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Response of Pre-pubertally Castrated Rams and Ewes to Artificial Photoperiod—Changes in Plasma LH and Prolactin

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Abstract

Castrate rams and ovariectomized ewes were maintained in the presence of entire rams and ewes and subjected to successive periods of alternating 6 h light : 18 h darkness ('short' days) and 18 h light : 6 h darkness ('long' days) preceeded by a period of 12 h light : 12 h darkness ('constant' light days). Plasma concentrations of LH and prolactin were measured in the castrate animals in order to determine how LH and prolactin secretion responded to (i) the artificial light regime and (ii) corresponding periods of elevated or depressed testicular and ovarian activity in the entire rams and ewes.

There was no variation in mean plasma LH concentrations or LH pulse frequency with either the changes in photoperiod or the phases of gonadal activity in the entire animals. However, there was a highly significant ($P < 0{\cdot}001$) relationship between prolactin secretion and the artificial photoperiod in both castrate groups with high and low levels coinciding with long and short days respectively. In addition, there was a marginally significant ($P < 0{\cdot}1$) relationship between prolactin secretion in the castrate ram and the stage of testicular activity in the entire rams with elevated levels associated with regressed activity. Prolactin secretion in the ovariectomized ewes was significantly ($P < 0{\cdot}05$) related to the phase of ovarian development with high levels associated with acyclic activity.

It is concluded that LH secretion and pituitary responsiveness to exogenous GnRH were not modified by the artificial light regime. However, the changing light pattern was physiologically 'perceived' by the castrate animals as indicated by a concomitant variation in plasma prolactin concentrations.

Introduction

It is well established that seasonal testicular function in rams and ovarian activity in ewes are regulated by photoperiod and that steroid feedback is involved in this mechanism (for reviews see: Yeates 1949; Ortavant *et al.* 1964; Lincoln and Short 1980). The gonadectomized ram and ewe model has thus proved an invaluable means of elucidating many of the physiological aspects of the seasonal sexual response of the entire animal (Robinson 1982).

In the present study, castrate and entire rams and ewes were maintained under identical conditions of artificial photoperiod thus affording an opportunity to investigate several endocrine parameters in the castrate animal associated with seasonal reproductive activity in the entire animals. The castrate animals were of the Dorset Horn × Merino breed and hormonal responses of these sheep were analysed with respect to the changing artificial photoperiod and testicular and ovarian activity of entire Dorset Horn rams and ewes. The parameters measured were (i) the pattern of LH secretion as determined by serial blood-sampling techniques, (ii) the degree of pituitary responsiveness to exogenous GnRH as monitored by a standard GnRH/LH test, and (iii) the nature of prolactin secretion as estimated by plasma prolactin concentrations.

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Materials and Methods

Experimental Design

Four castrate Dorset Horn × Merino rams and four ovariectomized Dorset Horn × Merino ewes were maintained with entire rams and ewes of each of the three breeds Romney, Dorset Horn and Merino in two controlled-environment rooms as detailed by Poulton and Robinson (1987). The crossbred animals were introduced to the artificial light regime at 6 months of age. The rams had been castrated at 6 weeks of age and the ewes were ovariectomized via mid-line incision at 10 months of age. Examination of the ovaries indicated that the ewes were pre-pubertal. Two rams and two ewes were allocated to each of the rooms. From their introduction on 4 May 1979, 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 a period of 120 weeks of three alternating 16-week and six alternating 12-week blocks of 'short' days (6 h light : 18 h darkness) and 'long' days (18 h light : 6 h darkness) to provide 32-week and 24-week 'years'. Each room had an identical light cycle, but each operated out of phase so as to exclude any residual effect of former photoperiod.

Plasma Collection

Blood (5 or 10 ml) samples were collected by jugular venepuncture using lithium heparin-coated Vacutainers (Becton Dickinson, New Jersey, U.S.A.) and the plasma stored at −20°C until hormone assay. During darkness, blood was collected under faint red illumination. From week 24 to 48, samples were collected at 2-hourly intervals for 12 h every 8 weeks (four episodes). From week 52 to week 112, samples were collected at hourly (rams) or two-hourly (ewes) intervals for 12 h every 4 weeks (16 episodes). Blood samples were collected from 0800 to 2000 h.

