Seasonal changes in LH and prolactin concentrations in ewes of two breeds*

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Summary. The current experiment was designed to investigate whether changes in prolactin concentrations might be involved in the seasonal change in responsiveness of the hypothalamic–pituitary axis to the negative feedback effects of oestradiol in the ewe. Twelve Dorset Horn ewes (long breeding season) and 12 Welsh Mountain ewes (short breeding season) were ovariectomized after insertion of empty subcutaneous implants ($N = 2$ per breed), or ones containing oestradiol-17 β ($N = 10$ per breed). Intact ewes, 10 of each breed, were used to monitor breeding activity. Mean weekly plasma LH concentrations in oestrogen-treated ovariectomized ewes of both breeds were elevated over the period corresponding to their respective breeding seasons, but were basal during anoestrus. The changes in prolactin concentrations, however, followed the same temporal pattern in both breeds and were approximately parallel to changes in daylength. These data support the suggestion of a seasonal change in negative feedback responses to oestradiol which corresponds to changes in breeding activity, but any involvement of prolactin in this response remains questionable.

Introduction

There is a marked seasonal variation in the responsiveness of the hypothalamic–pituitary axis to the negative feedback action of oestradiol on LH secretion in the ewe, which corresponds to the transitions between the breeding season and seasonal anoestrus (Legan, Karsch & Foster, 1977; Legan & Karsch, 1979; Goodman & Karsch, 1980). During anoestrus oestradiol is a potent inhibitor of LH secretion whereas during the breeding season it is much less effective. Furthermore, this pivotal neuroendocrine event governing seasonal breeding appears to be dictated by photoperiod (Legan & Karsch, 1980), the major environmental factor known to control the annual reproductive cycle of the ewe (Yeates, 1949; Hafez, 1952).

Since the negative feedback control of tonic LH secretion during the breeding season requires the combined presence of oestradiol and progesterone (Karsch, Legan, Ryan & Foster, 1980), it is possible that some other non-ovarian endocrine factor which shows seasonal variation may be involved in mediating this seasonal change in responsiveness of the hypothalamic–pituitary axis to oestradiol alone. For example, it has been shown that a seasonal fall in plasma prolactin concentrations, in response to shortening daylength, precedes the onset of reproductive activity in the ewe (Walton, McNeilly, McNeilly & Cunningham, 1977) and that hyperprolactinaemia may play a major role in suppressing gonadotrophin secretion during lactational anoestrus in this species (Kann & Martinet, 1975; Kann, Martinet & Schirar, 1977). Moreover, clinical studies in women have indicated that hyperprolactinaemia is associated with the absence of menstruation, ovulation and LH episodes (see McNeilly, 1980). In such cases restoration of normal prolactin levels with

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bromocriptine results in return of the characteristic episodic pattern of LH secretion, and cyclic activity then follows.

McNeilly (1980) also reviewed data from the rat which suggest that prolactin acts by increasing the sensitivity of the hypothalamus to the negative feedback effects of gonadal steroids, a feature identical to that observed in lactational amenorrhoea in women (Baird, McNeilly, Sawers & Sharpe, 1979), and during seasonal anoestrus in sheep (see Karsch, Goodman & Legan, 1980). The aim of the present study therefore was to investigate the possible role for prolactin in determining the seasonal changes in the negative feedback control of gonadotrophin secretion in the ewe, and whether differences in breeding season length might be mediated by breed differences in the seasonal changes in prolactin concentrations.

Materials and Methods

Animals and experimental design

Two breeds of ewe (Welsh Mountain and Dorset Horn) with breeding seasons widely different in length were used in an attempt to highlight any differences in response to daylength changes. Since the presence of oestrous cycles, and in particular progesterone, during the breeding season would confound the underlying changes in responsiveness to negative feedback, it was necessary to remove the influence of progesterone without affecting oestradiol levels. Twelve ewes of each breed were therefore ovariectomized. Immediately before ovariectomy, 10 ewes of each breed were given a subcutaneous implant containing oestradiol-17 β (Sigma Chemical Co., St Louis, Missouri, U.S.A.) (Karsch *et al.*, 1973) which was designed to maintain constant plasma oestradiol levels of 3–5 pg/ml (F. J. Karsch, personal communication). The other 4 ovariectomized ewes were treated with empty implants to act as controls. A further 10 ewes of each breed were left untreated and allowed to run with a colour-marked vasectomized ram. The colour was changed every 17 days and evidence of sexual activity was noted daily to obtain information about the length of the breeding season for each breed. Throughout the experiment, all animals were housed in breed groups under conditions of natural daylength and temperature. They had free access to fresh water and mineral licks and were given a maintenance ration of hay and 'indoor' sheep concentrates each day between 08:00 and 11:00 h. Blood samples were collected into heparinized containers by jugular venepuncture from all ewes 3 times each week between August 1979 and August 1980. Blood samples were centrifuged immediately after collection and the plasma stored at -20°C until required for hormone assays.

