Evidence that the onset of the breeding season in the ewe may be independent of decreasing plasma prolactin concentrations*

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Summary. Ten ewes of each of two breeds, Dorset Horn (long breeding season) and Welsh Mountain (short breeding season), were given subcutaneous oestradiol-17 β implants and then ovariectomized. Another 10 ewes of each breed were left intact. On 3 May 1982, all the ewes were housed in an artificial photoperiod of 16L:8D. After 4 weeks, half of the ewes of each breed and physiological state were abruptly exposed to a short-day (8L:16D) photoperiod while the others remained in long days (16L:8D). The time of onset of the breeding season was significantly ($P < 0.05$) advanced in ewes switched to short days (12 August $\pm$ 10 days) compared to those maintained in long days (4 September $\pm$ 14 days). Dorset Horn ewes began to cycle (20 July $\pm$ 7 days) significantly ($P < 0.001$) earlier than Welsh Mountain ewes (19 September $\pm$ 6 days). Disparities in the time of onset of cyclic activity in ewes of different breeds and daylength groups were echoed in disparities in the time at which plasma LH and FSH concentrations rose in oestrogen-implanted, ovariectomized ewes of the same light treatment group. Prolactin concentrations showed an immediate decrease in ewes switched to short days, but remained elevated in long-day ewes. Since the breeding season started in the presence of high prolactin concentrations in long-day ewes, it seems unlikely that prolactin is an important factor determining the timing of the onset of cyclic activity.

Introduction

It has long been accepted that the sheep is a short-day breeder, in which the onset of reproductive activity occurs as daylength shortens in the autumn. Many experiments have demonstrated that exposure of ewes to artificial short daylengths in spring or early summer results in premature onset of the breeding season (Ducker, Thwaites & Bowman, 1970; Newton & Betts, 1972; Walton, Evins, Fitzgerald & Cunningham, 1980). Furthermore, exposure of ovariectomized, oestrogen-implanted ewes to short days during the period of seasonal anoestrus results in a rapid increase in gonadotrophin concentrations (Legan & Karsch, 1980). It is thought that such changes in LH and FSH levels, which coincide temporally with the onset and end of the breeding season in intact ewes, reflect seasonal shifts in the responsiveness of the hypothalamic-pituitary axis to the negative

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feedback effects of oestradiol, and that these shifts in hypothalamic–pituitary responsiveness probably form the physiological basis of seasonal reproduction in the ewe (Legan, Karsch & Foster, 1977).

Several workers have suggested that daylength-mediated changes in prolactin concentrations could play a part in the photoperiodic control of seasonal breeding in the sheep (Walton, McNeilly, McNeilly & Cunningham, 1977; Lincoln, McNeilly & Cameron, 1978). In natural environments, this proposal fits well with the inverse relationship between prolactin concentrations and reproductive activity observed in most breeds of sheep. However, ewes of certain breeds, such as the Dorset Horn and the Merino, have been reported to begin cycling at or near the summer solstice (Yeates, 1956; Webster & Haresign, 1983). Furthermore, testicular growth in rams often begins before the longest day (Lincoln & Short, 1980). In view of such observations it seems unlikely that these animals could be responding to a decreasing photoperiod.

Webster & Haresign (1983) compared the patterns of LH and prolactin release throughout the year in ewes of two breeds with very different breeding season lengths. Although the coincidental changes in cyclic activity in intact ewes and increased LH concentrations in oestrogen-treated, ovariectomized ewes were seen about 2 months earlier in Dorset Horn than in Welsh Mountain ewes, temporal changes in prolactin were virtually identical in ewes of both breeds. Indeed, Dorset Horn ewes started to cycle when prolactin concentrations were at or near their seasonal peak value, while cyclic activity in Welsh Mountain ewes did not start until plasma prolactin concentrations were approaching their seasonal nadir. These data could be interpreted to indicate either that seasonal changes in prolactin do not underlie seasonal breeding in the ewe, or that there is a marked difference in breed sensitivity to the antigonadal effects of prolactin. The present experiment was therefore designed to manipulate the prolactin status of ewes by means of photoperiod, and hence to investigate its effect on cyclic activity in entire ewes and gonadotrophin concentrations in ovariectomized, oestrogen-implanted ewes of the same two breeds.

