrations of prolactin in peripheral plasma of 84 ± 18 and 71 ± 10 µg l⁻¹, respectively, over the first 50 days of the experiment (Fig. 4). This period corresponded to the natural long days of summer (16 to 18 h of light). Thereafter, prolactin concentrations declined in synchrony with the natural decrease in photoperiod to mean concentrations over the last 50 days of the experiment (October and November) of 11 ± 1.9 and 13 ± 3.4 µg l⁻¹ for the control and 'GnRH_a only' groups, respectively. Removal of the GnRH_a on day 50 had no effect on prolactin concentrations. In ewes receiving melatonin either alone or in combination with GnRH_a, prolactin concentrations declined to less than 10 µg l⁻¹ by day 18 of melatonin treatment ($P < 0.001$ for comparison with controls) and this effect was independent of the GnRH_a treatment. After approximately 80 days of melatonin treatment, prolactin began to

increase to values which, in the last 50 days of the experiment, were significantly higher than for those not receiving melatonin (25 versus 12 µg l⁻¹, $P < 0.05$).

Discussion

The results reported here demonstrate that the suppression of pulsatile LH release from the pituitary during the first 50 days of melatonin treatment does not interfere with the melatonin-induced advance of the breeding season in ewes. Thus, the initiation of oestrous cyclicity following two months of melatonin treatment (Arendt *et al.*, 1988; Kennaway, 1988) is neither dependent on the ovary being subjected to a progressive increase in the frequency of the pulsatile release of LH, nor of it being exposed to a continuous episodic pattern of LH release. Although an increasing pattern of LH release is seen following an increase in the duration of the daily melatonin signal in rams (Lincoln and Short, 1980) and female goats (Chemineau *et al.*, 1988), observations from melatonin-treated ewes suggest that the pituitary gonadotrophin response is expressed as an abrupt switch from the anoestrous to oestrous pattern just before the time of first ovulation (Poulton *et al.*, 1987; Robinson *et al.*, 1991).

By obliterating pulsatile LH secretion, the present experimental model mimics the ovariectomized/oestradiol-implanted ewe during seasonal anoestrus (Karsch *et al.*, 1984). Unlike the 'ovex-model', the isolation of the ovary from the pituitary is reversible

and therefore provides direct evidence that continuous exposure of the ovaries to the pituitary gonadotrophins is not essential in timing their response to melatonin. Thus, following the programming of the central timing mechanism by melatonin, the subsequent activation of the GnRH pulse generator, which is currently thought to be controlled by specific cellular secretions from the ovine pars tuberalis (Morgan *et al.*, 1992), occurs abruptly just before the first ovulation of the breeding season. In other situations, the nature of the target organ and its function are such that a more immediate translation of the melatonin signal to the pituitary gonadotrophs appears to be needed. For example, in rams, continuous exposure to pulsatile LH is essential for the maintenance of the short-day stimulus on testicular growth (Lincoln *et al.*, 1986). In this context the observation by Lincoln and Maeda (1992) that under a long-day photoperiod the insertion of melatonin implants into the mediobasal hypothalamus (an area adjacent to the pars tuberalis) caused a rapid activation of gonadotrophin secretion and testes growth in rams is noteworthy in view of the suggestion that the pars tuberalis acts as a relay in timing the activation of the GnRH pulse generator by melatonin (Morgan *et al.*, 1992).

In the study reported here, the frequency of LH pulses on day 6 following the removal of the GnRH_a was three times that of control ewes. This did not induce ovulation in a population of follicles, the growth of which was observed by laparoscopy, six days earlier, to have been restricted to less than 2.5 mm diameter by the GnRH_a treatment. Whether oestrous behaviour occurred at about this time is unknown as the ewes were not exposed to the ram. However, if it did occur, it was not followed by an increase of progesterone in the daily blood samples taken at this time. Between 7 and 11 days after the termination of a 29-day GnRH_a treatment during the natural breeding season, McNeilly and Fraser (1987) observed behavioural oestrus with no subsequent rise in progesterone at the anticipated time. In their study, progesterone concentrations had not increased by the time the experiment ended, 15 days after the removal of the GnRH_a, i.e. 3 days before the first observed increase of plasma progesterone in a melatonin plus GnRH_a-treated ewe in the present experiment.

