BIOLOGY OF REPRODUCTION 33, 324–334 (1985)

Seasonal Changes in Pulsatile Luteinizing Hormone (LH) Secretion in the Ewe: Relationship of Frequency of LH Pulses to Day Length and Response to Estradiol Negative Feedback[1]

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ABSTRACT

Seasonal changes in pulsatile luteinizing hormone (LH) secretion in ovariectomized ewes were examined over the course of 2 yr in relation to annual changes in environmental photoperiod, shifts in response to estradiol negative feedback control of LH secretion, and timing of the breeding season. Under natural environmental conditions, the frequency of LH pulses in individual ovariectomized ewes changed gradually and in close association with the annual cycle of day length. As days became shorter in late summer and autumn, LH pulse frequency increased; conversely, as day length increased in late winter and spring, frequency declined. Under artifical conditions in which ovariectomized ewes were exposed to different photoperiods, a similar inverse relationship was observed between day length and LH pulse frequency. The seasonal changes in frequency of LH pulses in ovariectomized ewes, although symmetric with the annual photoperiodic cycle, were not temporally coupled to the dramatic shifts in response to estradiol feedback inhibition of LH secretion at the transitions between breeding season and anestrus. The feedback shifts occurred abruptly and at times when LH pulse frequency in ovariectomized ewes was at, or near, the annual maximum or minimum. The tight coupling between LH pulse frequency and photoperiod leads to the conclusion that there is a photoperiodic drive to the LH pulse-generating system of the ewe. The temporal dissociation between changes in this photoperiodic drive and the seasonal shifts in response to estradiol negative feedback support the hypothesis that the neuroendocrine basis for these two phenomena is not one and the same.

INTRODUCTION

Seasonal changes in reproductive condition are associated with frequency modulation of the luteinizing hormone (LH) pulse generator, a neural oscillator that produces the episodic pattern of gonadotropin secretion characteristically observed in mammals (Lincoln and Short, 1980; Martin et al., 1983; Karsch et al., 1984). Changes in the activity of this LH pulse-generating system are evident both in the presence and in the absence of gonadal steroids. Moreover, in the ewe, for example, there is a marked seasonal change in the capacity of estradiol to inhibit the frequency of LH pulses; this is referred to as a seasonal shift in response to estradiol negative feedback (Legan et al., 1977; Goodman et al., 1982; Martin et al., 1983). In the absence of gonadal steroids, the frequency of LH pulses changes with season (Karsch et al., 1980a; Goodman et al., 1982). Because this effect does not require ovarian hormones, it is referred to as direct photoperiodic drive to the LH pulse generator.

Much is known about the regulation, and the significance, of the seasonal shifts in response to estradiol negative feedback in the ewe. These shifts have been shown to be temporally coupled to the onset and cessation of the breeding season; they are controlled by photoperiod; and they produce changes in frequency

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Accepted February 25, 1985.
Received October 5, 1984.

[1] Portions of this study were presented at the 15th and 16th Annual Meetings of the Society for the Study of Reproduction and appeared in Biol. Reprod. 26 (Suppl. 1):Abstr. 41 and Biol. Reprod. 28 (Suppl. 1):Abstr. 62. Supported by grants from the NIH (HD-11311 and HD-18337) and the Ford Foundation and by the CSIRO in Australia.

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of LH pulses that, in turn, determine the seasonal reproductive state (Legan et al., 1977; Goodman and Karsch, 1980a; Legan and Karsch, 1980; Karsch et al., 1984). In contrast, much less is known about direct photoperiodic drive to the LH pulse generator and whether a functional relationship exists between it and the shift in feedback response that underlies seasonal reproduction.

The present study focused on seasonal fluctuations in direct photoperiodic drive to the LH pulse generator. Specifically, we characterized the temporal relationship of the seasonal change in LH pulse frequency in the ovariectomized ewe to the annual photoperiodic cycle and the shift in response to estradiol negative feedback that governs the seasonal changes in estrous cyclicity. In addition, we tested whether the absolute frequency of LH pulses in the absence of gonadal steroid feedback can be set by photoperiod, the primary environmental variable governing seasonal reproduction in the ewe (Yeates, 1949; Hafez, 1952; Mauleon and Rougeot, 1962).

MATERIALS AND METHODS

Animals and Treatments

Two experiments were performed over the course of 2 years (1981–1983) on sexually mature ewes (purebred or predominantly Suffolk) maintained either under natural environmental conditions or in light control rooms at the Sheep Research Facility in Ann Arbor, Michigan (latitude 42°N). The animals kept outdoors were maintained on fresh pasture supplemented when necessary with hay; those housed indoors were fed hay. All ewes had free access to water and mineral licks. The four light control rooms (12.9 m^2) used in this study each held 6 ewes, and each was illuminated by four electronically timed 40-watt fluorescent bulbs that produced approximately 350 lux at the level of the sheep's head. None of the rooms had been occupied by animals prior to the experiment. Ovariectomy was performed 1–2 mo before the study began via midventral laparotomy under anesthesia induced with sodium pentobarbital (approximately 15 mg/kg).