Materials and Methods

Animals and experimental design

Mature Dorset Horn ewes (breeding season July–March) and Welsh Mountain ewes (breeding season October–February) were used. Half of the ewes of each breed ($N=10$ per group) were bilaterally ovariectomized after being given a subcutaneous oestradiol implant of a type designed to maintain plasma oestradiol concentrations at luteal phase levels of 3–5 pg/ml (Karsch *et al.*, 1973). The remaining ewes ($N=10$ per breed) were left entire and did not receive oestradiol implants.

On 3 May, before the summer solstice, the ewes were housed in one of two identical lightproof boxes in an artificial photoperiod similar to that prevailing outside, of 16 h light and 8 h dark (16L:8D). Lights-on and -off were at 04:00 and 20:00 h respectively. After 4 weeks, half of the ewes of each breed and physiological state were abruptly exposed to a short-day photoperiod (8L:16D), the remainder of the animals being kept in long days (16L:8D). For the ewes now in short days, the lights were switched on at 08:00 h and off at 16:00 h. Losses due to illness during the experiment meant that two groups were reduced in size ($N=3$ and 4). The animals were then kept on these two photoperiods for a further 21 weeks (ewes switched to short days) or 24 weeks (ewes kept in long days). Facilities for control of temperature and humidity did not exist, and these fluctuated in accordance with outside conditions. Lights were switched on and off by means of automatic time clocks, and light intensities at sheep head height were 85 and <1 lux during the light and dark phases, respectively. When housed, all ewes were fed a maintenance diet of indoor sheep concentrates and hay, with ad-libitum access to fresh water and mineral licks.

Blood samples were collected by jugular venepuncture during the light phase from all ewes 3 times a week throughout the experiment, and the plasma was stored at -20°C until required for LH, FSH, prolactin and progesterone determinations.

Hormone assays

Luteinizing hormone. Plasma LH concentrations were measured using the specific double-antibody radioimmunoassay of Foster & Crighton (1974), as modified by McLeod, Haresign & Lamming (1982). The limit of sensitivity of the assay within this study (defined as twice the standard deviation of blank values) was 0.3 ng NIH-LH-S17 equiv./ml plasma; the inter-assay coefficient of variation was 14%, and the intra-assay coefficient of variation for randomly selected duplicate pairs was 12%. There was negligible cross-reaction with other protein hormones (ovine prolactin, FSH, GH or TSH).

Prolactin. Plasma prolactin concentrations were determined by the specific double-antibody radioimmunoassay described by Howles, Webster & Haynes (1980) with the single modification that the first antibody was used at a working dilution of 1:25 000. The intra- and inter-assay coefficients of variation within this study were 9% and 13% respectively, and the limit of sensitivity of the assay was 1.6 ng NIH-P-S10 equiv./ml plasma. The assay showed negligible cross-reaction with other major protein hormones.

Progesterone. Plasma progesterone concentrations were measured by the radioimmunoassay method of Haresign *et al.* (1975). The assay showed negligible cross-reaction with other major steroid hormones. Within this study, the inter- and intra-assay coefficients of variation were 7% and 14% respectively, and the limit of sensitivity of the assay was 0.1 ng/ml. The mean extraction efficiency was $87 \pm 2\%$.

Follicle-stimulating hormone. FSH concentrations were measured by a heterologous double-antibody radioimmunoassay using an antiserum (M94) raised in rabbits against human FSH. The assay was based on the method described for bovine FSH by Webb, Lamming, Haynes & Foxcroft (1980), but with the following modifications.

Ovine FSH standards (0.8–50 ng NIH-FSH-S11/tube) were made up in 200 μ l assay buffer. For unknown plasma samples, 25 μ l plasma were diluted to 200 μ l with assay buffer immediately before measurement.