LH Assay

LH assays were performed on plasma samples collected over 20 serial blood-sampling episodes from week 24 to week 112 and estimated by a specific radioimmunoassay (RIA) based on the method of Gidley-Baird and Bindon (1976) as modified by Poulton and Robinson (1987). For the castrate rams, mean plasma LH concentration, LH pulse frequency and LH pulse amplitude, as defined by Poulton and Robinson (1987), were calculated for the last 16 blood-sampling episodes. Mean plasma LH concentrations were determined for the ovariectomized ewes over the 20 blood-sampling episodes. For both rams and ewes, plasma prolactin was estimated in samples pooled over the entire 0800 h–2000 h period for each of the 20 blood-sampling episodes for plasma LH. At weeks 94, 101, 109, 117, the ovariectomized ewes were bled at invervals of 20 min for 6 h (commencing 0800 h) for information on pulsatile LH release. A pulse was as defined for the castrate rams.

Pituitary responsiveness was determined from plasma LH concentrations from blood samples collected at invervals of 30 min from 0 (0830 h) to 4 h following a standard injection of 10 μg GnRH (lot 41-550-AL, Abbot Laboratories, Illinois, U.S.A.). GnRH and LH tests were performed at intervals of 4 weeks from week 3 to week 111 (28 episodes). Mean and peak plasma LH concentrations were calculated for all animals.

Plasma samples of 0·5, 1·0, 5·5 and 11·1 ng LH/ml had interassay coefficients of variation of 19·4, 16·7, 11·2 and 10·6% respectively and intra-assay coefficients of variation of 15·6, 10·3, 7·9 and 7·0% respectively. The limit of assay sensitivity, defined as the value of two standard deviations below maximum (zero standard) binding, was 0·3 ng/ml for 100 μl plasma assayed.

Prolactin Assay

Plasma prolactin was estimated by a specific RIA (Kennaway *et al.* 1981) as described by Poulton and Robinson (1987). The plasma samples were processed in two assays. Samples from serial bleeds 1–6 and 7–20 were included in assays 1 and 2 respectively. In assay 1, plasma samples of 25·5 and 83·0 ng prolactin/ml had intra-assay coefficients of variation of 9·8 and 22·0% respectively. In assay 2, intra-assay coefficients of variation at 26·0 and 71·0 ng prolactin/ml were 13·0 and 13·0% respectively. Assay sensitivity was reported to be 3 ng/ml.

Statistical Analysis

Analyses of variance were used. Data for the castrate animals were assumed to have been equally influenced by the experimental environment. Any variance between rooms and between animals in each room was accounted for in the analysis. Remaining variance was partitioned into a phase effect derived

from either (i) the respective phase of testicular or cyclic ovarian activity of the entire Dorset Horn rams and ewes of the same room, as detailed by Poulton and Robinson (1987), or (ii) the photoperiod with which the LH and prolactin data of the castrate animals were associated. As there were no significant differences in hormone measurements between rooms, data were pooled under a common light regime in order to facilitate further analysis.