Pulsatile secretion of LH in the absence of steroid feedback (direct photoperiodic drive) was assessed by assay of LH in samples of blood obtained from ovariectomized ewes at 6- or 12-min intervals (sampling begun between 0800 and 1000 h). Response to estradiol negative feedback was monitored by assay of LH in serum obtained twice weekly from ovariectomized ewes treated with a 3.0-cm s.c. Silastic estradiol implant constructed as described by Karsch et al. (1973). These implants release 7–8 μg estradiol/day and produce a concentration of serum estradiol (3–5 pg/ml) comparable to that in intact ewes during the luteal phase of the estrous cycle (Karsch et al., 1973; Legan et al., 1977; Karsch et al., 1980b). The onset of the breeding and anestrous seasons were determined in ovary-intact ewes by daily checks for estrous behavior using vasectomized rams (Karsch et al., 1980b). The occurrence of ovulation around the seasonal transitions was documented by assay of progesterone in samples collected twice weekly (Legan and Karsch, 1980). All blood samples (3–5 ml) were obtained by jugular venipuncture; serum was obtained and stored as described by Karsch and Foster (1975).

Assays

Concentrations of LH were determined by radioimmunoassay in duplicate in 25–200-μl aliquots of serum (Niswender et al., 1969; Hauger et al., 1977). When reassay was necessary to establish the concentration of LH at the peak of a pulse, both the peak and preceding nadir were reassayed. Assay sensitivity for 200 μl serum, determined as the 95% confidence interval from the buffer control, was 0.26 ± 0.1 ng/ml of NIH-LH-S12 (46 assays). Intraassay coefficients of variation (CV), evaluated from three standard sera determined 6 times in each assay and displacing labeled LH to approximately 30%, 40%, and 80% of the buffer control, averaged 4.7%, 4.7%, and 9.1%, respectively. Interassay CV for the same standard sera were 6.8%, 5.4%, and 19.5%, respectively. Progesterone was measured in duplicate in petroleum-ether extracts equivalent to 50 μl serum using a radioimmunoassay described by Niswender (1973) and Goodman et al. (1980).

Data Analysis

Pulses of LH were defined as abrupt increases in circulating LH followed by a decline and were identified using the following criteria as described by Goodman and Karsch (1980b): 1) the peak had to occur within two samples of the preceding nadir; 2) the amplitude (peak minus preceding nadir) had to exceed assay sensitivity; and 3) the peak had to exceed the 95% confidence interval of both the preceding and subsequent nadirs (determination of 95% confidence intervals described in Duddleson et al., 1972). After identification of pulses, a frequency for each collection period in each ewe was determined by calculating the average interval between pulses (interpulse interval). Luteinizing hormone pulse frequency was expressed as interpulse interval and, since interpulse interval decreases as frequency increases, data for interpulse interval were plotted on an inverted scale. Thus, the direction of changes in frequency matched the direction of changes in the plotted data. Relationships between interpulse interval and ambient photoperiod were identified by correlation analysis. Changes in interpulse interval over time were evaluated by analysis of variance using Duncan's new multiple-range test to determine differences among means. Statistical procedures were performed as described in Steel and Torrie (1960).