Hormone assays

Prolactin. This was a specific double-antibody radioimmunoassay performed using the technique and reagents described by Howles, Webster & Haynes (1980). The limit of sensitivity of the assay, defined as twice the standard deviation from the binding obtained with zero concentrations of prolactin, was 1.7 ng NIH-P-S10 equiv./ml. The intra-assay coefficient of variation (CV) between randomly selected duplicate pairs was 8.7% ($n = 100$) and the inter-assay CV of a standard reference plasma was 9.4%.

Luteinizing hormone. This was measured using the specific double-antibody radioimmunoassay method of Foster & Crighton (1974) with some minor modifications (McLeod, Haresign & Laming, 1982) to increase the limit of sensitivity of the assay to 0.3 ng NIH-LH-S17 equiv./ml. The intra-assay CV for randomly selected duplicate pairs was 11.0% over the range 0.3–7.5 ng/ml ($n = 50$) and 9.8% over the range 7.5–25 ng/ml ($n = 50$). The inter-assay CV of a standard reference plasma was 15.7%.

Progesterone. The onset and end of cyclic activity in untreated ewes was confirmed by progesterone measurements by the radioimmunoassay method of Haresign, Foster, Haynes, Crighton &

Lamming (1975). The reliability of the assay was similar to that reported previously (McLeod *et al.*, 1982), and within this study showed a limit of sensitivity of 0.1 ng/ml and inter- and intra-assay CVs of <10%. The mean extraction efficiency was $69.3 \pm 3.1\%$.

For all hormone measurements the plasma samples from any one ewe were measured in random order within the same assay.

Results

Breeding season

Data relating to the onset, duration and end of cyclic activity in the untreated ewes of each breed are presented in Table 1 and Text-fig. 1. Since the experiment started after the onset of cyclic activity in the Dorset Horn ewes, the start and end of the same breeding season could not be determined accurately. Data from the following year are therefore included in the calculation of breeding season length for this breed since annual differences in reproductive activity are likely to be negligible (Hafez, 1952).

Table 1. Breeding season data from ewes of each breed (based on progesterone determination)

Breed	Cyclic activity (days)			Mid-breeding season date
	Start	End	Duration	
Dorset Horn	July 13 $\pm$ 3.6*	April 3 $\pm$ 7.9	265 $\pm$ 9.4	22 November
Welsh Mountain	October 12 $\pm$ 2.6	April 11 $\pm$ 9.9	182 $\pm$ 8.1	10 January

Values are mean $\pm$ s.e.m.

* Start of 1980 breeding season.

LH concentrations

Seasonal changes in LH concentrations of ovariectomized ewes of each breed are shown in Text-fig. 1. LH patterns in ovariectomized ewes with empty implants were similar for both breeds and reflected the typical post-ovariectomy elevation with no apparent seasonal trends. In contrast to this, the LH levels in oestradiol-treated ovariectomized ewes showed a marked seasonal pattern, being high during the equivalent of the breeding season and basal during anoestrus. Moreover, since the duration of the breeding season (determined in the untreated ewes) differed for the two breeds, this resulted in a different temporal relationship in LH profiles between breeds. The onset of the breeding season in both breeds was reflected in an abrupt change in plasma LH concentrations in oestradiol-treated ovariectomized ewes, although the rate of change at the end of the breeding season was much more gradual.

Prolactin concentrations

Seasonal prolactin profiles for ovariectomized ewes with oestradiol implants are shown in Text-fig. 2(a). As with the LH levels, a marked seasonal trend was apparent. In both breeds the prolactin concentrations followed an identical temporal pattern which was approximately parallel to daylength. A similar profile was seen in untreated ewes (Text-fig. 2b) and ovariectomized ewes with empty implants. The abrupt decline in concentrations observed during the late May/early June was associated with shearing of the ewes.

Text-fig. 1. Mean ($\pm$ s.e.m.) plasma LH concentrations in (a) ovarioectomized, untreated and (b) ovarioectomized, oestrogen-implanted ewes throughout the year, and (c) cyclic activity of untreated ewes of the same breeds.

Discussion

The breeding season data for the entire Dorset Horn and Welsh Mountain ewes are in general agreement with those reported previously (Hafez, 1952; Williams, 1974), any slight discrepancies between this and other investigations being attributable to differences in climate, nutrition and age of ewes (Hammond, 1947), and the presence and activity of the rams (Schinckel, 1954). The earlier onset of cyclic activity in the Dorset Horn compared to the Welsh Mountain ewes resulted in an asymmetric distribution of the breeding season around the winter solstice for this breed, thus confirming the original observations of Kelley & Shaw (1943), Hammond (1947) and Hafez (1952).