After addition of 100 μ l of a 1:7500 dilution of the M94 first antibody in assay buffer containing 1:600 (v/v) normal rabbit serum, the tubes were incubated for 48 h at 4°C. This was followed by addition of 100 μ l 125 I-labelled rat FSH (NIAMD-Rat FSH-I-3) and a further 48 h incubation at 4°C before precipitation of the antibody-bound fraction with sheep anti-rabbit γ -globulin.

Table 1. Dates of onset of cyclic activity in entire ewes of the two breeds in relation to the first date of exposure to short days (7 June) for ewes changed onto this photoperiod

	Days from 7 June to first cycle		
	Constant long days (16L:8D)	Switched to short days (8L:16D)	Normal beginning of the breeding season*
Dorset Horn	51.4 $\pm$ 18.4 (28 July) N = 3	37.8 $\pm$ 3.5 (15 July) N = 5	13 July $\pm$ 3.6 days N = 10
Welsh Mountain	112.6 $\pm$ 8.2 (28 September) N = 5	95.3 $\pm$ 5.9 (10 September) N = 5	12 October $\pm$ 2.6 days N = 10

Values are group means for the number of ewes indicated; dates in parentheses are the mean date of onset of cyclic activity for the group.

*Included for comparison are the mean dates of onset of the breeding season in each breed under natural daylength conditions (data from Webster & Haresign, 1983).

The limit of sensitivity of this assay was 0.6 ng NIH-FSH-S11 equiv./tube (24 ng/ml plasma). Serial dilutions of a standard plasma sample ran parallel to the standard curve. The intra- and inter-assay coefficients of variation within this study were 8% and 12% respectively; cross-reaction with other major protein hormones (ovine GH, NIH-GH-S6; prolactin, NIH-P-S10 and LH, NIH-LH-S19) was < 1%.

Statistical analysis

Hormone concentrations measured in the 3-times weekly plasma samples were converted into weekly mean estimates for each animal, and subjected to a 3- or 4-factor split-plot analysis of variance as appropriate. For the purposes of analysis it was assumed that the variance in different treatment groups was of similar magnitude. This analysis generated separate values for the standard error of the difference (s.e.d.) for comparing mean hormone concentrations between weeks within a breed, and for comparing mean hormone concentrations between either breeds or daylength treatments within any particular week. The magnitudes of these s.e.d. values are indicated on each of the Text-figures.

Results

Cyclic activity of entire ewes

Mean values for the timing of the first cycle, estimated from plasma progesterone measurements, are given for each treatment group in Table 1. Entire ewes of both breeds switched to short days began cycling significantly ($P < 0.05$) earlier than those of the same breed maintained in long days. One ewe of the Welsh Mountain breed maintained in long days was still acyclic at the end of the experiment. Ewes of the Dorset Horn breed began to cycle significantly ($P < 0.001$) earlier than did Welsh Mountain ewes. Dorset Horn ewes changed to short days began to cycle at the normal time of year for the breed, whereas the breeding season was delayed in those kept in long days. By contrast, it was the long-day Welsh Mountain ewes that began cycling at the expected time for the breed, while individuals switched to short days became cyclic a month earlier than usual.

Prolactin concentrations

Text-figure 1 illustrates the long-term changes in prolactin concentrations in the various treatment groups. Ewes switched to short days showed a rapid fall in prolactin concentrations, whereas in those animals maintained in long days the prolactin levels only declined gradually; this effect of photoperiod was significant ($P < 0.01$) for both breeds. Although prolactin concentrations were initially higher ($P < 0.01$) in Welsh Mountain than in Dorset Horn ewes, by the end of the study ewes of all treatment groups showed broadly similar patterns of prolactin secretion. No significant differences in prolactin release were detected between entire and ovariectomized, oestrogen-treated ewes.