The mean interval from GnRH_a removal to progesterone increase for the melatonin plus GnRH_a ewes in the present experiment was 21 days (range 18 to 25), compared with 16 days (range 1 to 25) for those receiving melatonin only. The small spread in the values for the melatonin plus GnRH_a-treated ewes implies that for some of these ewes the agonist was not removed sufficiently early to allow their pituitary gonadotrophs to respond to the melatonin-induced activation of the GnRH pulse generator. On the basis that the first ovulation in the 'melatonin only' group occurred close to the time of GnRH_a removal from the melatonin plus GnRH_a group, the shortest observed interval from GnRH_a removal to the first increase in progesterone (18 days) may approximate to the minimum interval for the gonadotrophs and ovaries to recover naturally following the 50-day GnRH_a treatment. This is four to five times longer than the time taken for ovarian follicles from GnRH_a-treated ewes infused with FSH to grow from the 2.0 mm diameter seen during the period of GnRH_a treatment (Picton *et al.*, 1990) to an ovulatory size of 4–5 mm and with an ability to ovulate in response to human chorionic gonadotrophin (McNeilly *et al.*, 1991).

The 50-day GnRH_a treatment had no effect on the number of corpora lutea or the magnitude of the subsequent increase in progesterone. Within the melatonin-treated ewes, those that received GnRH_a for 50 days returned to anoestrus 21 days earlier ($P < 0.03$) than did their non-GnRH_a counterparts. We have no explanation for this finding. There was no evidence for a subtle difference between the two groups in circulating plasma prolactin concentrations coincident with their return to anoestrus. By this time, prolactin concentrations, which had initially been suppressed by melatonin, had increased to values that were significantly higher than those for the two groups not receiving melatonin. This inability of sustained melatonin treatment to suppress prolactin indefinitely has been observed by Robinson *et al.* (1992a), but whether the timing of the prolactin rise plays a role in the initiation of photorefractoriness and the return to anoestrus is uncertain; the observations of Worthy and Haresign (1983) suggest that it may play a minor or facilitatory role.

In conclusion, the results of the experiment reported here clearly demonstrate that 50 days of GnRH agonist-induced suppression of pulsatile LH release does not alter the timing of first ovulation in melatonin-treated ewes. Nor does it alter the numbers of corpora lutea or their function at first ovulation, 18 to 25 days after its removal. Thus temporary isolation of the ovaries from normal pituitary gonadotrophin production during melatonin treatment does not interfere with the ovarian response to the melatonin-induced activation of the GnRH pulse generator.

The authors thank M. Cheyne, I. Robertson and S. Wensvoort for skilled technical assistance, the NIADDK and L. E. Reichert for hormone preparations and antiserum and M. Franklin for advice on statistical analysis. The Rowett Research Institute is financed by the Scottish Office Agricultural and Fisheries Department.

References

- Arendt J, Symons AM, English J, Poulton AL and Tobler I (1988) How does melatonin control seasonal reproductive cycles? *Reproduction Nutrition Développement* **28** 387–397
- Backstrom CT, McNeilly AS, Leask RM and Baird DT (1982) Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the menstrual cycle *Clinical Endocrinology* **17** 29–42
- Bittman EL (1984) Melatonin and Photoperiodic Time Measurement: Evidence from Rodents and Ruminants. In *The Pineal Gland*, pp 155–192 Ed. Russel J. Reiter. Raven Press, New York
- Bittman EL, Kaynard AH, Olster DH, Robinson JE, Yellon SM and Karsch FJ (1985) Pineal melatonin mediates photoperiodic control of pulsatile luteinizing hormone secretion in the ewe *Neuroendocrinology* **40** 409–418
- Chemineau P, Martin GB, Saumande J and Normant E (1988) Seasonal and hormonal control of pulsatile LH secretion in the dairy goat (*Capra hircus*) *Journal of Reproduction and Fertility* **83** 91–98
- Djahanbakhch O, Swanston IA, Corrie JET and McNeilly AS (1981) Prediction of ovulation by progesterone *Lancet* **ii** 1164–1165
- Genstat 5 Committee (1990) *Genstat 5 Reference Manual* Clarendon Press, Oxford
- Karsch FJ, Bittman EL, Foster DL, Goodman RL, Legan SJ and Robinson JE (1984) Neuroendocrine basis of seasonal reproduction *Recent Progress in Hormone Research* **40** 185–232
- Kennaway DJ (1988) Short- and long-term effects of manipulation of the pineal/melatonin axis in ewes *Reproduction Nutrition Développement* **28** 399–408

