The response of rams and ewes of three breeds to artificial photoperiod

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Summary. Rams and ewes of the Romney Marsh (N = 6), Dorset Horn (N = 8) and Australian Merino (N = 8) breeds were subjected to 4 successive periods of alternating 6 h light/18 h dark ('short' days) and 18 h light/6 h dark ('long' days) preceded by 16 weeks of 12 h light/12 h dark. The initial period was of 32 weeks (16 weeks 'short' days; 16 weeks 'long' days) and the next 3 were of 24 weeks (12 weeks 'short' days; 12 weeks 'long' days).

Rams of all breeds showed a cyclic pattern of growth and regression of testes associated with plasma testosterone concentration, influenced by the change in light regimen 15–19 weeks previously. Sexual behaviour was also cyclic but lagged by some 6–7 weeks. The changes were greatest in the Romneys and least in the Merinos in which a higher degree of sexual activity was evident even when the testes were regressed ($P < 0.001$). This was the major breed difference.

All ewes of the Romney and Dorset breeds showed marked seasonality related to the imposed light regimen, whereas only 1 of the 4 Merinos did so. The mean peak of ovarian activity in the former 2 breeds coincided with that of maximum sexual activity of rams housed with them; that is, some 6 weeks after maximum scrotal volume.

The rams and ewes were subjected to serial blood sampling episodes for plasma LH and testosterone and tested for plasma LH release following GnRH administration. There was little variation between breeds in LH concentration. Testosterone concentration varied greatly in the ram, highest levels associated with the developed phase of the testes and with maximum LH pulse frequency. The LH response to GnRH changed with respect to the state of the gonads. Maximal responses were observed in the developing phase of testicular growth although this variation was greater in the Romney and Dorsets than in the Merinos ($P < 0.001$). In the ewes, maximal responses were seen in the follicular phase ($P < 0.001$), with no difference between the luteal and acyclic phases. There were no breed differences.

Plasma pooled from the serial blood sampling episodes was assayed for prolactin. Rams of each breed exhibited a cyclic pattern of plasma prolactin concentration, directly related to photoperiod ($P < 0.001$) and inversely related to testicular activity ($P < 0.001$). All Romney and Dorset ewes, but only the 1 'seasonal' Merino ewe, displayed a cyclic relationship between plasma prolactin and photoperiod ($P < 0.001$). Mean prolactin concentrations were greater in the Romney and Dorset rams ($P < 0.02$) and ewes ($P < 0.05$) than in the Merinos.

It is concluded that, although these 3 breeds followed a similar endocrinological pattern, there was a marked difference in the 'ultimate' responses (i.e. libido and oestrus) with the Merino displaying the least constraint to the imposed light regimen. This could reflect the natural and artificial selection pressures upon the Merino for unrestricted (year-round) breeding.

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Introduction

Although it is commonly accepted that the sheep is a 'short-day breeder', Robinson (1982) pointed out that most of the sheep in the world, the Merinos of Australia and South Africa, are mated in the summer, before the summer solstice. This is in the face of an overwhelming mass of evidence that sheep kept in a light-controlled environment start breeding activity after a decrease in daily light hours. Most such critical studies of photoperiodism and reproductive activity have used highly seasonal breeds such as the Suffolk (Yeates, 1949), Ile-de-France (Ravault, 1976), Préalpes (Thimonier, 1981), Clun Forrest (Ducker & Bowman, 1970a, b) and Soay (Lincoln *et al.*, 1981) and generally only one breed and sex at a time.

To examine the question of whether the Australian Merino is fundamentally different from sheep of breeds exhibiting more seasonality, an experiment was set up in which ewes and rams of several breeds were kept in the same artificially controlled photoperiod with constant temperature and nutrition. A number of endocrinological, anatomical and behavioural changes associated with changing photoperiod were measured in the highly seasonal Romney Marsh and the less seasonal Dorset Horn and Australian Merino.

The artificial photoperiod imposed was similar to that used in several recent investigations (Legan & Karsch, 1980; Kennaway *et al.*, 1983; D'Occhio *et al.*, 1984) and consisted of alternating blocks of 'long' and 'short' days.

Data recorded were scrotal sac volumes, plasma testosterone concentrations and sexual behaviour patterns in rams, and ovarian activity in ewes, based on weekly bleedings and observations. In addition, serial bleedings were carried out for comparison of the patterns of pulsatile release of LH and testosterone (Lincoln, 1976a, b; Lincoln & Davidson, 1977) and responsiveness to exogenous GnRH (Carr *et al.*, 1976; Lincoln, 1976a, 1979; Evans & Robinson, 1980b). Finally, in view of the controversy concerning the functional significance of seasonal changes in plasma prolactin and reproductive phenomena (Pelletier, 1973; McNeilly & Baird, 1977), serial bleedings were carried out at different periods of the photoperiodic regimen and the data for plasma prolactin concentration were related to those for reproductive phenomena.

Materials and Methods

Controlled environment

The two controlled environment rooms described by Evans & Robinson (1980a) were used. Each measured $7 \times 5 \times 3.5$ m. Internal lighting was provided by ceiling-mounted fluorescent tubes giving cool white illumination, at floor level, of 350–380 lux during the 'day' and total darkness during the 'night'. The rooms were continuously ventilated by a thermostatically regulated evaporative cooler and extractor fan. With the aid of ceiling-mounted radiators operating during the colder months, temperature was maintained close to 22°C.