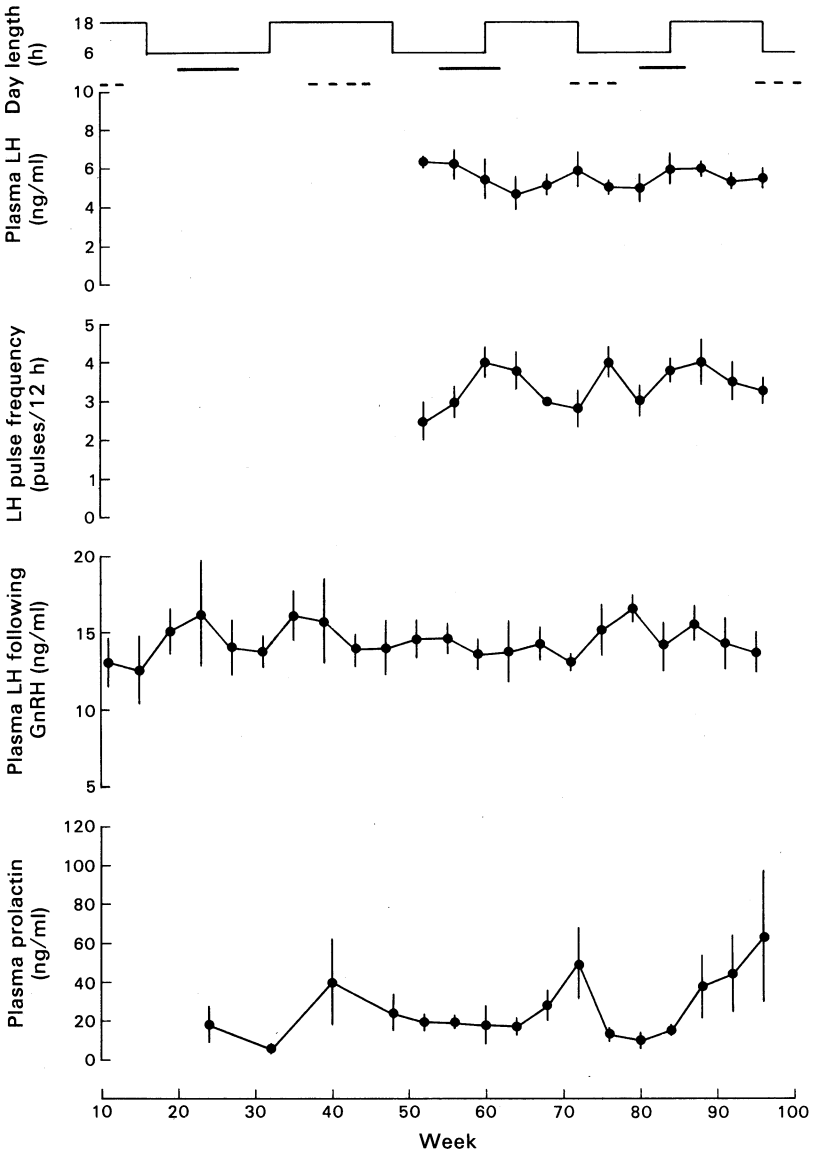

Fig. 1. Changes in mean plasma LH concentrations and LH pulse frequency, mean plasma LH concentrations following GnRH (10 μg) injection and plasma prolactin concentrations in castrate Dorset Horn × Merino rams in relation to artificial photoperiod. Each point is the mean ± s.e.m. (n = 4). Periods of 'developed' (———) and 'regressed' (- - -) phases of testicular activity of entire Dorset Horn rams (n = 4) maintained within the same environment are indicated.

Results

Castrate Rams

Plasma LH concentrations were pulsatile in nature. Data for mean LH concentrations and pulse frequencies are presented in Fig. 1. Mean LH concentrations, pulse frequency and pulse amplitude (data not shown) did not vary with the light regime. Moreover, there was no correlation between the pattern of LH secretion and the stage of testicular development in the entire rams (Table 1).

Table 1. Plasma LH serial blood-sampling measurements, plasma LH response following GnRH (10 μg) injection and plasma prolactin concentrations in castrate rams ($n = 4$) and ovariectomized ewes ($n = 4$)

Data were pooled with respect to the associated phase of testicular or ovarian activity of entire Dorset Horn rams and ewes maintained within the same environment. Corrected means (italics) with 95% fiducial limits are presented