LH concentrations in ovariectomized, oestrogen-treated ewes

LH levels were initially low in all oestrogen-implanted, ovariectomized ewes, but increased later in the experiment (Text-fig. 2). Dorset Horn ewes switched to short days showed a significantly ($P < 0.01$) earlier rise in LH concentrations than ewes of the same breed maintained in long days, although this was then followed by a decline towards the end of the experiment in short-day ewes. Peak LH concentrations were higher in long-day ewes than in those switched to short days.

The increase in LH concentrations in Welsh Mountain ewes changed to short days was much smaller than that seen in Dorset Horn ewes of the same photoperiod group and was only of short

Text-fig. 1. Weekly mean prolactin levels in groups of intact or ovariectomized, oestrogen-treated (Ovx + E₂) ewes of the Welsh Mountain (WM) and Dorset Horn (DH) breeds. Ewes were maintained in a 16L:8D photoperiod (LD; ●—●) or abruptly switched (arrow) to an 8L:16D photoperiod (SD; ○—○) in early June.

duration. Since there was some degree of variation in the timing of this event between individual ewes, the actual patterns of change observed are not altogether apparent from the mean profile depicted in Text-fig. 2. Welsh Mountain ewes kept in long days did, however, show a substantial rise in LH in the later part of the study. Disparities in the time of onset of cyclic activity in individual ewes of different breeds and daylength treatment groups were echoed in disparities in the time at which LH levels rose above basal in individual ovariectomized, oestrogen-implanted ewes of the same treatment group.

FSH concentrations in ovariectomized, oestrogen-treated ewes

Weekly mean FSH levels in ovariectomized, oestrogen-treated ewes are depicted in Text-figs 3(a) and 3(c). FSH concentrations were low in all groups at the beginning of the study. Although a rise in FSH was observed in all Dorset Horn ewes, this was of smaller magnitude and occurred significantly ($P < 0.01$) earlier in ewes changed to short days compared to those kept in long days. A decline in FSH concentrations was then seen in both photoperiod groups.

There was a significant ($P < 0.01$) difference in the patterns of change in FSH concentrations between the breeds, since ovariectomized, oestrogen-treated Welsh Mountain ewes showed little

Text-fig. 2. Weekly mean LH concentrations in groups of ovariectomized, oestrogen-treated ewes (Ovx + E₂) and cyclical activity in groups of intact ewes of the Dorset Horn (DH) and the Welsh Mountain (WM) breeds. Ewes were kept in a 16L:8D photoperiod (LD; ●—●) or abruptly switched (arrow) to an 8L:16D photoperiod (SD; ○—○) in early June.

change in FSH secretion during the experiment, except that FSH values were seen to rise during the last few weeks of the sampling period in some long-day ewes.

The increases in LH and FSH concentrations occurred at the same time in ovariectomized, oestrogen-implanted ewes of a given breed/light treatment group.

Gonadotrophin concentrations in entire ewes

Weekly mean FSH (Text-figs 3b & d) and LH concentrations remained low and stable throughout the study period in entire ewes of both breeds, and showed no significant breed or light treatment differences.

Discussion

The similarity in the timing of the changes in LH concentrations in ovariectomized, oestrogen-treated ewes and cyclical activity in entire ewes of the same breed within each light treatment group is consistent with previous suggestions that a change in responsiveness of the hypothalamic-pituitary

Text-fig. 3. Weekly mean FSH concentrations in groups of intact and ovariectomized, oestrogen-treated (Ovx + E₂) ewes of the Welsh Mountain (WM) and Dorset Horn (DH) breeds. Ewes were maintained in a 16L:8D photoperiod (LD; ●—●) or abruptly switched (arrow) to an 8L:16D photoperiod (SD; ○—○) in early June.

axis to the negative feedback effects of oestradiol on tonic LH secretion may form the basis of seasonal breeding in this species (Legan *et al.*, 1977; Legan & Karsch, 1980; Webster & Haresign, 1983). In addition, the observation that there was no significant increase in plasma FSH concentrations in entire ewes of either breed before the start of cyclic activity suggests that a deficiency in FSH secretion is not an important component of seasonal anoestrus.