From their introduction on 4 May 1979, early autumn in the southern hemisphere, the animals in both rooms were subjected to an adjustment period of 16 weeks of 'constant-light' days (12 h light/12 h darkness). This was followed by the 120-week long experimental period of 3 alternating 16-week and 6 alternating 12-week blocks of 'short' days (6 h light/18 h dark) and 'long' days (18 h light/6 h dark) to provide 32-week and 24-week light 'years'.

'Lights on' always occurred at 08:00 h during 'short' and 'long' days respectively. For convenience, the automatic light switch was routinely adjusted to NSW Eastern Summer ('daylight-saving') time.

Each room had an identical light cycle, but each operated out-of-phase to avoid residual or an external effect on the reaction pattern to the imposed photoperiodic regimen.

Experimental animals

Four mature rams and 4 maiden ewes of each of the breeds, Romney Marsh, Dorset Horn and Merino, were reared at Bathurst, 250 km west of Sydney, and at the same latitude (33°25'S), before being transported to Sydney in April 1979. Two Rams and 2 ewes of each breed were allocated to each of the rooms. One Romney Marsh ewe and one Romney Marsh ram died of unknown causes early in the experiment. Data from these animals were discarded.

Animals received a maintenance ration of lucerne and oaten chaff, supplemented by salt licks; they were vaccinated against clostridial infections (Vaxal 5 in 1; Websters, Sydney), regularly drenched (Ranide; Merck Sharp & Dohme, Sydney), shorn twice a year, and weighed every 4 weeks. They had free access to fresh water.

Data recorded for rams

Scrotal sac volume. The method was as described by Evans & Robinson (1980b). A 2 litre beaker was filled with lukewarm water and scrotal sac volume was estimated by the amount of water displaced by the immersion of the entire shaven sac, with the ram standing in the normal position. Volumes were measured, concurrently with bleedings for assay of plasma testosterone, during the 16-week settling-in period and from Week 0 to Week 118 of the experimental observation period.

Plasma testosterone. Rams were bled weekly, from Weeks 0 to 118 for assay for plasma testosterone concentration.

Sexual behaviour. Sexual behaviour tests were conducted at weekly intervals from Week 9 to Week 96, using the reaction of the ram to an ovariectomized ewe implanted with oestradiol benzoate. A ewe was introduced to the pen containing the 6 rams, in each of the 2 rooms, and was left for 15 min, and the subsequent behaviour of each ram was recorded. The behaviour pattern for each ram was classed as described by Banks (1964) and Lees (1965). In general, there was a sequential pattern which was scored on a 6-point scale, as follows: (0) no interest; (1) sniffing of genitalia; (2) aggression; (3) Flehmen; (4) courtship; (5) active attempts to mount.

Pituitary-hypothalamic activity. Assays for LH and testosterone were performed on plasma collected in 20 serial blood sampling episodes, spanning 4 cycles of alternating 'long' and 'short' phases of light, according to the following schedules. During Weeks 24–48 (4 episodes), there were 2-h bleeding intervals for 12 h (7 samples per ram; 77 per episode) every 8 weeks. During Weeks 52–112 (16 episodes), there were 1 h bleeding intervals for 12 h (13 samples per ram; 143 per episode) every 4 weeks. The latter, more intensive, bleedings were designed to detect peaks of LH and testosterone. More frequent bleeding was physically impossible as the ewes housed with the rams were bled concurrently, always in the same order. Hourly bleedings were judged to be adequate, based on the evidence of Lincoln & Peet (1977) and Lincoln *et al.* (1977) and of Pelletier *et al.* (1982) who calculated that hourly sampling would detect 90% of LH and testosterone peaks. Allowing for the admitted lower sensitivity of our LH assay, it was expected that the large numbers of episodes and the long period over which they were to be studied would yield a valid picture of events.

The data were expressed as: (1) mean plasma concentration; (2) basal plasma concentration; (3) pulse frequency; (4) mean pulse amplitude and (5) time of each pulse.

Mean and basal concentrations were defined as the average for all 13 bleeds and for the 6 with the lowest concentrations. A pulse was defined as an increase in the concentration of the hormone that exceeded the basal level by at least 2 standard deviations, was greater than the estimated intra-assay variation at that level, and which was followed by a decrease. The amplitude of a pulse was defined as the increase in hormone concentration above the basal level. A raised hormone concentration at the time of the first bleeding episode (08:00 h) followed by a decrease or an increase in hormone concentration at the time of the last bleeding episode (20:00 h) was considered a pulse. LH and testosterone pulses were considered coincident if a testosterone pulse occurred simultaneously or 1 h after an LH pulse.

Pituitary responsiveness to GnRH. Plasma LH concentrations were measured after a standard injection (i.v.) of 10 µg GnRH (lot 41-550-AL, Abbott Laboratories, IL, U.S.A.), at intervals of 4 weeks on 28 occasions. Bleeding was at intervals of 30 min from 0 (08:30 h) to 4 h after injection.

Plasma prolactin. Plasma was collected in 20 serial blood sampling episodes, commencing 24 weeks after the rams had entered the controlled environment rooms, by which time testicular activity was entrained to the imposed light regimen. For the first 4 sampling episodes, at intervals of 8 weeks, bleeding was at intervals of 2 h. For the remaining 16 sampling episodes, at intervals of 4 weeks, bleeding was at intervals of 1 h.

