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Seasonal Changes in LH Secretion in Normal Ewes and Ewes which Grazed Oestrogenic Clover

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Abstract

Plasma luteinizing hormone (LH) concentrations were measured in normal (control) Corriedale × Merino (comeback) ewes and in clover-infertile comeback ewes which had grazed oestrogenic Yarloop clover (*Trifolium subterraneum* L. cv. Yarloop) for more than 4 years. Plasma LH concentrations were measured in samples taken at 20-min intervals for 6 h during the dioestrous stage of the oestrous cycle in the breeding season (BS) and during the anoestrous season (AS). In the control ewes during BS, transitory elevation in plasma LH concentration (pulses) occurred, reflecting secretory episodes, with a frequency of one per 5·2 h. This frequency fell to one per 16·5 h during the anoestrous season. In clover-infertile ewes, LH pulses occurred with a frequency of one per 4·5 h during BS and one per 4·9 h during AS (difference not significant).

In the controls, plasma LH levels were higher ($P<0·05$) during BS (mean ± s.d. = 1·2 ± 0·4 ng/ml, $n = 9$) than in AS (0·7 ± 0·3 ng/ml, $n = 5$). In the clover-infertile ewes, plasma LH levels in BS (1·3 ± 0·6 ng/ml, $n = 12$) were similar to those of controls. During AS, plasma LH levels in the clover-infertile ewes (1·0 ± 0·6 ng/ml, $n = 10$) remained similar to their BS levels, being significantly ($P<0·05$) higher than LH levels in the controls at this time.

These studies indicate that the higher plasma concentrations of LH which have been reported in clover-infertile ewes arise from more frequent LH pulses. Furthermore, in contrast to normal ewes, average plasma LH, reflecting pulse frequency, is not reduced in AS. This supports the view that ingestion of phyto-oestrogens affects neural centres involved in regulating LH secretion.

Introduction

Clover infertility describes a condition in sheep which has arisen from sheep grazing cultivars of subterranean clover (*Trifolium subterranean* L.) containing high levels of oestrogenic isoflavones. Extensive investigations into the patho-physiology of this disease have been carried out and most often in such studies ewes which had grazed these oestrogenic pastures for several years (Findlay *et al.* 1973; Lightfoot *et al.* 1974; Adams 1976, 1977; Chamley *et al.* 1981) have been used.

A variety of physiological alterations and clinical manifestations have been described in ewes afflicted by clover infertility. These include uterine and cervical pathology (Adams 1976), the production of large volumes of cervical mucus (Smith 1971), increased variability in oestrous cycle length (Adams *et al.* 1975) and luteal function (Adams *et al.* 1981). An initial study with aged Merino ewes which had grazed Yarloop clover suggested that in such animals there was an undefined defect at the hypothalamic level (Findlay *et al.* 1973). This suggestion was based upon the failure of such ewes to release LH in response to intravenously injected oestradiol-17β, whereas there was an LH release when the ewes received synthetic gonadotrophin releasing hormone (GnRH). Subsequent

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studies failed to confirm this lack of pituitary response to oestradiol-17β (Rogers *et al.* 1980; Chamley *et al.* 1981), although the existence of a defect within the hypothalamic–pituitary axis was still indicated because of the elevated basal LH secretion (Rogers *et al.* 1980; Chamley *et al.* 1981), and changes in behavioural responses to oestrogen (Adams 1978).

In the study by Chamley *et al.* (1981) plasma LH concentrations were measured in clover-infertile and normal Border Leicester × Merino ewes before and after treatment with either GnRH or oestradiol-17β. These authors noted that basal plasma LH concentrations were higher in clover-infertile ewes during anoestrus than in normal ewes, and they suggested that LH secretion may not be subject to seasonal changes as is the case for normal ewes.

This paper now reports more detailed studies of LH levels during spring and autumn, in an attempt to define more precisely the nature of the neuroendocrine defect in ewes which are affected by clover disease.

