Can the transition into anoestrus in the ewe be accounted for solely by insufficient tonic LH secretion?

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ABSTRACT

It has been proposed that a seasonal increase in oestradiol negative feedback elicits anoestrus by preventing a key step in the preovulatory sequence of endocrine events, namely a sustained increase in tonic LH secretion. In the present study we compared the patterns of serum LH, FSH, oestradiol and progesterone after regression of the last corpus luteum of the breeding season, with their respective patterns during an ovulatory cycle in the late breeding season (samples obtained every 4 h from eight ewes). After regression of the last corpus luteum of the breeding season, serum LH and oestradiol showed distinct deviations from their respective late breeding season patterns. The rise in tonic LH secretion was curtailed. Further, there were no marked increases in oestradiol, despite a distinct, although brief, tonic LH rise; thus there were no gonadotrophin surges. If the hypothesis that the transition into anoestrus is caused solely by insufficient tonic LH secretion were correct, the brief increase in LH should have induced a transient rise in oestradiol. Since this was not the case, these results suggest that a decreased ovarian response to LH may also contribute to the termination of oestrous cyclicity at the transition to anoestrus.

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INTRODUCTION

Based on our current hypothesis for the neuroendocrine regulation of seasonal breeding in ewes, oestrous cycles cease during the transition to anoestrus because there is an increase in the negative feedback action of oestradiol on tonic luteinizing hormone (LH) secretion (Legan, Karsch & Foster, 1977). Considerable evidence suggests that this enhanced feedback action of oestradiol prevents oestrous cycles by eliminating a key step in the preovulatory sequence of endocrine events, namely a sustained increase in tonic LH secretion (Karsch, Goodman & Legan, 1980b). According to the hypothesis, the rapid decline in serum progesterone levels which accompanies regression of the corpus luteum permits serum LH concentrations to increase progressively due to removal of the negative feedback action of progesterone (Karsch, Legan, Ryan & Foster, 1980b). This sustained tonic LH rise, in turn, stimulates a parallel rise in oestradiol secretion (Goodman, Reichert, Legan et al. 1981b; McNeilly, O’Connel & Baird, 1982) which triggers the LH surge (Goding, Catt, Brown et al. 1969; Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971) and thus causes ovulation (Robertson, 1967).

During the transition to anoestrus, however, the hypothesis predicts that, following the decline in progesterone accompanying regression of the last
corpus luteum of the breeding season, serum LH would be held in check by the newly acquired negative feedback action of oestradiol. Preliminary data obtained in three ewes indicated this was the case, namely, there was no significant increase in serum LH or oestradiol after regression of the last corpus luteum of the breeding season (Legan, Goodman, Ryan et al. 1981). However results from the remaining five ewes, reported herein, are not in unqualified agreement. They indicate that in some cases a sustained increase in LH levels occurs, but there is no change in oestradiol, suggesting that a decreased ovarian response to LH may also play a role in the transition to anoestrus.

Some preliminary portions of this work have been reported previously (Legan & Karsch, 1979; Karsch et al. 1980a; Legan et al. 1981).

MATERIALS AND METHODS

Sixteen adult ewes with regular oestrous cycles (either pure-bred Suffolk or predominantly Suffolk) were pastured in natural environmental conditions in Ann Arbor, MI (42°N), and were fed brome-grass hay supplemented with grain; water was available ad libitum. Beginning in mid-January, the incidence of oestrus was monitored every 4 h during the daylight hours with the use of a raddled vasectomized ram.