Data recorded for ewes

Ovarian activity. Blood plasma was collected and stored for progesterone assay twice weekly during the 16-week settling-in period of 12 h light/12 h dark and the subsequent 120 weeks of experimental observations. A period of ovarian activity/cyclicity was indicated by a block of fluctuating plasma progesterone concentrations representing a series of oestrous cycles. An oestrous cycle was defined as at least 2 consecutive progesterone values > 1.0 ng/ml followed by a decrease, with 2 or more such cycles constituting a period of ovarian cyclicity. It was not possible to monitor oestrous behaviour within the confines of the environment rooms.

Pituitary-hypothalamic activity. Assays for LH were performed on plasma collected at intervals of 2 h in 20 serial blood sampling episodes, each of 12 h spanning 4 cycles of alternating 'long' and 'short' phases of light, from Weeks 24 to 112. In addition, LH assays were performed on plasma samples collected from the Romney and Merino ewes, at intervals of 20 min, over a period of 6 h, in 4 serial blood sampling episodes at Weeks 94, 101, 109 and 117. The data were as defined above for the rams. The Romneys and Merinos were chosen as representing extremes of seasonality.

The first plasma sample of each bleeding episode was also assayed for progesterone in order to define ovarian activity.

Pituitary responsiveness to GnRH. This was tested as described above for rams.

Plasma prolactin. Blood was collected for measurement of prolactin concentrations as described above for rams.

Bleeding procedure

Blood (10 ml) was collected by jugular venepuncture using a 21-gauge needle and the blood was withdrawn into evacuated sodium heparinized tubes (Vacutainer; Becton-Dickinson, NJ, U.S.A.). During dark hours, samples were

taken using the faint illumination of a red-filtered headlamp. All samples were immediately centrifuged and the plasma pipetted into plastic storage vials which were stored at -20°C until required for assay.

Assay procedure and validation

Testosterone. The radioimmunoassay was as described by Evans & Robinson (1980b), based on the method of Corker & Davidson (1978), except for the source and preparation of antiserum and standard solutions.

Testosterone antiserum (code 457/1208130976) was kindly provided by Dr R. I. Cox and Mr N. Hinks of the CSIRO Division of Animal Production, NSW, Australia, and was used at a final concentration of 1:80 000. Cross-reactions were: 5 α -dihydrotestosterone, 98%; 4-androsten-3 β ,17 β -diol, 47%; 4-androsten-17 β ,19-diol-3-one, 3.6%; androsterone, 1%; other steroids, <1%. Testosterone (4-androsten-17 β -ol-3-one, Cat.No.213-1, Research Plus Steroid Laboratory, Denville, NJ, U.S.A.) was used to prepare standards in plasma from ovariectomized ewes treated with dexamethasone phosphate (Roussel Uclaf, France) which provided testosterone values indistinguishable from those of buffer and water blanks.

The extraction efficiency of the assay was tested on 5 occasions and had a mean $\pm$ s.e.m. value of $94 \pm 4\%$. Non-specific binding was consistently <5%. Control samples of 0.5 and 5.7 ng testosterone/ml had inter-assay coefficients of variation of 14.3 and 11.2% and intra-assay coefficients of variation of 8.6 and 6.9% respectively. The limit of assay sensitivity, expressed as the value of 2 standard deviations below maximum (zero testosterone standard) binding was 30 pg/ml for 100 μ l plasma assayed.

LH. Plasma LH was estimated by radioimmunoassay based on the method of Gidley-Baird & Bindon (1976) as described by Evans & Robinson (1980b), except for the preparation of antiserum, label and standard solutions.

LH antiserum (UWA 3B) was kindly provided by Professor D. R. Lindsay and Dr G. B. Martin, Department of Animal Science and Production, University of Western Australia. Cross-reactions were: thyroid-stimulating hormone (NIH-S8), 9.4%; follicle-stimulating hormone (NIH-S12), prolactin (NIH-S12), growth hormone (NIH-S11) <1%. Diluted antiserum (1:40 000 in Tris-NaCl (1 M-NaI) buffer) was added (0.5 ml) to assay tubes and incubated at 37°C for 2 h. Ovine LH (oLH 22-NIAMDD; Bethesda, MD, U.S.A.) was used to prepare standards (0–40 ng/ml) in plasma from rams treated with oestradiol benzoate which provided LH titres indistinguishable from buffer and water blanks. ^{125}I -labelled LH (0.16 ng LH/ml; 12 000 c.p.m.) was added (0.1 ml) to all tubes.

Non-specific binding was always <3%. Control samples of 0.5, 1.0, 5.5 and 11.1 ng LH/ml had inter-assay coefficients of variation of 19.4, 16.7, 11.2 and 10.6% and intra-assay coefficients of variation of 15.6, 10.3, 7.9 and 7.0 respectively. The limit of assay sensitivity, expressed as the value of 2 standard deviations below maximum (zero LH standard) binding, was 0.3 ng/ml for 100 μ l plasma assayed.