	Castrate rams Phase of testicular activity		Ovariectomized ewes Phase of ovarian activity	
	Regresseed	Developed	Acyclic	Cyclic
Mean plasma LH concn (ng/ml)	5·2–*5·9*–6·6	4·6–*5·2*–5·9	5·4–*6·0*–6·7	4·9–*5·3*–5·8
LH pulse frequency (pulses/12 h)	2·8–*3·4*–4·0	2·7–*3·2*–3·8	–	–
LH pulse amplitude (ng/ml)	2·0–*2·3*–2·6	2·0–*2·3*–2·6	–	–
Mean plasma LH concn, following GnRH (ng/ml)	12·1–*13·4*–14·8	12·5–*13·9*–15·4	9·1–*10·0*–11·1	9·7–*10·7*–11·8
Peak LH amp. following GnRH	24·8–*27·5*–30·6	28·1–*31·5*–35·2	21·9–*23·5*–25·7	22·8–*24·5*–26·4
Plasma prolactin concn (ng/ml)	18–*26*–38	9–*14*–20	32–*46*–67	17–*23*–31

An injection of GnRH evoked a rapid increase in plasma LH with peak concentrations generally occurring in 30 min falling to basal levels within 4 h. Mean and peak (data not shown) LH concentrations did not vary with changes in photoperiod (Fig. 1) nor with the corresponding stage of testicular development (Table 1).

There was a highly significant ($P < 0{\cdot}001$) relationship between plasma prolactin concentrations and photoperiod (Fig. 1) with highest and lowest prolactin levels associated with long and short days respectively (pooled mean ± s.e.m. values: 38 ± 6 and 15 ± 2 ng/ml). There was a marginally significant ($P < 0{\cdot}1$) relationship between prolactin concentrations and the stage of gonadal activity of the entire rams, with enhanced secretion associated with regressed activity (Table 1).

Ovariectomized Ewes

Blood sampling at intervals of 2 h for 12 h provided no information on pulsatile LH release. There was no relationship between mean plasma LH concentrations and the stage of light cycle (Fig. 2) or the presence or absence of cyclic ovarian activity of the entire ewes (Table 1). When plasma samples were collected at intervals of 20 min, plasma LH was found to be highly episodic in nature. However, there was no relationship between pulse frequency of the ovariectomized ewes and

photoperiod nor with the phase of ovarian activity in the entire ewes [pooled mean pulse frequency (pulses/6 h) ± s.e.m. values for cyclic phase 5·5 ± 0·54 and for acyclic phase 5·3 ± 0·48].

Plasma LH release following GnRH administration was similar to that in the castrate rams in that peak values occurred at the first sampling interval (30 min post-injection) falling to basal levels within 4 h. Likewise, mean and peak LH concentrations (data not shown) changed neither with time (Fig. 2) nor with the associated phase of ovarian activity (Table 1).