- Lincoln GA and Maeda K-I (1992) Reproductive effects of placing microimplants of melatonin in the mediobasal hypothalamus and preoptic area in rams *Journal of Endocrinology* **132** 201–215
- Lincoln GA and Short RV (1980) Seasonal breeding: nature's contraceptive *Recent Progress in Hormone Research* **36** 1–52
- Lincoln GA, Fraser HM and Abbott MP (1986) Blockade of pulsatile LH, FSH and testosterone secretion in rams by constant infusion of an LHRH agonist *Journal of Reproduction and Fertility* **77** 587–597
- McNeilly AS and Andrews P (1974) Purification and characterisation of caprine prolactin *Journal of Endocrinology* **60** 359–367
- McNeilly AS and Fraser HM (1987) Effect of gonadotrophin-releasing hormone agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe *Journal of Endocrinology* **115** 273–282
- McNeilly AS, Jonassen JA and Fraser HM (1986) Suppression of follicular development after chronic LHRH immunoneutralization in the ewe *Journal of Reproduction and Fertility* **76** 481–490
- McNeilly AS, Picton HM, Campbell BK and Baird DT (1991) Gonadotrophic control of follicle growth in the ewe *Journal of Reproduction and Fertility Supplement* **43** 177–186
- Martin GB (1984) Factors affecting the secretion of luteinizing hormone in the ewe *Biological Reviews* **59** 1–87
- Morgan PJ, Barrett P, Davidson G and Lawson W (1992) Melatonin regulated synthesis and secretion of several proteins by pars tuberalis cells of the ovine pituitary *Journal of Neuroendocrinology* **4** 557–563
- Picton HM, Tsonis CG and McNeilly AS (1990) FSH causes a time-dependent stimulation of preovulatory follicle growth in the absence of pulsatile LH secretion in ewes chronically treated with gonadotrophin-releasing hormone agonist *Journal of Endocrinology* **126** 297–307
- Poulton AL, English J, Symons AM and Arendt J (1987) Changes in plasma concentrations of LH, FSH and prolactin in ewes receiving melatonin and short-photoperiod treatments to induce early onset of breeding activity *Journal of Reproduction and Fertility* **112** 103–111
- Robinson JJ, Wallace JM, Aitken RP and McNeilly AS (1989) Can melatonin time the onset of oestrous cyclicity in the ewe in the absence of pulsatile LH secretion? *Journal of Reproduction and Fertility Abstract Series No. 3*, p. 12
- Robinson JJ, Wigzell S, Aitken RP, Wallace JM, Ireland S and Robertson IS (1991) The modifying effects of melatonin, ram exposure and plane of nutrition on the onset of ovarian activity, ovulation rate and the endocrine status of ewes *Animal Reproduction Science* **26** 73–91
- Robinson JJ, Wallace JM, Aitken RP and Wigzell S (1992a) Effect of duration of melatonin treatment on the onset and duration of oestrous cyclicity in ewes *Journal of Reproduction and Fertility* **95** 709–717
- Robinson JJ, Wigzell S, Aitken RP, Wallace JM, Ireland S and Robertson IS (1992b) Daily oral administration of melatonin from March onwards advances by 4 months the breeding season of ewes maintained under the ambient photoperiod at 57°N *Animal Reproduction Science* **27** 141–160
- Worthy K and Haresign W (1983) Evidence that the onset of seasonal anoestrus in the ewe may be independent of increasing prolactin concentrations and daylength *Journal of Reproduction and Fertility* **69** 41–48