Prolactin. For the first 6 sampling episodes, separate assays were performed on samples pooled from the 08:00–14:00 h and the 14:00–20:00 h bleedings. For the remaining episodes, assays were performed on plasma pooled for the entire bleeding episode. The assays were kindly carried out by Dr D. J. Kennaway, Department of Obstetrics and Gynaecology, University of Adelaide, using the double-antibody radioimmunoassay (RIA) described by Kennaway *et al.* (1981) and an antibody to NIH-prolactin-S10 and sheep anti-rabbit serum. NIH-prolactin-S10 was used as a standard and also for iodination. Cross-reactivity with ovine LH (NIH-LH-S19), FSH (NIH-FSH-S10) and TSH (NIH-TSH-S8) was <1%.

Plasma samples (in duplicate) were processed in 2 assays. Samples from serial bleeds 1–6 were included in the first assay and samples from serial bleeds 7–20 were included in the second. In Assay 1, intra-assay coefficients of variation at 25.5 and 83.0 ng/ml were 9.8 and 22.0% respectively. In Assay 2, intra-assay coefficients of variation at 26.0 and 71.0 ng/ml were 13.0 and 13.0% respectively. Assay sensitivity was 3 ng/ml.

Progesterone. Plasma progesterone was estimated by radioimmunoassay, as described by Pearce & Robinson (1985), based on the method of Thorneycroft & Stone (1972).

Progesterone antiserum was prepared by Olivia Gaston-Parry in our laboratory and was used at a final concentration of 1:12 000. Cross-reactions were: 17 α -hydroxyprogesterone, 3.4%; 2 α -dihydroprogesterone, 1%; other steroids, <1%. Progesterone (4-pregnene-3,20-dione, Cat.No. P-0130, lot 25c-0106, Sigma, MO, U.S.A.) was used to prepare standards in buffer. The extraction efficiency of the assay was tested on 6 occasions and had a mean $\pm$ s.e.m. value of $81 \pm 3\%$. The extracted water blank was always <45 pg progesterone/tube and non-specific binding was consistently <4%. Control samples of 0.6 and 5.3 ng progesterone/ml had inter-assay coefficients of variation of 14.8 and 11.5% and intra-assay coefficients of variation of 11.3 and 9.1% respectively. The limit of assay sensitivity, expressed as the value of 2 standard deviations below maximum (zero progesterone standard) binding was 0.27 ng/ml for 100 μ l plasma assayed.

Statistical analyses

Time lag regression analysis, as used by Evans & Robinson (1980b), was applied to the data for scrotal sac volume and testosterone concentration. Analysis of variance was applied to all ram data which, for each breed, were assumed to be equally influenced by the experimental environment. Variance between rooms and between animals of the same room was accounted for in the analysis. Overall variance for the 'time' component was partitioned into 'treatment effects' based upon the phase of testicular activity with which the data were associated and an orthogonal comparison was made between 'effects'.

The pattern of testicular development and regression induced by the imposed photoperiod was classified, according to Lincoln (1976a), into 'regressed', 'developing', 'developed', and 'regressing' phases for some analyses.

Analysis of variance was applied to all ewe data as described for the rams. Remaining variance was partitioned into a 'phase effect' derived from the phase of ovarian activity with which the data were associated.

Split-plot analysis of variance after $\log_{10}(x + 1)$ transformation was applied to the ram and ewe data for plasma prolactin to partition time and breed effects. Reconverted pooled means, with 95% fiducial limits, were calculated for 'long' and 'short' days.

Pooled ram and ewe data were subjected to non-orthogonal analyses of variance to examine breed effects.

Results

Rams

Testicular activity. During the 16-week settling-in period, scrotal sac volume and plasma testosterone concentrations increased. With the imposition of the 'short' or 'long' day regimen, rams in both rooms rapidly entered a cyclic pattern which was indistinguishable between the two rooms, thereby enabling pooling of the data (Fig. 1). Four complete cycles are illustrated for each of the 3 breeds.

Fig. 1. Mean patterns of response to artificial photoperiod of scrotal sac volume, plasma testosterone and sexual behaviour of rams of 3 breeds, showing the deviations from the overall mean (—): Romney Marsh ($N = 3$) (---); Dorset Horn ($N = 4$) (-.-.); Australian Merino ($N = 4$) (....). Only the sexual behaviour score of the three breeds was significantly different ($P < 0.001$).

Table 1. Interval (weeks) between former photoperiod and maximum response of two measures of testicular activity

Breed	Scrotal sac volume		Plasma testosterone	
	32-week light cycle	24-week light cycle	32-week light cycle	24-week light cycle
Romney	17	17	17	17
Dorset	16	19	16	16
Merino	15	17	14	15
Mean	16	18	16	16

Periods of testicular development coincided with alternating blocks of 'long' and 'short' days with the peak in scrotal sac volume about the centre of each of the blocks of 'short' days during the 32-week light cycle and towards the end of each of the blocks of 'short' days during the 24-week cycle. Plasma testosterone concentrations followed a similar pattern. These patterns were exhibited by rams of each breed but the amplitude of the peaks and troughs appeared largest and best defined in the Romney and least in the Merino. Analysis of the data for the periods when the testes were 'regressed' or 'developed' showed a significant 'seasonal' or time effect ($P < 0.001$) for scrotal sac volume and plasma testosterone concentration and a marginally higher scrotal sac volume for the 32-week light cycle compared with the 24-week cycle ($P < 0.1$). There were no significant effects of breed and no breed $\times$ time interactions.

