Does the first LH surge of the breeding season initiate the first full-length cycle in the ewe?

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Summary. To determine whether the first LH surge of the breeding season initiates a transient rise in progesterone in most ewes, serum progesterone (daily) and LH (every 4 h) concentrations were measured in samples collected from 7 ewes between 19 July and first oestrus or 8 September, whichever came first. In 6 of the 7 ewes, the first LH surge of the breeding season was followed within 5 days by a transient, 2-day rise in progesterone. Within less than 5 (N = 4), or 9 (N = 1) or 10 (N = 1) days later, a second LH surge occurred, which was similar in maximum amplitude and duration to the first surge, and which initiated the first full-length luteal phase of the breeding season. In the remaining ewe, the first LH surge of the breeding season induced an abbreviated (9 days) and insufficient (maximum progesterone, 0.94 ng/ml) luteal phase. These results demonstrate that most ewes have more than one LH surge before the first full-length luteal phase, the first surge inducing a transient rise in progesterone. Therefore, although the seasonal decrease in response to oestradiol negative feedback is sufficient for initiation of the first LH surge of the breeding season, additional endocrine mechanisms may be necessary to induce the first full-length luteal phase.

Keywords: seasonal transition; LH surge; serum LH; short luteal phases; progesterone

Introduction

According to the current hypothesis for the endocrine control of the transition to breeding season, a short-day-induced decrease in response to oestradiol negative feedback results in an abrupt increase in LH pulse frequency (Karsch, 1980; Goodman & Karsch, 1981). This in turn stimulates a rise in oestradiol secretion, which ultimately attains the threshold required to elicit an LH surge, resulting in ovulation, and thus the beginning of the breeding season (Karsch *et al.*, 1980b). However, in the majority of ewes the first rise in progesterone which occurs at the transition from anoestrus to the breeding season is not a full-length luteal phase. Rather, one or more transient rises in progesterone occurs between 1 and 4 weeks before the first full-length cycle (Yuthasastrakosol *et al.*, 1975; Walton *et al.*, 1977; I'Anson & Legan, 1988). If the first brief rises in progesterone observed during the transition to breeding season are caused by the first LH surge, then the above sequence of events may be sufficient for induction of LH surges, but not full-length cycles, indicating that additional endocrine mechanisms may be necessary to induce the first full-length luteal phase of the breeding season.

That there is an LH surge before each transient rise in progesterone is supported by the observations that serum LH concentrations were 19.1 ± 8.2 ng/ml (N = 8) 3 days before a transient progesterone rise (Walton *et al.*, 1977) and that serum LH concentrations were >20 ng/ml (N = 3) about 1 week before the first full-length luteal phase (Yuthasastrakosol *et al.*, 1975). Although the daily sampling regimen used in these 2 studies was too infrequent to detect an 8–12 h-long LH surge reliably, part of an LH surge was observed 5 days before the first transient increase in progesterone

at the beginning of the breeding season in one of 8 ewes sampled every 15 min for 8 h (I'Anson & Legan, 1988). Finally, additional supportive evidence is provided by the observations that when oestrous cycles are induced in anoestrus, the first LH surge initiates a short luteal phase in most ewes (Oldham & Martin, 1978; McNatty *et al.*, 1982; McLeod *et al.*, 1982a, b; Pearce *et al.*, 1985; Legan *et al.*, 1985a, b). Therefore, the following study was designed to determine whether most first LH surges at the transition to the breeding season induce transient rises in progesterone rather than full-length luteal phases.

Materials and Methods

Animals. Thirteen Suffolk or Suffolk-crossbred ewes were maintained in an outdoor pen at the Sheep Research Facility in Ann Arbor, Michigan (42°N latitude), in the presence of rams. All animals were fed hay and had free access to mineral licks and water.

Experimental procedure and blood samples. To determine when LH surges occurred during the transition to breeding season, blood samples (7 ml) were obtained by jugular venepuncture every 4 h from 19 July to first oestrus or 8 September, whichever came first. Oestrous behavior was monitored daily using a raddled vasectomized ram, which was introduced on the 2nd day of this study (20 July) (Karsch *et al.*, 1980a). To establish occurrence of the first luteal phase of the breeding season and any antecedent rises in progesterone, serum progesterone concentrations were measured in daily blood samples obtained throughout the previously-defined experimental period.

Blood samples were stored overnight at 4°C. Sera were separated and stored at -20°C until assayed.