An abrupt exposure to short days in early June significantly advanced the beginning of the breeding season in Welsh Mountain but not Dorset Horn ewes. Hafez (1952) was able to advance the onset of cyclic activity in Dorset Horns using a similar switch from long to short days. However, this difference may be attributable to the fact that ewes were switched to short days much later in the year in the current experiment.

The short-day light regimen also caused a rapid fall in prolactin concentrations in ewes of both breeds, similar to that reported for Blackface-cross ewes exposed to an 8L:16D photoperiod in summer (Walton *et al.*, 1980). As high levels of prolactin were not maintained throughout in ewes kept in long days, it was not possible to discount an involvement of prolactin in determining the timing of the onset of cyclic activity. However, the delay in the onset of reproductive activity in Dorset Horn ewes kept in constant long days was unlikely to be due to elevated prolactin concentrations compared to the ewes of the same breed in short days, because Dorset Horns normally start cycling in the presence of high prolactin concentrations (Webster & Haresign, 1983). Long-day Welsh Mountain ewes began to cycle at the normal time of year for the breed, despite prolactin

concentrations which were considerably higher than those recorded at the beginning of the breeding season under natural daylength conditions (Webster & Haresign, 1983). It therefore seems unlikely that the differences in the timing of the onset of reproductive activity between Welsh Mountain ewes in long and short days resulted from differences in the prolactin status of these treatment groups. Such a conclusion is consistent with the observation that suppression of plasma prolactin concentrations in ovariectomized ewes treated with oestradiol during the period of seasonal anoestrus does not reduce responsiveness of the hypothalamic-pituitary axis to the negative feedback effects of oestradiol (Wright, Findlay & Anderson, 1981).

Data of this kind raise questions about the accuracy of the classical view, based largely on data from hyperprolactinaemic women (Bohnet, Dahlen, Wuttke & Schneider, 1976) and rats (McNeilly, Sharpe, Davidson & Fraser, 1978), that high prolactin concentrations cause hypogonadism. It is uncertain whether such observations can be usefully compared to data relating to seasonal variations in prolactin concentrations in normal animals. In addition, experiments involving pharmacological manipulation of prolactin concentrations by means of dopamine agonists should be viewed with some caution, since these agents may act upon the general level of secretion of hypothalamic releasing hormones rather than specifically upon factors regulating the release of prolactin.

This experiment also examined the role of photoperiodic change in determining the timing of the onset of the breeding season. It was surprising to find that Dorset Horn ewes kept in long days showed a delay in the start of the breeding season compared to ewes kept under natural daylength conditions. This indicates that the onset of cyclic activity under natural daylength conditions is probably initiated by shortening daylength, even in this breed in which cyclic activity begins in July. For this to occur, ewes must normally be able to detect the very small changes in photoperiod that occur between the summer solstice and the onset of the breeding season in July. Studies of mammals of other species have shown that changes in photoperiod of as little as 0.25 h can cause large-scale reproductive responses (Elliott, 1976; Goss, 1977). The question then arises of whether Welsh Mountain ewes also respond to such minor changes in photoperiod, and, if not, whether this could form part of the mechanism by which the onset of reproductive activity is delayed in this breed. However, even an abrupt switch to 8L:16D in early June did not advance the breeding season of Welsh Mountain ewes to a date comparable with that seen in Dorset Horns. It therefore appears that it is not the need for relatively short days to induce photostimulation, but rather the fact that available photoperiodic information is not rapidly translated into effects on breeding activity which is responsible for the later onset of the breeding season in the mountain breed. Furthermore, the fact that LH and FSH showed a late rise in ovariectomized, oestrogen-treated Welsh Mountain ewes compared to Dorset Horns suggests that the slow response to daylength changes in the former breed is not due to a failure of the ovaries to respond to gonadotrophic stimulation, but rather to the need for a longer period before an increase in hypothalamic-pituitary activity is initiated.