Time lag regression analysis was carried out separately on the 32- and 24-week cycles. For both, scrotal sac volume and plasma testosterone concentration of all breeds were influenced by the light regimen of some 15–19 weeks previously (Table 1).

The mean values for testicular volume (ml) and for plasma testosterone concentration (ng/ml) did not differ with breed. The means, with 95% fiducial limits, were: 1176 (973–1422) and 3.2 (2.3–4.4) for Romneys, 1249 (1053–1480) and 2.4 (1.8–3.1) for Dorsets, and 1153 (973–1367) and 2.5 (1.8–3.2) for Merinos.

Sexual behaviour. Figure 1 illustrates the pattern of behaviour relative to scrotal sac volume and plasma testosterone concentration. Maximum sexual activity in all 3 breeds of rams was associated with the end of the 'developed' phase and the beginning of the 'regressing' phase of testicular activity, with an interval of about 6–7 weeks between maximum scrotal sac volume and peak sexual response. Overall mean $\pm$ s.e.m. values of sexual behaviour tests pooled over the regressed and developed phases of testicular development were 0.3 ± 0.12 and 1.2 ± 0.19 (Romney), 0.9 ± 0.14 and 1.9 ± 0.16 (Dorset) and 1.9 ± 0.20 and 2.4 ± 0.16 (Merino). There were significant differences between 'regressed' and 'developed' stages and between breeds ($P < 0.01$) with a linear progression from the Romney through the Dorset to the Merino. During the regressed phase, the Romneys displayed little, if any, sexual activity. By contrast, the Merino at this stage exhibited activity comparable to that of the Romneys at their peak. The overall difference between rams of these breeds (Fig. 1) was significant ($P < 0.001$).

Pituitary-hypothalamic activity. Secretion of LH and of testosterone was pulsatile, with 453 pulses of LH and 510 of testosterone recorded, of which 74.4% were coincident.

Figure 2 presents data for mean concentrations and pulse frequencies of LH and testosterone, relative to the light regimen and the stage of testicular development. Mean concentrations of plasma LH varied little with time. By contrast, testosterone varied greatly ($P < 0.01$), highest concentrations being associated with the 'developed' phase of the testes. This was associated with increased LH pulse frequency during the 'developing' and 'developed' phases compared with the 'regressing' and 'regressed' phases ($P < 0.05$). Basal testosterone concentrations followed a similar pattern.

Fig. 2. Mean plasma LH (●—●) and testosterone (×—×) concentrations of Romney Marsh (N = 3), Dorset Horn (N = 4) and Australian Merino (N = 4) rams in relation to artificial photoperiod and consequent testicular development (overall mean profile of scrotal sac volume measurements redrawn from Fig. 1). Periods of 'developed' (—) and 'regressed' (---) phases of testicular activity are indicated.

Fig. 3. Plasma LH concentrations (mean $\pm$ s.e.m.) after an injection of GnRH (10 μ g) to Romney Marsh (N = 3), Dorset Horn (N = 4) and Australian Merino (N = 4) rams, in relation to phase of testicular activity. The number of individual hormone profiles per phase is shown in parentheses. The between-phase variation is significant for the Romneys and Dorset ($P < 0.001$) but not the Merinos.

The LH pulse amplitude appeared generally greater during the 'developing' phase than at other phases, but this was significant only in the Romneys and Merinos ($P < 0.05$). Overall, the Dorset rams had slightly higher basal and mean plasma LH concentrations than did the rams of the other 2 breeds ($P < 0.05$), associated with higher LH pulse frequency. This difference was not reflected in the concentration of plasma testosterone.

Pituitary responsiveness to GnRH. LH concentrations after administration of GnRH were pooled for each breed under the common light regimen and grouped according to the phase of testicular activity (Fig. 3). Overall, there were no breed differences in mean or peak concentrations of plasma LH or in the time of the peak which, with 95% fiducial limits, were 3.2 (2.8–3.6) ng/ml for the mean, 6.1 (5.3–6.9) ng/ml for the peak, and 1.2 (1.0–1.4) h for the time. Plasma LH rose to peak concentrations within 0.5–2.5 h and fell to basal levels by 4 h.

There was a significant difference in response to GnRH related to phase of testicular activity. Maximum LH response occurred during the 'developing' phase and minimum response during the 'regressing' phase in all breeds. This was significant only in the Romneys and Dorsets ($P < 0.001$); the between-phase difference in the Merinos was much less evident. There was also a significant

Fig. 4. Plasma LH concentrations (mean $\pm$ s.e.m.) after an injection of GnRH (10 μ g) to Romney Marsh (N = 3), Dorset Horn (N = 4) and Australian Merino (N = 4) rams in relation to artificial photoperiod and consequent testicular development (overall mean profile of scrotal sac volume measurements redrawn from Fig. 1). Periods of 'developed' (—) and 'regressed' (---) phases of testicular activity are indicated.

Table 2. Plasma prolactin concentrations (ng/ml) of rams pooled with respect to photoperiod

Breed	Photoperiod				Overall	
	<i>n</i>	'Short days'	<i>n</i>	'Long days'	<i>n</i>	By breed
Romney	28	20 (15–27)	32	76 (57–101)	60	41 (31–53)
Dorset	39	17 (13–21)	37	66 (53–82)	76	33 (26–41)
Merino	40	9 (6–14)	40	32 (23–44)	80	17 (13–23)
Overall	107	14 (11–17)	109	53 (44–63)	216	27 (23–32)

Values are means with 95% fiducial limits in parentheses; *n* = no. of samples.

difference in the pattern of release of LH related to phase; release was much more rapid during the 'regressed' phase and the decline faster than during the 'developed' phase in all breeds ($P < 0.01$).