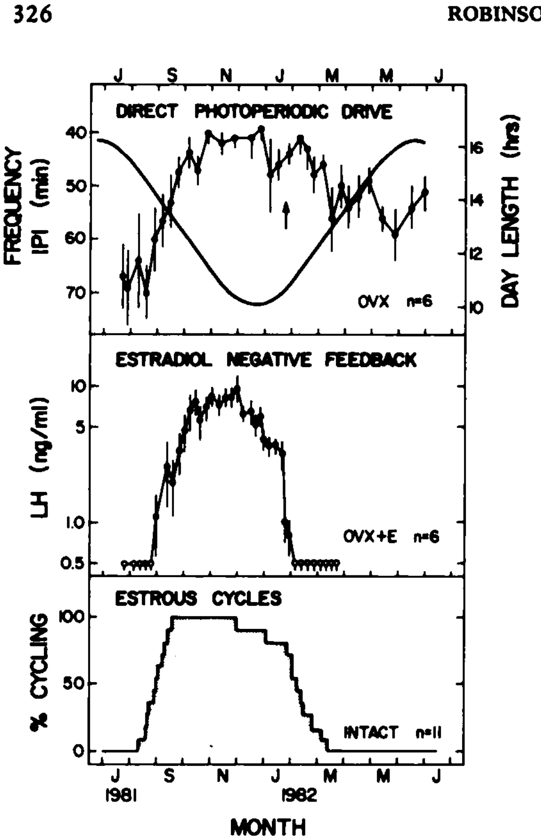

FIG. 1. Changes in three parameters of reproductive competence (direct photoperiodic drive, estradiol negative feedback, and estrous cycles) in Suffolk ewes exposed to naturally changing day lengths (*smooth solid curve, top panel*) from July, 1981 until July, 1982. *Top*: Mean (± SEM) frequency of LH pulses (plotted as interpulse interval, *IPI*) in ovariectomized (*OVX*) ewes from which blood was obtained every 12 min for 6 h on 31 occasions. *Middle*: Mean (± SEM) serum LH concentrations in OVX ewes treated with s.c. Silastic estradiol implants (*OVX + E*). Each *point* represents alternate values from samples taken twice per week. *Open circles* depict undetectable LH levels. *Bottom*: Percentage of intact ewes exhibiting estrous cycles. Number of ewes in each group indicated by *n*. *Arrow in top panel* corresponds to the time when mean LH levels in OVX + E ewes fell below 1 ng/ml.

RESULTS

Experiment 1: Relationship of Seasonal Changes in Direct Photoperiodic Drive to Day Length, Estradiol Negative Feedback, and Estrous Cycles

To assess changes in direct photoperiodic drive, blood samples for analysis of LH pulses were obtained at 12-min intervals for 6 h from each of 6 ovariectomized ewes. Collections were made every 2–3 wk for 1 yr beginning in late anestrus (22 July 1981; 31 total collection periods). Concurrently, response to estradiol negative feedback was determined in 6 ovariectomized ewes treated with estradiol implants, and the incidence of estrous cycles was monitored in 11–15 intact ewes. All animals were maintained under natural environmental conditions. Composite data for all animals are presented in Fig. 1; selected LH pulse patterns for representative ovariectomized ewes are illustrated in Figs. 2 and 3.

During the course of the year, the interpulse interval in the ovariectomized ewes varied in a characteristic fashion that was closely related to photoperiod (Fig. 1, top panel). At the start of the study, during the long days of July 1981, LH pulses were relatively infrequent (average interpulse interval of 67 min). Frequency then

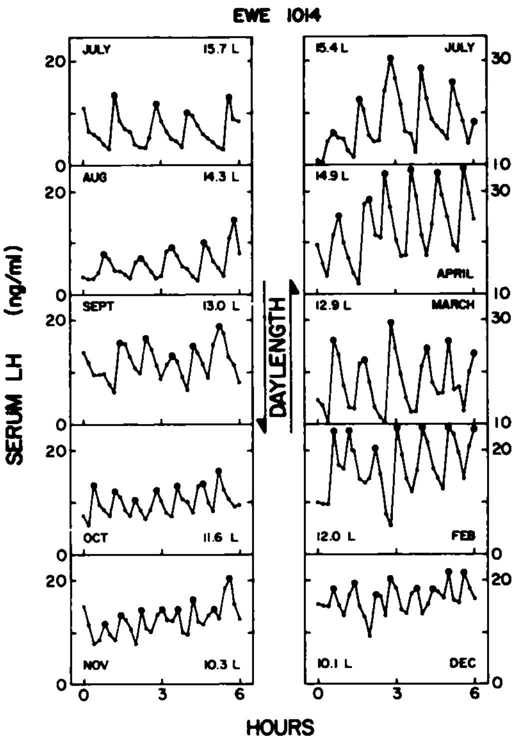

FIG. 2. Patterns of pulsatile LH secretion exhibited by a representative ewe (#1014) on the day lengths, and during the months, indicated in each panel. Shown are five representative patterns each for decreasing (*left*) and increasing (*right*) natural day lengths. Samples were obtained every 12 min for 6 h. The peak of a pulse is indicated by a *large solid circle*. Note that a well-organized pattern of pulses was always observed in this animal and that LH pulse frequency increased as day length decreased.

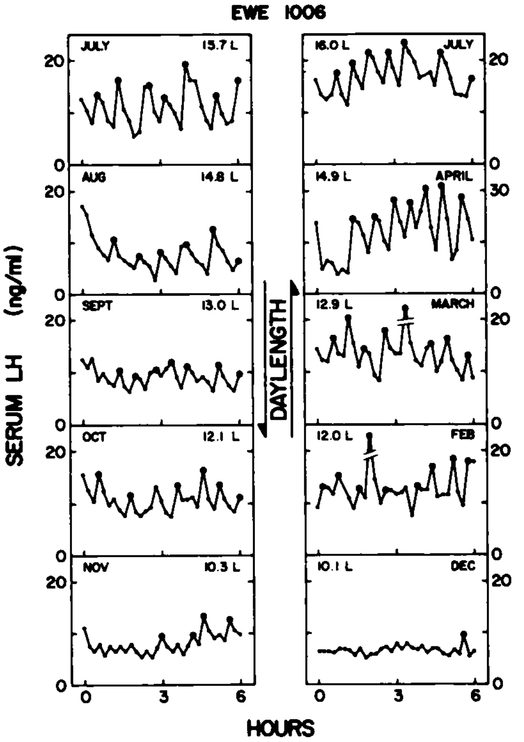

FIG. 3. Representative patterns of pulsatile LH secretion exhibited by OVX ewe #1006 in which well-organized pulses of LH were not seen during the shorter photoperiods. Samples were obtained every 12 min for 6 h. See text and legend to Fig. 1 for further details.