The LH profiles for the oestradiol-treated and control ovarioectomized ewes are consistent with those reported by Legan *et al.* (1977), Legan & Karsch (1979) and Goodman & Karsch (1980). A temporal relationship between oestrous cycle activity in the entire ewes and plasma LH concentrations in the ovarioectomized ewes with oestradiol implants is clearly evident; the transition to

Text-fig. 2. Mean ($\pm$ s.e.m.) plasma prolactin concentrations throughout the year in (a) ovariectomized, oestrogen-implanted, and (b) untreated ewes of two breeds.

ovarian activity coincided with an elevation of plasma LH concentrations and the transition to seasonal anoestrus occurred after plasma LH had returned to basal concentrations. This trend was apparent in both breeds, although the Dorset Horn sheep commenced breeding activity long before the Welsh Mountain sheep.

The dependence of this seasonal change in LH concentrations on oestradiol-17 β was demonstrated by the absence of such a change in the ovariectomized ewes with empty implants. Although mean oestradiol levels were not measured in this experiment, the oestradiol implants were identical to those used by Legan *et al.* (1977) which were shown to maintain constant physiological concentrations (3–5 pg/ml) throughout a 2-year experimental period. Thus it can be assumed that the seasonal shift in circulating LH in the oestradiol-treated ovariectomized ewe reflects a seasonal change in the ability of oestradiol to suppress tonic LH secretion. During the breeding season

oestradiol has a weak negative feedback effect, since mean plasma LH concentrations in these ewes were relatively high, though significantly lower than in non-steroid-treated ovariectomized ewes. During anoestrus oestradiol was much more potent and LH concentrations were basal.

Seasonal changes in tonic LH secretion have also been reported in the ram, in numerous other mammals, and in birds (Davis & Meyer, 1973; Turek, Elliott, Alvis & Menaker, 1975; Pelletier & Ortavant, 1975; Garcia & Ginther, 1976; Lincoln, 1976; Follett, 1978; Lincoln & Kay, 1979; Urbanski & Follett, 1982). The studies in the ewe and red deer stag, however, are the only ones in which little or no seasonal change in gonadotrophins has been observed in the absence of gonadal steroids (Text-fig. 1; Legan *et al.*, 1977; Lincoln & Kay, 1979). Although the existence of a separate, steroid independent, modulation of tonic LH secretion cannot be precluded by the present study (see Karsch *et al.*, 1980), these data suggest that such a mechanism is likely to be overshadowed by the profound seasonal change in LH responsiveness of the hypothalamic-pituitary axis to oestradiol.

The role of prolactin in this response remains unclear. The prolactin profiles in the untreated ewes and the ovariectomized ewes with oestradiol or empty implants showed the same temporal pattern of change for both breeds, remaining approximately parallel to changes in photoperiod, and indicating that seasonal changes in prolactin concentrations are largely steroid independent. The lower levels of prolactin observed in June compared with those in May or July have been reported previously (Jackson & Davis, 1979) and may be due to a direct effect of shearing on prolactin secretion (Fisher & Lapwood, 1981).

In support of previous work by Walton *et al.* (1977) and Rhind, Robinson, Chesworth & Crofts (1980), the breeding season was characterized by low or basal levels of prolactin whereas these became elevated during seasonal anoestrus. However, because of the difference in the onset of the breeding season between the two breeds, the levels of prolactin encountered during the transition to reproductive activity were much higher for the Dorset Horn ewes. The observation of a temporal relationship between plasma prolactin concentrations and breeding activity led Walton *et al.* (1977) to suggest that prolactin was antigonadotrophic in the ewe, and this supported earlier work of Kann & Martinet (1975) who found such an effect during lactational anoestrus. However, if this relationship is causal, it is clear from the present study that the sensitivity of each breed to prolactin at the onset of the breeding season must be very different, which seems unlikely. Rather, the data suggest little or no involvement of prolactin in determining between-breed differences in the timing of the onset of the breeding season.

Since ewes of both breeds stopped cycling at approximately the same time, it is possible to argue that prolactin has an antigonadotrophic effect during the transition to seasonal anoestrus but that the length of this effect, and hence anoestrus, may vary between breeds. However, if as was suggested by Land (1980), sheep start to cycle for reasons opposite to those which cause them to stop, the current data do not support a major role for prolactin in the control of seasonal breeding in the ewe. Indeed, they indicate that the inverse relationship between prolactin concentrations and oestrous cycle activity may be coincidental rather than causally related, as suggested by Jackson & Davis (1979). Further study is therefore required to determine the reasons for breed differences in seasonal breeding patterns in the ewe.

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