The mean and peak concentrations of plasma LH declined with time and the passage from the 32-week to the 24-week light cycle ($P < 0.001$) but remained linked throughout with the light/dark pattern and the associated changes in testicular growth and regression (Fig. 4).

Plasma prolactin. There was no significant diurnal effect upon plasma prolactin concentrations in any breed and no apparent change in overall prolactin release after the transition from the 32-week to the 24-week light cycle.

Table 2 presents data for mean plasma prolactin concentrations, partitioned into 'long' and 'short' day periods and breed. There was a significant relationship with the light regimen ($P < 0.001$) in that highest plasma prolactin concentrations were associated with long daylength and lowest with short daylength. Figure 5 presents the data for all rams, pooled under the common light regimen and illustrates this relationship and also that with scrotal sac volume. High and low plasma prolactin concentrations were associated with low and high degrees of testicular activity with a significant difference between the 'developed' and 'regressed' phase in all breeds ($P < 0.001$). Mean plasma prolactin concentrations, in the Romney and Dorset rams were higher than in the Merino rams ($P < 0.02$).

Ewes

Ovarian activity. During the 16-week settling-in period of 12 h light/12 h dark, all 4 Merino ewes exhibited continuous cyclic ovarian activity and this continued for 31–41 weeks after the imposition of alternating photoperiod. By contrast, the Romneys and Dorsets exhibited a variable pattern during the initial 16-week period and fell into a 'seasonal' pattern related to the imposed light regimen within 20 weeks (Fig. 6). Individual ewes displayed up to 5 discrete periods of cyclic ovarian activity which coincided with alternating blocks of 'long' and 'short' days. Cyclic behaviour generally began during the period of 'short' days, peaked early during the 'long' days and terminated towards the end.

The Merino ewes exhibited extremely variable ovarian responses. One ewe had a pattern similar to that of the Romneys and Dorsets. Of the remaining 3, 1 exhibited 2 prolonged periods of cyclic ovarian activity and 2 had 3 such periods separated by a brief period of anoestrus. These periods bore no obvious relationship to the imposed light regimen.

The initial period of ovarian activity appeared rather longer in the Dorsets than in the Romneys, but thereafter there was no apparent difference. The interval between the mean peaks of activity decreased from 34 weeks after the 32-week light cycle to 24 weeks after transition to that of a 24-week cycle (Fig. 6).

Pituitary-hypothalamic activity. Bleeding at intervals of 2 h for periods of 12 h revealed little indication of pulsatile LH release and no relationship with the presence or absence of cyclic

Fig. 5. Plasma prolactin concentrations (mean $\pm$ s.e.m.) of Romney Marsh ($N = 3$), Dorset Horn ($N = 4$) and Australian Merino ($N = 4$) rams in relation to artificial photoperiod and consequent testicular development (overall mean profile of scrotal sac volume measurements redrawn from Fig. 1). Periods of 'developed' (—) and 'regressed' (---) phases of testicular activity are indicated. The overall mean plasma prolactin concentration for the Merinos is less than for the Romneys and Dorsets ($P < 0.02$).

Fig. 6. Mean patterns of response to artificial photoperiod of cyclic ovarian activity, as determined by plasma progesterone concentrations, of ewes of 3 breeds. The vertical lines indicate the mean centres of activity for the 'seasonal' Romney Marsh ($N = 3$) and Dorset Horn ($N = 4$) ewes. Only one of the Australian Merinos ($N = 4$) was 'seasonal'.

activity. There were no differences in basal and mean plasma LH concentrations between the 32- and 24-week light cycles or within oestrous cycles as indicated by the phase of ovarian activity. There were no effects of breed.

More frequent bleeding at intervals of 20 min indicated a pulsatile mode of secretion of LH which occurred at random throughout the sampling period. Again, there was no significant effect of phase of ovarian activity on basal or mean plasma concentration of LH or on LH pulse frequency or amplitude.

Pituitary responsiveness to GnRH. LH concentrations after administration of GnRH were pooled for each breed and grouped according to the phase of ovarian activity (Fig. 7). Plasma LH rose to peak concentrations within 0.5 to 2.0 h falling to basal values after 4.0 h. Overall, the release of LH was extremely variable both within and between periods of cyclic and acyclic activity. This pattern was similar in all ewes and was related to the phase of ovarian activity. Maximal responses were observed in the follicular phase ($P < 0.001$) in all breeds and in particular in the Romneys and Merinos. Responses in the luteal and acyclic phases were similar. There were no differences between breeds in mean or basal concentrations of plasma LH and no effect of duration of the light cycle.

Plasma prolactin. As for the rams with which the ewes were housed, there was no diurnal effect on plasma prolactin concentration.

Fig. 7. Plasma LH concentrations (mean $\pm$ s.e.m.) after an injection of GnRH (10 μ g) to Romney Marsh ($N = 3$), Dorset Horn ($N = 4$) and Australian Merino ($N = 4$) ewes, in relation to phase of ovarian activity. The number of individual hormone profiles per phase is shown in parentheses. The differences between phases were significant ($P < 0.001$) but there was no effect of breed.