increased progressively in association with the autumnal decrease in day length, climbing to 1 pulse/40 min by late October. No further consistent changes in mean frequency of LH pulses were detected from then until late February. At that time the frequency declined together with the springtime increase in photoperiod. Although the absolute frequencies in the summer of 1982 were not as low as in the preceding year, a reduction in frequency was observed in each ewe during the late winter-spring of 1982 (e.g., see representative ewe #1014, Fig. 2). The correlation of interpulse interval and day length was significant for 5 of the 6 ewes (r ranging from 0.49 to 0.71, P<0.01–0.001). In the remaining ewe (#1006, Fig. 3), such a relationship was not observed, probably because of an insufficient sampling interval (clarified below).

The time course of the seasonal changes in response to estradiol negative feedback (serum LH in estradiol-treated ovariectomized ewes) was virtually identical to that of the seasonal changes in estrous cyclicity in intact ewes (Fig. 1, bottom 2 panels). However, the curves describing these two indices of reproduction were markedly asymmetric with respect to the annual cycle of photoperiod, a finding consistent with previous reports (Hafez, 1952; Robinson and Karsch, 1984). Thus, the seasonal changes in feedback response and estrous cycles were also skewed with respect to the changes in direct photoperiodic drive to the LH pulse generator.

Although the time course of the change in LH pulse frequency was symmetric with respect

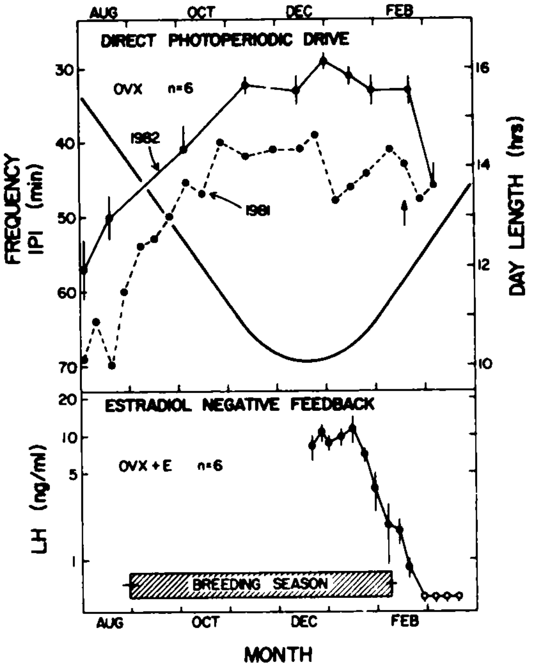

FIG. 4. *Top:* Changes in LH pulse frequency (*solid line between points:* mean ± SEM interpulse interval, *IPI*) from August to March, 1982 in 6 ovariectomized (*OVX*) ewes maintained outdoors. Samples were taken every 6 min for 3–6 h beginning in October. For comparison, the pulse frequencies of the same 6 ewes for the same period in 1981 are shown as the *dashed line* (samples collected every 12 min for 6 h). The *smooth solid curve* represents duration of the natural photoperiod. *Bottom:* Mean (± SEM) serum LH concentrations around the transition into anestrus of 6 OVX ewes treated with s.c. Silastic estradiol implants (*OVX + E*). The *cross-hatched bar* depicts the timing of the breeding season in intact ewes in 1982. For reference, the *arrow (top panel)* indicates the time when mean serum LH levels in OVX + E ewes fell below 1 ng/ml.

to the annual photoperiodic cycle, a close relationship between interpulse interval and photoperiod was not obvious in the 3-mo period surrounding the winter solstice (Figs. 1 and 2, Nov–Feb). It is noteworthy, however, that the LH pulse patterns generally became less distinct during this time, and in one ewe obvious pulses were not apparent (Fig. 3, Nov and Dec). The question thus arose as to whether the frequency of LH pulses increased further but the 12-min sampling regimen was insufficient to detect them, much as during the follicular phase of the cycle when frequency rises to 1 pulse/30 min (Karsch et al., 1983). The study was thus continued on the same ewes into the second year, with the blood collection regimen being intensified to 1 sample every 6 min (for 3–6 h) beginning in October and continuing through early March. Composite data are presented in Fig. 4; representative pulse patterns are shown in Figs. 5 and 6 (same ewes as in Figs. 2 and 3 for the first year).