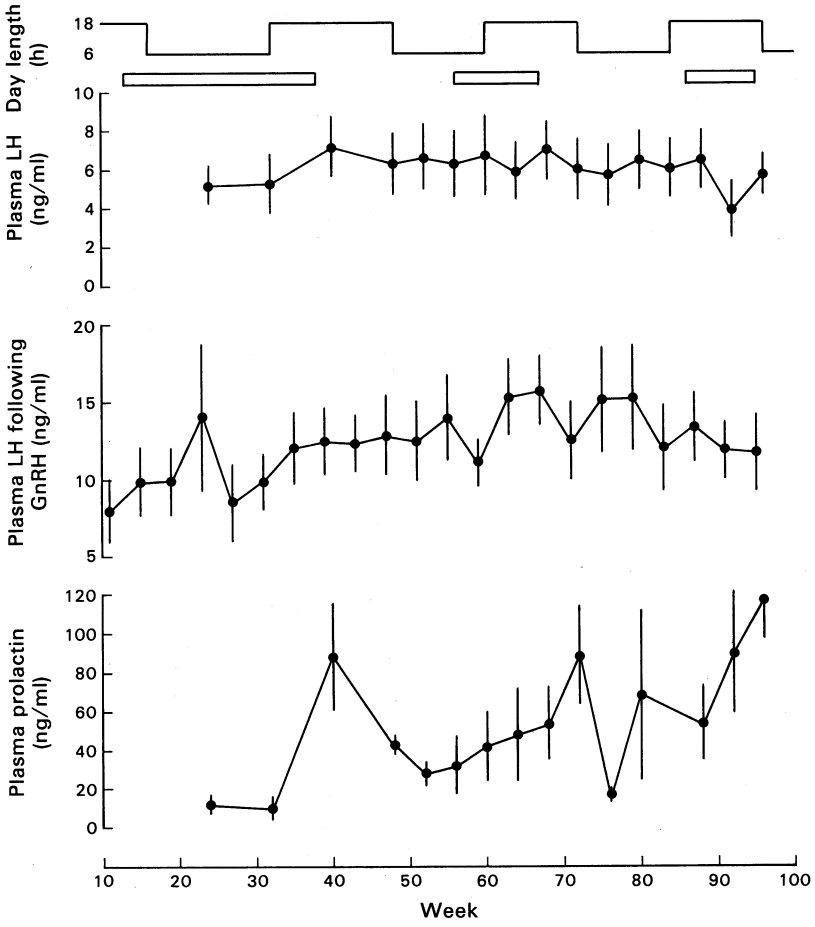

Fig. 2. Changes in mean plasma LH concentrations, mean plasma LH concentrations following GnRH (10 μg) injection and plasma prolactin concentrations in ovariectomized Dorset Horn × Merino ewes in relation to artificial photoperiod. Each point is the mean ± s.e.m. (n = 4). Periods of pooled phases of cyclic ovarian activity of entire Dorset Horn ewes (n = 4) maintained within the same environment are indicated (open rectangles).

The relationship between photoperiod and plasma prolactin concentrations was highly significant ($P < 0·001$) with elevated and depressed levels corresponding with long and short days respectively (pooled mean ± s.e.m. values: 73 ± 9 and 30 ± 8 ng/ml). There was a significant ($P < 0·05$) relationship between prolactin

secretion and the phase of ovarian activity of the entire ewes: high levels were associated with the acyclic state (Table 1).

Discussion

It has been suggested that photoperiod can influence hypothalamic-hypophyseal activity to regulate gonadotrophin secretion in castrate rams devoid of interference of any steroid feedback (Pelletier and Ortavant 1975; Lincoln and Short 1980). These authors found relatively higher plasma LH concentrations of castrate rams to be associated with short rather than with long photoperiod, indicating that enhanced plasma LH concentrations may coincide with breeding activity of entire rams of the same breed. It appears that basal plasma LH concentrations and pituitary responsiveness are affected rather than the frequency of pulsatile LH release which does not vary with long or short photoperiod (Schanbacher 1980).

The present investigation confirmed no seasonal variation in LH pulse frequency in castrate rams, but in contrast to the authors cited above, revealed no change in either mean LH concentrations or LH pulse amplitude. Likewise, there was no evidence of any seasonality in pituitary responsiveness to exogenous GnRH. Breed effect is commonly cited as a possibility to such endocrinological differences but the recent observations of Poulton and Robinson (1987) discount this. More probable is that the Ile de France rams of Pelletier and Ortavant (1975) and the Soay rams of Lincoln and Short (1980) had been previously exposed to their respective light regimes as entire rams and some form of photoperiodic conditioning upon the LH secretory mechanism has persisted. Such conditioning was improbable in the present study as the animals were not exposed to the experimental light regime until some 5 months after castration.

Mean LH concentrations and LH pulse frequency and amplitude in the castrates were elevated compared to corresponding values in entire Dorset rams within the same environment (Poulton and Robinson 1987). Furthermore, administration of GnRH elicited a greater and more rapid increase in plasma LH concentrations. These observations support the evidence for a negative feedback action of testicular steroids upon gonadotrophin synthesis and/or release in the ram (Riggs and Malven 1974; Schanbacher and Ford 1977; Lincoln and Short 1980).