Materials and Methods

Sheep used in these studies were taken from two flocks in Victoria, all being progeny from South Australian Merinos. Control ewes, which had not grazed oestrogenic pasture and were aged 6–8 years, were purchased from north-eastern Victoria. Comeback ewes of similar age and condition, which were identified as being clover-infertile, were purchased from a property in central Victoria. These had been exposed to Yarloop clover for at least 4 years before purchase and, at the time of purchase, the lambing percentage for the flock was less than 35%. Lambing percentage in control ewes had been in excess of 100% over the 3 years preceding these experiments. Both flocks were mated during autumn. All experiments were carried out within 1 year of sheep being acquired. The ewes were run together on non-oestrogenic pasture with a vasectomized ram fitted with a sire-sine harness to record oestrous periods.

Each series of experiments was conducted according to a fixed schedule. On the day before experimentation, animals were brought to an enclosed shed and one jugular vein was fitted with an indwelling Silastic cannula (Dow-Corning, U.S.A.). The cannula was kept patent by flushing with 0·154 M NaCl solution containing heparin (75 units/ml).

In each series of experiments, blood (10 ml) was sampled from the jugular vein every 20 min during a period of 6–7 h. After collection, the plasma was harvested and stored at −12°C until it was assayed for LH. Ewes were bled according to this schedule during the breeding season (May) and during anoestrous (September). When ewes were sampled in the breeding season, the day of sampling represented day 8–10 of an oestrous cycle (dioestrous). Stage of cycle was verified in retrospect by measuring progesterone in a random selection of plasma samples. Ewes were considered to be in the luteal phase of a cycle if their plasma progesterone concentrations were greater than 1·5 ng/ml. All ewes which were studied during the anoestrous season had peripheral progesterone concentrations of less than 0·5 ng/ml at the time of experimentation.

Plasma progesterone concentrations were measured using the assay described by Hossian *et al.* (1979). In this assay, within-assay variation was <20% over the range of 0·5–4·0 ng/ml. Between-assay variation for internal standards, which measured 1·23 and 3·56 ng/ml, were 8·0 and 17·0% respectively.

LH concentration was determined in all plasma samples by a double antibody radioimmunoassay as described by Lee *et al.* (1976), with all samples taken from a single ewe being measured in one assay. Sensitivity of the assay was 0·1 ng/ml and within-assay variation was <20% over the range 0·8–20 ng/ml. The between-assay coefficients of variation for pools of plasma, which were included with each assay as internal standards, were 20% at 0·1–1·3 ng/ml, 20·9% at 3–5 ng/ml, and 10% at 9–11 ng/ml.

In the present study, a secretory episode (pulse) was identified when the concentration of LH in one sample exceeded the concentration in the previous sample by at least two standard deviations of the assay value for that previous sample. This definition of the start of a secretory episode was adopted previously by Rogers *et al.* (1980). A secretory episode was considered to be finished when the plasma LH level returned to the pre-pulse level. Pulse frequency and pulse magnitude (the maximum LH concentration measured during a secretory episode) was analysed by χ^2 test of independence and Student's *t*-test respectively. In attempting to analyse pulse magnitude, it was realized that the values determined for this might often be underestimates because the precision of the description of a peak would depend on the frequency of blood sampling (Martin

et al. 1983). Differences in overall mean LH concentrations and basal LH concentrations were tested by analysis of variance and Student's *t*-test. Basal LH samples represented those samples taken when there was no secretory episode.

Results

Patterns of pulsatile LH secretion were evident for individual ewes from either group in both the breeding (BS) and the anoestrous season (AS). In control ewes during dioestrus in BS, LH pulse frequency was one per 5·2 h (range 1–3 pulses per 6–7 h, $n = 9$ ewes) and this was reduced significantly ($P < 0{\cdot}01$) to one per 16·5 h (range 0–1 pulse per 6–7 h, $n = 9$) during AS. In clover-infertile animals, pulse frequency remained relatively constant, being one per 4·5 h ($n = 12$) and one per 4·9 h ($n = 10$) in BS (dioestrus) and AS respectively. There were no significant differences in pulse frequency between the two types of sheep during BS.

There were no significant differences in the magnitude of the LH pulse with respect to ewe type or season (2·0 ± 0·5, mean ± s.d., $n = 11$ pulses, 2·1 ± 0·5, $n = 17$, for normal and clover-infertile ewes, respectively, in BS; 1·4 ± 0·3, $n = 12$, 2·3 ± 1·0, $n = 14$, for normal and clover-infertile ewes, respectively, in AS), but the variability in the magnitude of the LH pulses was greater (Student's *t*-test, $P < 0{\cdot}001$) in clover-infertile ewes than in controls during AS. This variability suggests that the influence of season on the pituitary function of these clover-infertile ewes was less than for control ewes.