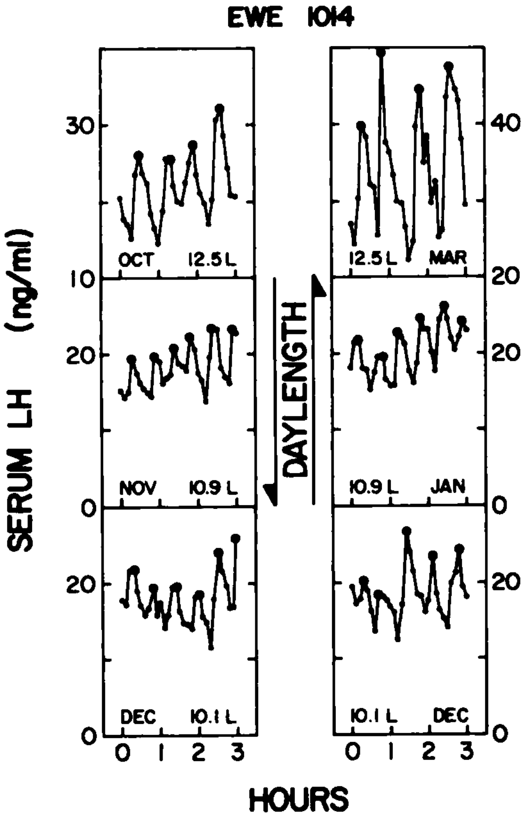

FIG. 5. Representative pulsatile patterns of serum LH in OVX ewe #1014, which was maintained outdoors. Luteinizing hormone pulse patterns observed during the previous year in the same ewe using a 12-min sampling interval are shown in Fig. 2. Further details in legend to Fig. 2.

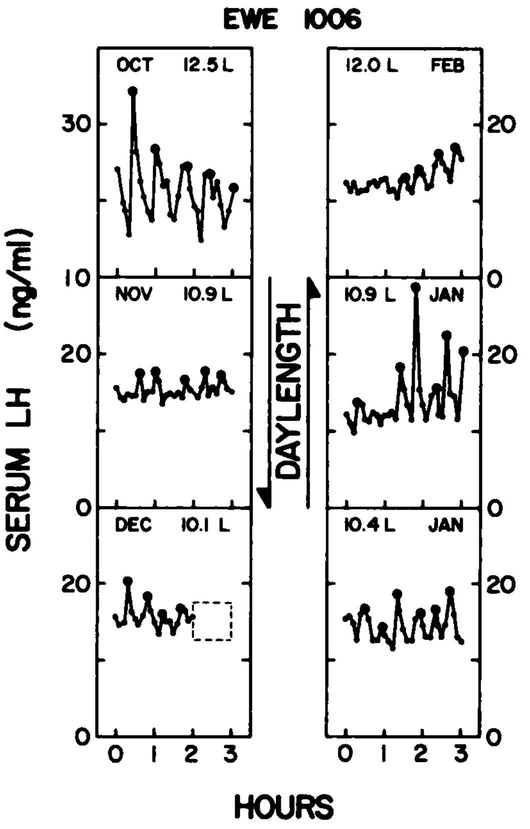

FIG. 6. Representative pulsatile patterns of serum LH in OVX ewe #1006, which was maintained outdoors. Samples were collected every 6 min for 3 h except in December, when samples were obtained over 2 h (*dotted box* delineates the missing hour of data). Luteinizing hormone pulse patterns observed during the previous year in the same animal using a 12-min sampling interval are shown in Fig. 3. Note that the 6-min sampling frequency allowed detection of LH pulses around the winter solstice; such pulses were not seen using the less frequent sampling regimen (see Fig. 3). Further details in legend to Fig. 2.

With the 6-min sampling regimen, the interpulse interval was again found to change with photoperiod (Fig. 4, top). The correlation between day length and interpulse interval was highly significant (*r* ranging from 0.80 to 0.95, $P<0.001$), and it was greater ($P<0.05$) than that during the first year in the 5 ewes for which a significant correlation was observed with 12-min samples. For ewe #1006, in which this relationship between day length and frequency was not seen in the first year, a significant correlation was disclosed during the second year ($r=0.84$, $P<0.001$). Furthermore, in this ewe and all others unambiguous LH pulses were evident in the period spanning the winter solstice (Figs. 5 and 6). Even with the 6-min samples, however, occasional pulses still seem to have been missed (Fig. 6; example of missed pulses at 10.9 L in Nov and Jan).

Another relationship in this experiment is noteworthy; there was an unambiguous temporal dissociation between the shift in estradiol negative feedback and the change in direct photoperiodic drive at the end of the breeding season. In both years of the study, the average date on which the serum LH concentration in estradiol-treated ovariectomized ewes had plummeted to the anestrous level of less than 1 ng/ml was not different from the date of last ovulation in intact ewes ($P>0.1$, determined by t-test). At the time of this fall in LH, and in both years, a decrease in LH pulse frequency in individual ovariectomized ewes was either not yet evident or had just begun (Figs. 1 and 4, Jan-Feb).