To determine which step in the preovulatory sequence fails at the end of the breeding season, patterns of serum gonadotrophins and steroids were evaluated in blood samples obtained by jugular venepuncture according to the following schedule. Beginning in mid-January, samples were collected once daily until day 10 of the cycle (oestrus = day 0). Blood samples were then collected every 4 h from day 10 of each oestrous cycle until 24 h after the next oestrus. If no oestrus occurred, the 4-h blood samples were continued for an additional 8 days to verify the absence of a corpus luteum (serum progesterone levels less than 3·1 nmol/l (1 ng/ml). This indicated that a 'silent' ovulation (ovulation without oestrus) did not occur, and established the end of the breeding season. All blood samples were obtained under natural environmental conditions without the aid of artificial light. Once eight ewes had stopped their cycles, the experiment was terminated. In all cases but one, the same animals provided data for the ovulatory cycle late in the breeding season and the transition into anoestrus. In the one exceptional ewe, anoestrus began during the first 4-h blood collection period; thus no data are available for the late breeding season in this ewe.

Sera were separated and stored at -20°C. Concentrations of LH were determined in duplicate in 25–200 µl aliquots of serum by means of a radioimmunoassay (Niswender, Reichert, Midgley & Nalbandov, 1968) modified as described previously (Hauger, Karsch & Foster, 1977). The limit of detection (95% confidence limit of buffer control) averaged 0·2 µg/l for 200 µl serum. The intra- and interassay coefficients of variation (C.V.) averaged 7·5 and 10·0%, respectively. Concentrations of LH are expressed in terms of NIH-LH-S12, which has a biological activity of 0·82 × S1 as determined by the ovarian ascorbic acid depletion assay (Reichert & Wilhelmi, 1973). Follicle-stimulating hormone (FSH) was measured in duplicate in 100 µl aliquots of serum by a radioimmunoassay described previously (Goodman, Pickover & Karsch, 1981a). The limit of detection averaged 9·0 ng/ml and the interassay C.V. for serum pools containing 69 or 78 µg/l averaged 6·6 and 10·6%, respectively. The intraassay C.V. were 4·7 and 6·2%, respectively. Results are expressed in terms of NIH-FSH-S8. Concentrations of serum oestradiol were determined in duplicate in chromatographed benzene extracts of 1 ml serum by a modification (Goodman, Legan, Ryan et al. 1980) of a radioimmunoassay described previously (England, Niswender & Midgley, 1974). The limit of detection averaged 0·7 pmol/l (0·2 pg/ml) and the intra- and interassay C.V. averaged 8 and 13%, respectively. Serum progesterone concentrations were measured in duplicate in petroleum ether extracts of 60 µl serum using a modification (Hauger et al. 1977) of a radioimmunoassay described previously (Niswender, 1973; Foster, Lemons, Jaffe & Niswender, 1975). The limit of detection of the assay averaged 0·12 nmol/l (0·04 ng/ml) and the intra- and interassay C.V. averaged 5·6 and 11·0%, respectively.

To determine the beginning of the fall in progesterone at luteolysis, we first calculated the 90% confidence limits of the mean of at least six values before an obvious decline in progesterone. The beginning of the fall in progesterone was defined as the first value below the lower 90% confidence limit of this mean.

Statistical analyses were performed using repeated measures analysis of variance, with time after the progesterone fall as the repeated measures factor and season as the grouping factor. Undetectable hormone concentrations were assigned a value equivalent to the limit of detection of the assay. Missing values were assigned the mean of all other values in the group. Having determined by the repeated measures analysis that the season by time interaction was significant (P < 0·0001), we performed a trend analysis for two within factors, season and time, using the BMDP Statistical Software, 1983 (SAS Institute, Inc., Raleigh, NC, U.S.A.), to determine whether the difference was in the linear, quadratic or other components of the LH secretion pattern.
RESULTS

Mean serum LH, FSH, progesterone and oestradiol concentrations during the preovulatory period in the late breeding season are compared with those following regression of the last corpus luteum of the breeding season in the same animals in Fig. 1. As shown previously (Baird & Scaramuzzi, 1976; Karsch, Foster, Legan et al. 1979) the decline in serum progesterone levels at luteolysis in the late breeding season was

![Graph showing endocrine events during luteolysis](image)