Table 3. Plasma prolactin concentrations (ng/ml) of ewes pooled with respect to photoperiod and phase of ovarian activity

Breed	<i>n</i>	Phase of ovarian activity				Overall			
		'Short days'	<i>n</i>	'Long days'	<i>n</i>	Cyclic	<i>n</i>	Acyclic	<i>n</i> By breed
Romney	28	31 (23–41)	32	99 (76–129)	31	48 (35–66)	29	70 (50–98)	60 58 (43–75)
Dorset	40	28 (21–38)	40	73 (54–98)	45	39 (29–53)	35	56 (40–78)	80 45 (35–57)
Merino	40	24 (17–33)	40	33 (24–46)	56	24 (19–30)	24	40 (28–57)	80 28 (23–34)
Overall	108	28 (24–33)	112	60 (51–71)	132	33 (28–39)	88	55 (45–67)	220 41 (34–48)

Values are means with 95% fiducial limits in parentheses; *n* = no. of samples.

Fig. 8. Plasma prolactin concentrations (mean $\pm$ s.e.m.) of Romney Marsh ($N = 3$), Dorset Horn ($N = 4$) and Australian Merino ($N = 4$) ewes in relation to artificial photoperiod and consequent ovarian activity. Periods of cyclic activity are indicated (redrawn from Fig. 6). The overall mean plasma prolactin concentration for the Merinos is less than for the Romneys and Dorsets ($P < 0.05$).

Table 3 presents data for plasma prolactin concentrations, partitioned into photoperiod, cyclic or acyclic phases of ovarian activity and breed. There was distinct evidence of cyclicality but it was difficult to determine whether this was associated with light regimens or individual cycles of ovarian activity. Significantly higher plasma prolactin concentrations were therefore associated with 'long' days than for 'short' days for all Romney and Dorset ewes ($P < 0.001$) but for only one of the Merinos, the ewe that exhibited cyclic ovarian activity entrained to photoperiod. Overall, concentrations were higher during acyclic than cyclic ovarian activity ($P < 0.02$) and particularly so in the Merinos. Mean prolactin concentrations in the Romney and Dorset ewes were higher than in the Merino ewes ($P < 0.05$).

These relationships are illustrated in Fig. 8 in which the data for all ewes are pooled under the common light regimen.

Discussion

All rams of all breeds showed a similar pattern of testicular growth and regression under the influence of the photoperiodic regimen, with an associated pattern of change in mean plasma testosterone. All showed a pulsatile pattern of synchronous secretion of LH and testosterone. The 74.4% coincidence of peaks accords well with the estimate of 90% of Pelletier *et al.* (1982) as attainable with hourly bleedings, bearing in mind the lower sensitivity of our LH assay. Furthermore, as reported by Sanford *et al.* (1974, 1977) and others, there was an increase in pulse frequencies with transition to higher breeding activity. As observed by Lincoln (1976a) for Soay rams, the greatest frequency of LH and testosterone pulses was associated with the fully developed phase of the testes. The amplitude of testosterone pulses was also greatest, when testicular size was maximal, as shown by Lincoln (1976a) and Schanbacher & Ford (1977). There was no evidence of a breed effect on the seasonal pattern of episodic release of LH and testosterone.

The relatively greater response of rams of all breeds to exogenous GnRH during the regressed and developing stages of the scrotum than during the developed and regressing stages was in general agreement with the results of Lincoln (1976a) for the very seasonal Soay rams and of Evans & Robinson (1980b) for the less seasonal Dorsets.

Previous attempts to establish breed differences with respect to pituitary activity have, for the most part, been unsuccessful (Bindon *et al.*, 1974; Hanrahan *et al.*, 1981). However, D'Occhio *et al.* (1984) have studied several characteristics similar to those reported here, under a similar photoperiodic environment: their results were similar to ours in some, but not all, respects. In both studies, all breeds exhibited greater pituitary/testicular activity during short than long days and plasma prolactin concentration studied was higher during long than short days. The Rambouillet rams studied by D'Occhio *et al.* (1984) behaved as did our Merinos in that prolactin concentrations were lower than in other breeds, but D'Occhio *et al.* (1984) reported more frequent and higher peaks of LH and testosterone, and of FSH, in the more seasonal than the less seasonal breeds during the periods of short days (but not the long) and they concluded that there were distinct breed differences in hormone secretion, related to seasonality. We also observed breed differences in plasma LH but, although significant, they were not large. Further, with one exception, they cannot be related to seasonality of reproduction. It is difficult, therefore, to reconcile the relatively high basal and mean plasma LH concentrations observed in the Dorset rams with characteristic seasonality. On the other hand, the greatest variation in mean LH release after administration of exogenous GnRH related to testicular activity, was apparent in the very seasonal Romney rams and the least was seen in the Merinos. These results suggest a possible difference in pituitary responsiveness between rams of breeds such as the Romney with a short breeding season and those such as the Merino with a long season. The distinct breed difference in plasma prolactin concentrations observed in both studies, we consider to be an index of general sensitivity to photoperiod rather than a causal factor in the regulation of seasonal reproduction.