Experiment 2: Manipulation of LH Pulse Frequency by Artificial Photoperiod

The strong correlation between natural day length and LH pulse frequency in ovariectomized ewes led to the hypothesis that photoperiod can set, in a graded fashion, the rate at which LH pulses occur. For example, the gradually decreasing day lengths of autumn may provide a progressively more intense stimulus to the LH pulse generator and cause frequency to increase progressively. To test this hypothesis, a study was designed in which ewes were subjected to one of a number of decrements in artificial photoperiod, beginning around the time of the summer solstice and continuing until early autumn.

In early anestrus (23 April 1982), 23 ovariectomized ewes were transferred from their natural environment to a long artificial photoperiod of 16L:8D. After a 68-day priming period on this inhibitory day length, LH pulse frequency was monitored and verified to be at the low rate typical of mid-anestrus. The animals were then allocated to 4 groups and exposed to one of the following four day lengths beginning on June 30: 1) large decrease to a short day length (8L:16D); 2) intermediate decrease to an intermediate day length of 11.5L:12.5D; 3) small decrease to an intermediate day length of 13.5L:10.5D; and 4) no decrease in day length (16L:8D, control group). Blood samples for pulse analyses were obtained on 6 occasions at approximately 18-day intervals until the end of the study, on Day 91 after the light shift (Sept. 29). Samples were drawn every 12 min for 3 h except for Day 91, when blood was collected every 6 min for 6 h. Alternate samples (12-min intervals) on Day 91 were used for comparison with data at other times; every sample on Day 91 was used to correlate interpulse interval with day length.

The switch from 16L:8D to each of the three shorter day lengths caused LH pulse frequency to increase ($P<0.05-0.01$) within 54 days (Fig. 7, left 3 panels). This increase was sustained, with frequency on Day 91 being greater than that on Day 0 regardless of the magnitude of the decrease in photoperiod ($P<0.01$). Furthermore, the interpulse interval at the end of the study was correlated with photoperiod. Thus, the shorter the day length, the shorter the interval between LH pulses on Day 91 ($r=0.52$, $P<0.01$; Fig. 8). Finally, it is noteworthy that LH pulse frequency in control ewes that remained on the inhibitory day length of 16L:8D tended to decline between Days 0 and 36 (Fig. 7, right panel, $P<0.05-0.10$). Thereafter, despite the absence of a change in the photoperiod, pulse frequency increased ($P<0.05$) in these control ewes by the end of the study in early autumn (Sept. 29).

DISCUSSION

The present observations confirm earlier reports of seasonal changes in the activity of the LH pulse generator in the ovariectomized ewe (Karsch et al., 1980a; Goodman et al., 1982). In addition, our findings expand those studies by: 1) defining the pattern of these changes during the course of the year; 2) describing the pattern of these changes relative to the annual photoperiodic cycle and the changes in response to estradiol negative feedback; and 3) providing evidence using artificial lighting that the changes in LH pulse frequency are controlled in a graded fashion by the gradual shift in ambient day length.

Under natural environmental conditions, a close temporal coupling was observed between the length of the day and the frequency of LH pulses in ovariectomized ewes. Thus, as days became shorter in late summer and autumn, there was a stronger photoperiodic drive to the LH pulse generator and frequency increased.

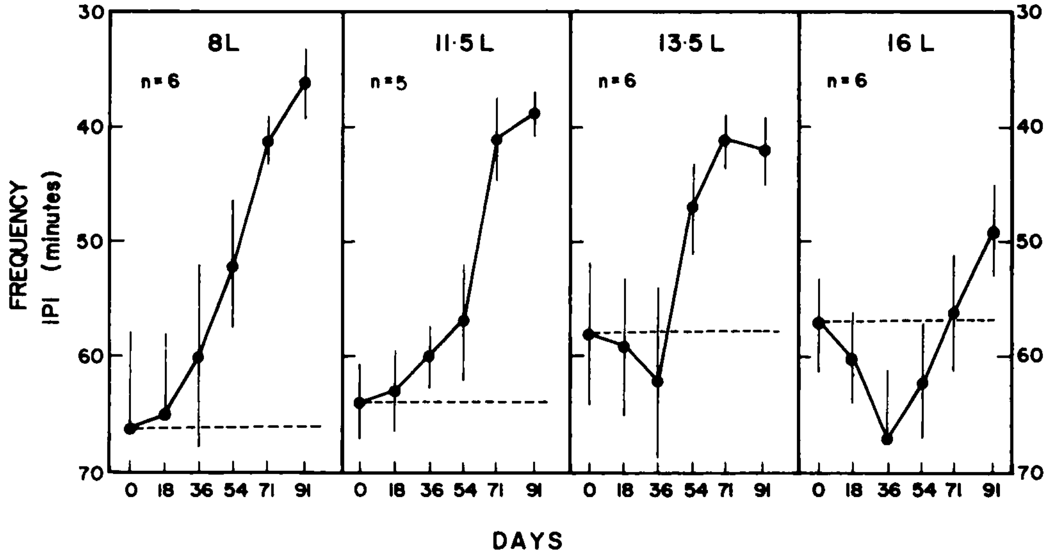

FIG. 7. Changes in LH pulse frequency (mean ± SEM, plotted as interpulse interval, *IPI*) in ovariectomized ewes exposed to four different day lengths. Samples for pulse analysis were taken on six occasions following transfer from 16L:8D to 8L:16D, 11.5L:12.5D, or 13.5L:10.5D (designated *8L*, *11.5L*, and *13.5L*, respectively). Control ewes remained on 16L:8D throughout the study (designated *16L*). *n* denotes the number of animals in each group.