**FIGURE 1.** Comparison of the endocrine events accompanying luteolysis in (a) the late breeding season with those during (b) the transition to anoestrus. Mean ± S.E.M. (shaded areas) serum progesterone (P), LH, FSH and oestradiol (OE$_2$) concentrations measured every 4 h are depicted during a 4- to 5-day period around the time of luteal regression in a late breeding season cycle (a, seven ewes) and around the time of regression of the last corpus luteum of the breeding season (b, eight ewes). All hormone concentrations are normalized to the first decrease in serum progesterone concentrations (see Materials and Methods), depicted by the broken vertical line. Data are not available for one ewe in the late breeding season because regression of the last corpus luteum of the breeding season occurred during the first series of 4-h blood collections. Serum progesterone and oestradiol concentrations are expressed in molar terms on the left axis, and in terms of mass on the right axis. Note the logarithmic scales.
accompanied by sustained parallel increases in LH and oestradiol \((P < 0.05)\). These increases persisted until the onset of the preovulatory LH surge 56.0 ± 1.5 h after the start of the fall in progesterone. Serum FSH concentrations did not change until onset of the FSH surge.

After regression of the last corpus luteum of the breeding season, serum progesterone and basal FSH levels were similar to their respective late breeding season values, but LH and oestradiol showed distinct deviations. The most notable differences were: (1) complete absence of gonadotrophin surges, (2) no increment in serum oestradiol and (3) a difference in the linear, but not quadratic or other, components of the regression describing the tonic LH secretory pattern between days 0 and 2 \((P < 0.0001)\). Although mean LH increased during the first 20 h after progesterone fell, and these increased LH levels were similar to those during the early follicular phase of breeding season cycles, they gradually returned to basal levels (approximately 0.5 µg/l).

Profiles of serum progesterone, oestradiol and FSH in individual ewes were similar to their respective mean patterns (not illustrated). The individual time course of LH, however, revealed a spectrum of patterns reflecting marked differences among ewes (Fig. 2). In some ewes, the duration of the tonic LH rise at the transition into anoestrus was almost as long as that associated with the progesterone fall during an ovulatory cycle late in the breeding season (e.g. 429 and 6041). In other ewes, however, serum LH concentrations during the transition to anoestrus increased for only about 24 h (e.g. 373 and 376), very briefly (e.g. 379 and 432), or not at all (e.g. 7017). Regardless of the duration of the tonic LH rise, no oestradiol rise was seen in any ewe.

**DISCUSSION**

According to the hypothesis that seasonal breeding in the ewe results from a change in potency of oestradiol negative feedback on tonic LH secretion, it is predicted that after regression of the last corpus luteum of the breeding season there would not be a typical sustained rise in circulating LH; thus there would be no oestradiol rise and no gonadotrophin surge (Legan et al. 1977). The present study clearly indicates that there is no oestradiol rise and no gonadotrophin surge at the transition to anoestrus. The question thus becomes, is there a typical rise in tonic LH secretion? Clearly, LH did increase at this time in most ewes. In none of the animals, however, was this increase typical because it was not sustained for the full 56-h period which, in ovulatory cycles, spans the time from the progesterone fall to the LH surge. Nevertheless, LH patterns in individual ewes ranged from near-normal increases (e.g. 6041, Fig. 2) to no increase at all (e.g. 7017). Such individual profiles must be interpreted with caution, however, because of the pulsatile nature of tonic LH secretion and the 4-h sampling interval which is insufficient to characterize LH pulses. Because any single pattern reflects values obtained at random phases of the pulse rhythm, we consider the mean time-course to be most useful in interpreting the results. This time-course appears to support our hypothesis for the transition into anoestrus.

The temporal relationships among mean hormone concentrations raise several questions. First, why was the brief LH rise in some ewes during the transition to anoestrus as large as that during an ovulatory cycle and why was this LH rise transient? Further, in those ewes in which LH appeared to increase markedly (e.g. 373), why was there no oestradiol rise?