Despite the close similarity between breeds in their qualitative and quantitative endocrinological changes, under the influence of changing photoperiod, there was a marked difference in libido. Merino rams maintained a consistently higher sexual behaviour score than did the Romneys, despite plasma testosterone concentrations indistinguishable between breeds at all stages of testicular development. This suggests either that the Merinos were more sensitive to endogenous testosterone than the Romneys or that some factor additional to testosterone was involved in the behavioural response. In this context, Sanford *et al.* (1982) have suggested that oestradiol is associated with mating activity of rams, so that increased conversion of testosterone to oestradiol is one possible explanation.

There is a considerable time lag between the almost coincident time of maximum testicular volume and plasma testosterone concentration and of subsequent maximum sexual activity. In this study, and in those of Lincoln & Davidson (1977) and Evans & Robinson (1980b), maximum plasma testosterone concentration coincided with or slightly preceded by about 2 weeks, that of

maximum scrotal sac volume. On the other hand, maximum sexual activity did not develop until some 6 or 7 weeks later.

Care must be exercised in drawing conclusions about breed characteristics from such a small sample. However, the results for the ewes (and for the rams) are so clear-cut and fit such a long-standing set of field and other observations that some generalities are justified.

First of all, there was a basic pituitary-hypothalamic response to changed photoperiod in all ewes of all breeds. Despite this, there was a marked breed difference in the response of the gonads and, as a consequence, the pattern of cyclic activity. The Merino ewes showed little evidence of being entrained to the light regimen, whereas ewes of the other breeds were closely controlled. In the rams, a cyclic pattern of testicular growth and regression was associated with marked changes in libido of Romneys but not of Merinos, with Dorsets intermediate.

Secondly, a time lag between peak testicular activity and ovarian cycles has been reported by Evans & Robinson (1980b), and this is now confirmed. There is a similar time lag between maximum scrotal sac volume and aggressiveness (Lincoln & Davidson, 1977) and maximum sexual activity. The interval approximates to the duration of the spermatogenic cycle (Ortavant, 1959), in that maximum numbers of available spermatozoa could be expected after maximum size of the testes had been attained and at the peak of ovarian activity. This coincidence of expected maximum numbers of spermatozoa and sexual drive in rams and ovarian activity in ewes is particularly evident in the more seasonal breeds. In the Merino there appears to be much more latitude.

Thirdly, as for the rams, there was no measurable breed difference in the pulsatile pattern of release of LH, and there were no differences between the follicular (pro-oestrus), luteal and acyclic phases of ovarian activity. This observation differs from some earlier reports, reviewed by Baird & McNeilly (1981), that plasma concentrations of LH are relatively high during the follicular phase. This apparent anomaly may be related to differences in bleeding interval (Martin *et al.*, 1983). On the other hand, there was a clear relationship between the response of the pituitary to injected GnRH and the phase of ovarian activity. More LH was released during the follicular phase than during the luteal or acyclic phases, as reported by Reeves *et al.* (1971) and Foster & Crighton (1976). However, there were no changes in the pattern of release during the cyclic or acyclic phases to indicate the imminent termination or onset of cyclicity, nor were there any breed differences.

Fourthly, the role of prolactin in seasonal breeding has been the focal point of a vast amount of research. In the current study, high and low prolactin concentrations were significantly associated with long and short days in the Romney and Dorset ewes, and this correlated with 'seasonal' ovarian activity, a finding in accord with that of Kennaway *et al.* (1983) for Finn $\times$ Dorset/Rambouillet ewes housed under conditions similar to those used for the present ewes and which were equally seasonal. By contrast, our Merino ewes showed no such regulatory influence of photoperiod upon prolactin secretion. However, the 'unseasonal' pattern of ovarian response displayed by these ewes could be correlated with plasma prolactin concentrations. This does not resolve the question of causality, but it does indicate that plasma prolactin concentration is a remarkably accurate index of a ewe's sensitivity to photoperiod. Additional evidence is that, as with the rams, average peak plasma prolactin concentrations in the Romney and Dorset ewes, associated with long photoperiod, and mean values were significantly higher than in the Merinos. This accords with the observation of Carr & Land (1982) that rams of the highly seasonal Finnish Landrace breed produced more prolactin than those of the Blackface or Merino, but with a similar temporal pattern related to photoperiod. While all ewes of the two breeds which were highly seasonal in their ovarian response to photoperiod showed marked seasonality of prolactin production, following a pattern similar to that of the rams, only the one Merino ewe showed evidence of being entrained to the light regimen. It is concluded, therefore, that while prolactin secretion is regulated by photoperiod the process is different from that which regulates seasonal breeding activity.

The reason for the failure of the Australian Merino ewe and ram to adhere rigidly to the constraints imposed by photoperiod upon breeding activity in other breeds remains an enigma. The general pattern of response of the pituitary-hypothalamus to photoperiod or to challenge from

exogenous GnRH was similar, but appeared to be less defined in the Merino than the more seasonal Romney and Dorset breeds. On the other hand, the practical manifestations in terms of breeding activity were quite clear-cut. The combination of relatively low natural selection pressure for a restricted breeding season in the progenitors of the modern Merino, as postulated by Hammond (1952), coupled with artificial selection in Australia for autumn lambing, has resulted in considerable freedom from fundamental constraints imposed by photoperiod. Accurate definition of all the endocrinological factors involved appears not yet to be possible.

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