Conversely, as days became longer in the spring, there was a decreased drive and pulse frequency declined.

Although these changes in photoperiodic drive were observed in each of the 2 years of our study, it is of interest that the minimal frequency observed during the summer of 1981 was not as low as that during the summer of 1982 (approximately 1 pulse/59 min vs. 1 pulse/70 min). A similar trend was observed in

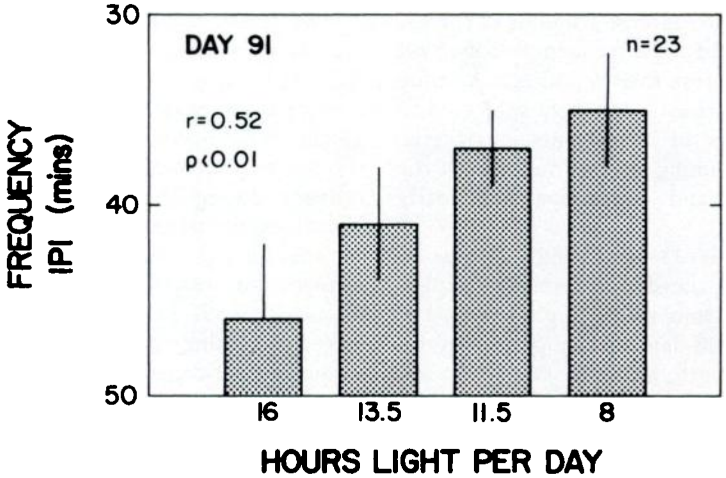

FIG. 8. Mean (± SEM) frequency of LH pulses (plotted as interpulse interval, *IPI*) in ovariectomized ewes 91 days after transfer from 16L:8D to one of three different photoperiods, 13.5L:10.5D, 11.5L:12.5D, or 8L:16D. Control ewes remained on 16L:8D throughout the study (*left bar*). Samples were obtained every 6 min for 6 h. Note the significant inverse correlation between day length and the frequency of LH pulses in the 23 ewes in this study.

another recent report (Montgomery et al., 1985). That study, which used artificial lighting, led to the conclusion that LH pulse frequency in ovariectomized ewes is a function of season, previous exposure to the stimulatory effects of season, and time after ovariectomy. Time from ovariectomy, in itself, is unlikely to have caused the changes in pulse frequency observed in our study. We and others have observed that the major increase in frequency occurs by Day 4 (Goodman et al., 1982; Wright et al., 1983; F. J. Karsch, unpublished). Furthermore, it is important to the interpretation of our study to reiterate that the same pattern of change in frequency occurred in the same ewes over the course of two successive years. Thus, the minimum frequency of the second summer was preceded by a gradual decrease during the spring and, much as in the first year, this nadir was followed by a marked increase in the autumn (see Figs. 1 and 4). Thus, whatever the basis for the difference in absolute number of LH pulses between years in our study (time from ovariectomy, previous stimulatory season, or perhaps an environmental variable other than photoperiod), the underlying season change in activity of the LH pulse generator was not masked. Furthermore, it is important to stress that the ovariectomies in our study were performed at the same time in ewes given estradiol implants and those that remained untreated after ovariectomy. Therefore, regardless of what factor(s) contributed to the difference in absolute number of LH pulses between years, comparisons between these groups should be valid.

Full recognition of the maximal frequency of LH pulses around the time of the winter solstice required that blood samples be collected more frequently than every 12 min, the interval used in the first year. Even with the 6-min sampling interval of the second year, however, a highly organized LH pulse pattern was occasionally not seen around the shortest day of the year. Although it is possible that more frequent samples would have disclosed a more regular pulse profile in these cases, another possibility is equally plausible. There is an upper limit with regard to how frequently the pituitary gland can be stimulated by gonadotropin-releasing hormone (GnRH) and still respond, at least in the rhesus monkey (Wildt et al., 1981). Furthermore, in the ewe, there is an inverse relationship between the frequency of GnRH stimulation and the amplitude of the resultant pulses of LH; these pulses become exceedingly small during high-frequency GnRH stimulation (Clarke et al., 1984; Kaynard and Karsch, 1983). It is possible, therefore, that the frequency of hypothalamic GnRH discharge around the time of the winter solstice increases to such an extent that it exceeds, in some ewes, the capacity of the pituitary gland to generate detectable and discrete pulses of LH.