The castrate rams displayed a variation in prolactin secretion related to photoperiod and analogous to that of the entire Dorset rams maintained in the same environment (Poulton and Robinson 1987). Such a response appeared better aligned to changing photoperiod than to corresponding phases of testicular activity in the entire rams. Average peak prolactin concentrations, associated with long photoperiod, were somewhat lower than corresponding levels reported in castrate Ile de France rams by Pelletier (1973), Clun Forrest rams by Parrott and Hills (1979) and Finnish Landrace, Scottish Blackface and (Tasmanian) Merino rams by Carr and Land (1982).

The present study revealed no seasonal variation in mean plasma LH concentrations of ovariectomized ewes associated with changes in the light regime. Similar results were obtained in the earlier study of Legan *et al.* (1977). More recently, however, evidence has been presented that ovariectomized ewes may display enhanced pulsatile LH secretion during the breeding season, indicating a direct effect of photoperiod upon hypothalamic activity (Goodman and Karsch 1981; Robinson *et al.* 1982;

Robinson 1983). Although there is a constant year-round mean plasma LH concentration, pulse amplitude has been reported as increasing while pulse frequency decreases during the non-breeding season (Goodman *et al.* 1982). The present study provided no evidence of such a change in LH pulse frequency and is in line with the observations of Martin *et al.* (1983) and Platt *et al.* (1983) who found no seasonal variation in the frequency of the LH pulses in ovariectomized Merino and Colombia ewes.

Furthermore, there was no significant effect of the light regime on the state of pituitary responsiveness to exogenous GnRH. By contrast, the observations of Goodman *et al.* (1982) imply that photoperiod may also have some direct regulatory influence upon pituitary activity as indicated by variation in LH pulse amplitude. The situation is further confused since Land *et al.* (1979) and Evans and Robinson (1980) have reported a seasonal pattern of pituitary responsiveness to injected GnRH in ovariectomized ewes. However, results from such studies are conflicting as Land *et al.* (1979) associated maximum LH release with the season of ovarian activity whilst Evans and Robinson (1980) found it to be coincident with the period of anoestrus in entire ewes.

In parallel to the aseasonal plasma LH response in the castrate rams, it is conceivable that the experimental ewes were likewise gonadectomized and introduced to the artificial light regime before any persistent endogenous rhythm in plasma LH secretion could be generated.

Plasma prolactin concentrations paralleled that of entire Dorset ewes kept under the same conditions (Poulton and Robinson 1987) and were more closely aligned with changing photoperiod than with the presence or absence of ovarian activity.

There was no seasonal pattern in plasma LH concentrations or pituitary responsiveness in either the castrate rams or the ovariectomized ewes. It is noted that, due to the relatively small groups of experimental animals used ($n = 4$) and the fact that comparison with entire animals of the same breed would have been preferable, the strength of the experimental design is tempered. However, in defence of the model, the animals were intensively monitored for over 2 years under rapidly changing photoperiod, thus allowing a thorough examination of 'seasonal' trends. Secondly, under field conditions, the duration of breeding activity in the Dorset Horn and its Merino cross is similar (approximately 6 and 7 months) with the midpoint of such activity occurring in the autumn (Hafez 1952; Robinson 1982).

Regardless of the asynchronous pattern of plasma LH concentrations, the marked 'seasonal' rhythm of prolactin secretion in the castrate ram and ewe indicates the ability of these animals to perceive changes in photoperiod. This might imply that, in these sheep, photic stimuli (via changing photoperiod) were interacting with the neuroendocrine axis at a level regulating prolactin secretion but not at one modulating the frequency and amplitude of LH release.

The variation in these results and those from studies in which gonadectomized ewes and rams maintained a seasonal rhythm in gonadotrophin secretion (Pelletier and Ortavant 1975; Lincoln and Short 1980; Goodman and Karsch 1981; Robinson 1983) may be due to some genotypic difference in endocrine activity between Merino crossbreds and the more seasonal European breeds (Martin 1984) but, as noted above, this seems unlikely. A more likely explanation is the fact that, as the animals from the above experiments were castrated as mature adults, 'memory' of the previous photoperiod persisted, imprinted when steroid feedback mechanisms were intact.

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