As ewes of both breeds kept in long days did begin to cycle, it seems that inhibitory long days cannot in general prevent the eventual onset of cycles in the ewe. Similarly, constant long days delayed but did not prevent the onset of puberty in ewe lambs (Smith, 1967) and the onset of testicular growth in Soay rams (Almeida & Lincoln, 1984). These data parallel the observations for both ewes and rams that photostimulatory short days cannot prolong the breeding season indefinitely (Howles, Craigon & Haynes, 1982; Worthy & Haresign, 1983).

An unexpected observation from the present study was the early decrease in LH and FSH levels in the ovariectomized, oestrogen-implanted Dorset Horn ewes that had been switched to short days. Even though gonadotrophin levels in oestrogen-treated, ovariectomized ewes usually begin to fall well before the onset of seasonal anoestrus in entire ewes of the same breed (Legan & Karsch, 1980; Webster & Haresign, 1983), the timing of these gonadotrophin changes would seem to indicate that the breeding season in the short-day ewes would have been significantly shorter than usual. This is in agreement with the data of Thimonier, Ravault & Ortavant (1978) which

demonstrate that Ile-de-France ewes switched to short days in early summer showed an advancement of the breeding season associated with an unusually short breeding season. It therefore appears that an abrupt exposure to short days in spring/summer, while providing a potent stimulus for advancing the onset of the season, in some way disrupts the mechanisms timing the length of the season. This may be due to the appearance of a photorefractory or insensitive condition which is normally only reached in late autumn after the animals have been exposed to shortening days for several months, and could be the result of rapid exposure to what would normally be a protracted daylength change.

The more striking reduction in gonadotrophin release observed in short-day ovariectomized, oestrogen-implanted Welsh Mountain ewes may reflect a real component of breed difference. Several lines of evidence suggest that steroid feedback from the ovaries during anoestrus may be relatively stronger in mountain breeds than in Merino types (Land, Wheeler & Carr, 1976; Scaramuzzi & Baird, 1979). This could indicate that a relatively greater shift in the responsiveness of the hypothalamic-pituitary axis to oestradiol negative feedback is necessary in mountain breeds before a given photoperiodic event can be translated into effects on the reproductive status of the animal. In the current experiment, identical implants were used in breeds with widely different bodyweights. It is therefore possible that breed differences in suppression of gonadotrophin secretion could be to some extent attributable to the implants used. However, the data of Webster & Haresign (1983) showed that seasonal changes in cyclic activity in entire ewes and LH concentrations in ovariectomized, oestrogen-treated ewes were synchronous in the same two breeds when identical implants were used, indicating that seasonal shifts in the responsiveness of the hypothalamic-pituitary axis to the negative feedback effects of oestradiol were large enough in both breeds to overcome the inhibitory feedback effects of the steroid present.

The results from this and previous studies (Worthy & Haresign, 1983) indicate that both the onset and end of the breeding season in the ewe can occur in the absence of an appropriate photoperiodic stimulus, although if the stimulus is given an increased synchrony and precision of the reproductive response is seen. It appears that an endogenous circannual rhythm of reproductive change may exist in seasonally breeding animals, which is normally entrained by photoperiodic cues, but is not driven by them. One possibility is that seasonal shifts in the perception of a given photoperiod could underlie this rhythm. This implies that the critical photoperiod may depend upon the reproductive state and recent photoperiodic experience of the animal rather than having fixed characteristics: hence the critical daylength for photostimulation may differ from that which causes cessation of reproductive function. This mechanism would allow seasonal patterns of breeding to persist in a constant photoperiodic environment. Such shifts in the critical photoperiod required to induce gonadal growth and regression have been described in Japanese quail kept in fixed or varying photoperiods (Follett, Robinson, Simpson & Harlow, 1981). Clearly, further work is required to elucidate the critical daylength of sheep at different times of the year, and to determine whether this component is responsible for breed differences in season length.

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