Table 1. Plasma LH concentrations in control and clover-infertile ewes during the breeding and anoestrous seasons

Values are means ± s.d.; those with the same superscript do not differ significantly at $P < 0{\cdot}05$. The number of LH estimations are given in parentheses

Ewe group	No. of ewes	Breeding season Overall LH concn (ng/ml)	Breeding season Basal LH concn[A] (ng/ml)	No. of ewes	Anoestrous season Overall LH concn (ng/ml)	Anoestrous season Basal LH concn[A] (ng/ml)
Normal	9	$1{\cdot}16 \pm 0{\cdot}4^{a,x}$ (113)	$1{\cdot}10 \pm 0{\cdot}4$ (91)	5	$0{\cdot}67 \pm 0{\cdot}3^{b,y}$ (100)	$0{\cdot}66 \pm 0{\cdot}3^{b}$ (92)
Clover-infertile	12	$1{\cdot}32 \pm 0{\cdot}6^{a,x}$ (94)	$1{\cdot}14 \pm 0{\cdot}5$ (70)	10	$1{\cdot}01 \pm 0{\cdot}6^{c,x}$ (205)	$0{\cdot}90 \pm 0{\cdot}5^{c}$ (141)

[A] LH concentration in plasma samples which were not included in an LH pulse.

Mean LH concentrations were calculated for all samples collected from each ewe and for those samples not included in an LH pulse (basal LH). During BS, the overall mean LH concentration for clover-infertile ewes was not different from controls, while during AS both the overall means LH concentration and the mean basal LH concentration were significantly higher ($P < 0{\cdot}01$) in clover-infertile ewes than in control ewes (Table 1). Whereas there was a significant change ($P < 0{\cdot}01$) in overall mean LH concentrations, between seasons, in control ewes, no change occurred in clover-infertile ewes.

Discussion

The seasonal LH secretory pattern in clover-infertile ewes was clearly different from the pattern in the control ewes. This could not be due to any differences in progesterone levels during the breeding season and therefore indicates that clover-infertile ewes did

not respond to seasonal events in the same manner as did the control ewes. In accordance with previous observations (Adams 1978; Rogers *et al.* 1980), the present data indicate a neuroendocrine lesion in clover-infertile ewes.

Secretory discharges of LH are more frequent in BS than in AS in the ram (Lincoln and Short 1980) and in the ewe, and there are more frequent pulses in the dioestrous stage of the oestrous cycle than in AS (Baird *et al.* 1976). Mean LH concentrations in the blood of ewes are higher in BS than in AS (Roche *et al.* 1970; Chamley *et al.* 1981). The present observations in control ewes are in agreement with these earlier findings. The seasonal difference in mean LH levels reflect, in part at least, the additional contributions which are being made to the total circulating pool of LH by each quantum of pituitary hormone which is released during a pulse. In contrast to the control ewes, the LH pulse frequency in clover-infertile ewes remained high throughout the year. In spite of this, the overall mean LH concentrations was significantly lower in AS, while basal LH secretion remained high irrespective of season.

These observations extend earlier knowledge about the nature of the neuroendocrine defect which develops in clover-infertile ewes (Rogers *et al.* 1980; Chamley *et al.* 1981).

The observations raise some interesting questions in relation to reported studies of ovarian function in clover-infertile ewes. Adams *et al.* (1979) reported that ovulation rate was higher in clover-infertile ewes than in normal ewes throughout BS. This difference in ovulation rate did not reflect any difference in liveweight, nor in the numbers of primordial follicles in ovaries from both types of sheep. Cahill and Mauleon (1980) have estimated that the growth time for an ovarian follicle in the sheep is approximately 170 days, meaning that follicles which ovulate in BS were activated to grow in AS. Thus the clover-infertile ewe might be a useful model to study the question of whether basal secretion of LH influences the dynamics of ovarian follicle growth by decreasing the incidence of atresia or by accelerating follicle growth rate or both.

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