Radioimmunoassays. LH concentrations were determined in duplicate in 2.5 to 200 µl volumes of serum by a radioimmunoassay (Niswender *et al.*, 1969), which was modified as described previously (Legan *et al.*, 1985a). The limit of detection (95% confidence limit of maximum binding) averaged 0.18 ng/ml for 200 µl serum. The intra- and inter-assay coefficients of variation (CV) for 200 µl samples of a serum pool containing 1.02 ng/ml LH ($n = 18$), which inhibited binding of labelled ligand to 50.6%, averaged 9.3% and 15.8%, respectively. LH concentrations were expressed in terms of the NIAMDD ovine LH reference preparation, Batch 21, which has a potency of 1.86 times that of NIH-LH-S12, as we determined by radioimmunoassay.

Progesterone concentrations were measured in duplicate petroleum ether extracts of 150 µl serum by a modification (Legan *et al.*, 1985a) of a radioimmunoassay described previously (Gibori *et al.*, 1977). Assay sensitivity (95% confidence limits of maximum binding) averaged 0.14 ng/ml for 150 µl serum, and ether and water blanks were routinely below this value. Intra- and inter-assay CVs averaged 14.1% and 24.7% ($n = 22$), respectively, for 100 µl samples of a serum pool from intact ewes which inhibited binding of labelled ligand to about 50.7% and contained 3.54 ng/ml progesterone.

Statistical analysis. Statistical analyses were performed using Student's *t* tests, paired *t* tests, or within-subjects, repeated-measures analyses of variance (BMDP Statistical Software, 1983; SAS Institute, Inc.) with time as the repeated-measures factor. The relationship between mean serum LH and time was also analysed using linear regression. Undetectable hormone concentrations were assigned a value equivalent to the limit of detection of the assay. Results are presented as mean $\pm$ s.e.m.

Results

All ewes were exposed to new rams, which were moved into an adjacent pen about 1 week before the beginning of this study. In 9 of the 13 ewes either a transient rise in progesterone ($N = 3$) or a full-length luteal phase ($N = 6$) was detected during the next 1–2 weeks. To avoid any possible effect of these ram-induced endocrine events on the onset of cycles, the results from 6 ewes, whose first full-length cycle began less than 3 weeks after the end of these ram-induced luteal phases, were excluded from the study.

The mean date of onset of the breeding season (onset of first full-length cycle) for those ewes included in the study was 28 August $\pm$ 2 days ($N = 7$). This date is similar to that reported previously for Suffolk ewes (3 September $\pm$ 5 days, Robinson & Karsch, 1984; 3 September $\pm$ 3 days, I'Anson & Legan, 1988).

Serum progesterone concentrations remained low in all 7 ewes (0.17 ± 0.02 ng/ml) until after the first LH surge of the breeding season (Fig. 1). In one ewe, the first cycle lasted 12 days (progesterone > 0.4 ng/ml for 6 days) and was insufficient as defined by a maximum serum progesterone value less than 1.00 ng/ml (Fig. 2a). In the remaining 6 ewes, during the 2-week period before the

Fig. 1. Mean ($\pm$ s.e.m.) serum LH ($\circ$) and progesterone ($\bullet$) concentrations during the transition into the breeding season. In 6 of 7 ewes, the first LH surge of the breeding season was followed by a brief increment in progesterone. Data from Days -23 to -3 were normalized to the first LH surge; those thereafter to the second surge.

Fig. 2. Serum LH concentrations in samples obtained every 4 h ($\circ$), and daily circulating progesterone concentrations ($\bullet$) during the transition to the breeding season in 3 representative ewes. See text for further details.

first full-length luteal phase, there was a transient rise in progesterone lasting for 2.00 ± 0.36 days, the maximum concentration averaging 0.81 ± 0.13 ng/ml. In 4 of these 6 ewes, the transient rises in progesterone occurred 2.25 ± 0.63 days before the LH surge which led to the first full-length luteal phase (one representative ewe, Fig. 2b), while in the other two ewes, they were observed 9 and 10 days before this surge (one representative ewe, Fig. 2c).

An LH surge was observed within 3.17 ± 0.17 days ($N = 6$) before each transient rise in progesterone and within 3.43 ± 0.37 days ($N = 7$) before each first full-length luteal phase (Fig. 1). There was no difference between the first and second LH surges in either the maximum amplitude (47.64 ± 6.20 and 39.08 ± 5.80 ng/ml, 6 ewes) or duration (10.67 ± 0.84 and 12.67 ± 1.60 h, LH > 5.0 ng/ml, 6 ewes).