With regard to the amplitude of the brief increase in LH, it is well documented that withdrawal of progesterone negative feedback causes a prompt increase in LH (Baird & Scaramuzza, 1976; Karsch et al. 1979). Further, progesterone withdrawal in mid-anoestrus results in a brief, but marked, LH rise identical to that observed in our study (Goodman & Karsch, 1980). Thus, the LH rise following regression of the last corpus luteum of the breeding season can be explained by the withdrawal of progesterone. The subsequent fall in LH is most likely to be due to oestradiol, which becomes a potent negative feedback hormone at the transition to anoestrus (Legan et al. 1977). The variable duration of the LH rise among ewes (Fig. 2).
may reflect differing intermediate degrees of oestradiol feedback potency during the shift from minimum (breeding season) to maximum (anoestrus). Thus the relatively long LH rise in ewe 429 might indicate that oestradiol negative feedback was barely sufficient to terminate cyclicity when the last corpus luteum of the breeding season regressed. In contrast, the extremely brief LH rise in ewe 379 may reflect a more highly developed negative feedback response. This interpretation implies that the neuroendocrine changes which lead to anoestrus develop gradually as the end of the breeding season approaches, rather than abruptly during 1 or 2 days at the end of the last oestrous cycle.

With regard to the second question of why there was no oestradiol rise in response to the brief LH increments, one possible explanation is that the follicle becomes less responsive to LH in anoestrus. Clearly, follicles can respond to physiological increments of LH in anoestrus, as is indicated by studies examining the ovarian response to endogenous LH pulses (Scaramuzzi & Baird, 1977), to exogenous LH pulses (McNeilly et al., 1982), and to LH infusions (Goodman et al., 1981b). Nevertheless, there are no data available which compare the response of oestradiol to the same physiological LH stimulus in both seasons. This comparison is critical for the determination of whether ovarian response decreases in anoestrus.

A second possibility, based on the apparent similarities in hormonal control of follicular maturation in sheep and rats (Webb & England, 1982), namely that FSH induces LH receptors in oestrogen-primed follicles (Richards, 1979), is that a deficiency in FSH could cause a reduced response to LH. Although the basal FSH level observed at the transition into anoestrus was not different from that at a comparable time in a late breeding season cycle, we cannot exclude a possible deficiency in FSH at a crucial, earlier stage of follicular maturation. In this regard, serum FSH concentrations are raised in the early luteal phase of the cycle during the mid-breeding season (Salamonsen, Jonas, Burger et al., 1973; Pant, Hopkinson & Fitzpatrick, 1977; Goodman et al., 1981a). Further, as anoestrus approaches, the negative feedback action of oestradiol on FSH secretion has clearly been shown to increase (Legan & Karsch, 1980); this may attenuate the early luteal phase increase in FSH, although this was not determined in the present study, and results of earlier studies are inconclusive regarding whether or not there is inadequate FSH in anoestrus (Findlay & Cumming, 1976; Walton, McNeilly, McNeilly & Cunningham, 1977; Walton, Evans, Fitzgerald & Cunningham, 1980).

A third possibility is that the pulsatile pattern of tonic LH secretion was altered in such a way that the rise in LH did not stimulate ovarian oestradiol secretion. For example, pulse amplitude may have increased and pulse frequency decreased. Thus, in some ewes, tonic LH levels may have appeared to increase but the ovary may not have been stimulated by such a secretion pattern. The sampling frequency was not high enough in this study to distinguish between these two possibilities. However, if there is an increase in tonic LH levels in some ewes, the complete absence of a concomitant oestradiol rise strongly supports the possibility that there is a decrease in ovarian response to LH at the transition to anoestrus, which must ultimately be explained.

The foregoing observations support the hypothesis that the mechanism for transition into anoestrus includes an increased potency of oestradiol negative feedback control of LH secretion. Nonetheless, our findings suggest that in some ewes, a decrease in ovarian response to LH may also play a role in onset of anoestrus. To resolve this issue, it remains to be determined whether ovarian response to physiological patterns of gonadotrophin secretion is diminished during this transition.

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