Mean serum LH remained at basal concentrations (1.08 ± 0.22 ng/ml, 7 ewes) during the transition to the breeding season until ~ 44 h before the first LH surge, when concentrations began to rise from 0.94 ± 0.28 to 2.32 ± 0.36 ng/ml (7 ewes) just before the onset of the surge. The preovulatory rises in mean tonic LH concentrations which occurred during the 44 h before the first and second surges were not different (1.03 ± 0.32 to 2.21 ± 0.41 ng/ml, slope = 0.14, and 1.08 ± 0.47 to 2.18 ± 0.54 ng/ml, slope = 0.09, 6 ewes, Fig. 1).

Mean tonic LH concentrations during the transient progesterone rises which occurred within 4 days of onset of the second LH surge were not different from those during an equivalent period (of both duration and time) before the first LH surge, when progesterone values were basal (1.37 ± 0.22 and 1.53 ± 0.51 ng/ml, 4 ewes).

Oestrous behaviour was detected in all ewes after the first full-length luteal phase, and in the ewe in which the luteal phase was insufficient (Ewe 430, Fig. 2a). In contrast, oestrous behaviour was not detected after the transient rises in progesterone.

Discussion

The foregoing results support previous observations that transient rises in progesterone occur just before the first full-length luteal phase of the breeding season (Yuthasastrakosol *et al.*, 1975; Walton *et al.*, 1977; I'Anson & Legan, 1988). Such transient progesterone rises are a common phenomenon at transitions from acyclicity to cyclicity, for example, after induction of LH surges in anoestrous ewes (Pearce *et al.*, 1985; Legan *et al.*, 1985a; Hunter *et al.*, 1986), at puberty in lambs (Foster & Ryan, 1979; Berardinelli *et al.*, 1980; Keisler *et al.*, 1983; Foster *et al.*, 1986), heifers (Gonzalez-Padilla *et al.*, 1975), rhesus monkeys (Foster, 1977; Nass *et al.*, 1979) and humans (Doring, 1969), at resumption of cycles in post-partum cows (Pope *et al.*, 1969) and women (Gray *et al.*, 1987), and at onset of the breeding season in dairy goats (BonDurant *et al.*, 1981). The foregoing results also extend previous observations by demonstrating that, in most ewes, the first LH surge of the breeding season leads to such a transient rise in progesterone rather than a full-length luteal phase. Thus, they suggest that the seasonal decrease in oestradiol negative feedback, which initiates the events leading to the first LH surge, may not be sufficient in most cases for initiation of full-length oestrous cycles, and that additional endocrine mechanisms are required.

Transient rises in progesterone play a key role in the initiation of full length cycles. In anoestrous ewes, an antecedent, transient progesterone rise similar to those occurring at the beginning of the breeding season ensures incidence of a full-length luteal phase following induction of an LH surge (Oldham & Martin, 1978; Legan *et al.*, 1985b). Similarly, at the transition to the breeding season as reported herein, all LH surges which occurred immediately after a transient increase in progesterone initiated a full-length luteal phase. It has been suggested that progesterone may ensure full-length luteal phases by synchronizing maturation of a follicle with the LH surge (Legan *et al.*, 1985b). This hypothesis is based on the finding that follicular development occurs throughout anoestrus in ewes (McNatty *et al.*, 1984), and on the proposal that short luteal phases result from ovulation of immature follicles (McLeod *et al.*, 1982b).

How might an antecedent increment in progesterone synchronize follicular maturation with an LH surge and thereby ensure initiation of a full-length luteal phase? One possible mode of action may be by altering tonic LH concentrations preceding the LH surge. However, our finding that both the mean basal serum LH concentrations and the preovulatory increments in serum LH before the first and second LH surges were similar indicates that the transient rises in progesterone do not ensure initiation of full-length cycles by altering tonic LH levels.

Another possible mode of action of progesterone in this regard might be to alter the amplitude, duration or time of onset of the LH surge. The similarity in the amplitude and duration of the first and second LH surges observed in this study indicates that brief increments in progesterone do not affect the amplitude or duration of the subsequent LH surge, as has also been shown in GnRH-treated anoestrous ewes (Legan *et al.*, 1985b). With regard to an action of progesterone on the time of onset of the LH surge, it has been shown that, in anoestrous ewes, induction of LH surges from 24 to 72 h after progesterone pretreatment ensures full-length cycles (McLeod & Haresign, 1984; Legan *et al.*, 1985b; Pearce *et al.*, 1987). However, it remains to be determined whether the brief increments in progesterone which occur before the first full-length cycle at the beginning of the breeding season delay the LH surge.

Another mechanism whereby progesterone might ensure initiation of full-length luteal phases by an ensuing LH surge is to increase ovarian response to circulating gonadotrophins. This possibility receives support from the observation that administration of progesterone to anoestrous ewes before induction of an LH surge increased in-vitro oestradiol production and binding of ^{125}I -labelled human chorionic gonadotrophin to granulosa cells from follicles which were removed just before and 2 h after onset of the LH surge (Hunter *et al.*, 1986). Although the results presented herein indicate that preovulatory serum LH concentrations were not different before a transient progesterone rise from those observed before a full-length luteal phase, it remains to be determined whether the preovulatory rise in oestradiol which occurs before a transient rise in progesterone is less than that which precedes a full-length cycle.

Progesterone might also act by modulating tonic FSH concentrations during or immediately after the transient rise in progesterone, such that follicular maturation is optimal at the next LH surge, and hence a full-length luteal phase ensues. Finally, progesterone may exert an indirect luteotrophic effect on the newly-formed corpus luteum by inhibiting release of prostaglandin (PG) F-2 α from the uterus or by decreasing ovarian response to PGF-2 α . Whatever the mechanism whereby progesterone ensures full-length luteal phases, its site of action in this regard also remains to be determined.

Although the observed rise in mean serum LH just before the first LH surge was not different from that which preceded the second surge, both of these 2-fold increments in tonic LH were lower than the 4–5-fold increase observed during the late follicular phase in the mid-breeding season (Karsch *et al.*, 1979). Most of this difference can be accounted for by the difference in potency of the respective ovine LH standards used in the assays, our standard being 1.86 times more potent. In addition, some of the difference may reflect the seasonal modulation of the response to oestradiol negative feedback, such that during the transition period and early breeding season the negative feedback action of oestradiol, though diminished, is still more evident than in the mid-breeding season.

In 2 ewes, a transient rise in progesterone was observed 9 or 10 days before the first full-length luteal phase. This delay between the first short cycle and the second LH surge may reflect a short-lived rebound in the response to oestradiol negative feedback such that the next rise in tonic LH and subsequent LH surge was prevented for a time. This is supported by the observation that serum LH concentrations fluctuate markedly over a 17-day period as they increase in individual ovariectomized, oestradiol-treated ewes, at a time coincident with the transition from anoestrus to the breeding season in intact ewes (Legan *et al.*, 1977).

The occurrence of an insufficient luteal phase at the transition to breeding season is rare. In most cases, whenever the first increase in progesterone concentrations lasts longer than 2 days,

serum progesterone concentrations remain elevated above at least 2 ng/ml for about 10–12 days (Yuthasastrakosol *et al.*, 1975; Walton *et al.*, 1977; I'Anson & Legan, 1988).

In conclusion, these results suggest that, although the seasonal decrease in oestradiol negative feedback is sufficient for initiation of the first LH surge of the breeding season, additional endocrine mechanisms may be necessary to induce the first full-length luteal phase. The current working hypothesis for the neuroendocrine control of onset of the breeding season may therefore be expanded to include the findings reported herein. Namely, the seasonal decrease in oestradiol negative feedback which occurs during the transition into breeding season allows an increase in tonic LH secretion which stimulates a rise in serum oestradiol causing an LH surge and subsequent ovulation (Legan & Karsch, 1979). If there is a mature follicle present at the time of the LH surge, a full-length luteal phase will ensue; if not, which is generally the case, a transient rise in progesterone or short luteal phase will follow. The response to oestradiol negative feedback will either increase once again to a level high enough to prevent another increase in tonic LH for a period of time following this first luteal phase or it will remain low, allowing another rise in tonic LH secretion. If this second rise in tonic LH occurs soon after the first luteal phase and is sustained long enough to initiate an LH surge, it will always initiate a full-length cycle. Once the decrease in response to oestradiol negative feedback is sustained for more than a few days, full-length oestrous cycles will recur. Since follicular development during anoestrus is random in relation to the photoperiod-induced decrease in response to oestradiol negative feedback, the onset of full-length cycles at the beginning of the breeding season will be immediately preceded by a short luteal phase in the majority of ewes.

We thank Dr Gordon D. Niswender and Dr Leo E. Reichert, Jr, for graciously providing reagents for radioimmunoassay; Dr F. J. Karsch, Dr D. L. Foster, Dr R. L. Goodman and Dr K. D. Ryan for expert assistance in the design and execution of this study; Mr D. Doop for invaluable help in the conduct of the experiment and animal husbandry; and Ms Marilee Van Arsdall for performing RIAs. This work was supported by NIH-HD-14039 and NSF-PCM-8302959.

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Received 11 August 1987