Recent studies have shown that the frequency of LH pulses in ovariectomized ewes is susceptible to regulation by artificial photoperiod (Pau and Jackson, 1983; Bittman et al., 1985). Those findings, and the close temporal association between changes in natural day length and pulsatile LH secretion observed in our first experiment, led to the hypothesis that photoperiod can set, in a graded fashion, the frequency of the LH pulse generator. Support for this hypothesis was derived from our second experiment, in which ovariectomized ewes were transferred from inhibitory long days to one of a number of shorter artificial day lengths. Not only did LH pulse frequency increase following transfer to short days, but the shorter the day length the greater this increase in frequency. Furthermore, there was a tendency for pulse frequency to increase more rapidly in ewes exposed to the shorter day lengths, although our study did not contain a sufficient number of time points for statistical documentation of this trend (see Fig. 7). This trend for a more rapid inductive effect with the shorter photoperiods is consistent with earlier observations of Ducker et al. (1970), who used estrous behavior as an index of reproductive condition.

It was also observed in our second experiment that the LH pulse frequency in control ewes held on the artificial long photoperiod increased at the end of the study. This rise began in September and was coincident with the autumnal increase in frequency observed in ewes maintained outdoors during the same year. Thus, while seasonal changes in the LH pulse generator are normally controlled by photoperiod, their occurrence may not depend upon it. With regard to this possibility, it is of interest that in the golden-mantled ground squirrel, a seasonal breeder that does not utilize photoperiod to time the breeding season, there appears to be a circannual rhythm of LH secretion that occurs independently of ovarian steroid feedback and that is generated endogenously (Zucker and Licht, 1983).

Given the characteristic changes in photoperiodic drive to the LH pulse generator under natural conditions, important questions arise as to the neuroendocrine mechanisms that produce these changes and their physiologic importance. Earlier studies have shown that the pineal gland, through its patterned and light-sensitive secretion of melatonin, mediates the effects of artificial photoperiods on the frequency of LH pulses in ovariectomized ewes (Bittman et al., 1985). Furthermore, it is now known that each pulse of LH in the ovariectomized ewe is preceded by a discharge of GnRH from hypothalamic neurons (Clarke and Cummins, 1982; Levine et al., 1982). These observations are consistent with the hypothesis that day length determines the pattern of pineal melatonin secretion and that this, in turn, directs the activity of those neurons that either generate or control the episodic release of GnRH (Bittman et al., 1985).

A similar pineal-based mechanism has also been shown to mediate the effects of day length on the seasonal swings in response to estradiol negative feedback that produce the seasonal changes in ovarian cyclicity in the ewe (Bittman et al., 1983a,b). This similarity is compatible with the hypothesis that the seasonal swings in steroid negative feedback are actually secondary to the level of photoperiodic drive to the LH pulse generator, the steroids being potent negative feedback agents when the drive is low and relatively ineffective when drive is high (Lincoln and Short, 1980; Goodman and Karsch, 1981; Goodman et al., 1982). If this were true, then changes in the level of direct photoperiodic drive to the LH pulse generator would be the primary determinant of the seasonal reproductive state (Lincoln and Short, 1980; Goodman and Karsch, 1981). Pertinent to this hypothesis is evidence that steroid-independent changes in gonadotropin secretion underlie the annual reproductive cycle of certain other seasonal breeders such as the Japanese quail and golden-mantled ground squirrel (Follett, 1978; Zucker and Licht, 1983).

However, several observations in our present study are not consistent with the hypothesis that the seasonal swings in response to estradiol negative feedback in the ewe are merely a simple reflection of the level of direct photoperiodic drive to the LH pulse generator. The changes in LH pulse frequency in ovariectomized ewes occur gradually and the curve describing them is symmetric with respect to the annual photoperiodic cycle. In contrast, the feedback shifts at the transitions between breeding season and anestrus occur abruptly and are not symmetric with the changes in day length. As a consequence, there is a marked discrepancy in the absolute frequency of LH pulses at the time of the two seasonal shifts in response to estradiol negative feedback. Specifically, frequency at the onset of the breeding season is low; that at the entry into anestrus is high, being at or near the annual maximum. Thus, the transitional shifts in estradiol negative feedback do not simply occur when the level of photoperiodic drive exceeds a certain threshold in the late summer and then drops below that threshold in late winter. In fact, it would seem that the frequency of LH pulses in ovariectomized ewes is not an accurate indicator of either the potency of estradiol negative feedback in ovariectomized ewes or of reproductive competence in ewes with intact ovaries.

The temporal dissociation between the changes in direct photoperiodic drive and the response to estradiol negative feedback do not necessarily imply that the two phenomena are functionally unrelated; rather, they suggest that the mechanisms that underlie these processes are not one and the same. Such a mechanistic separation is also suggested by recent evidence that different neurotransmitter systems mediate the effects of photoperiod on response to steroid feedback and direct drive (Meyer and Goodman, 1984). Furthermore, there is now evidence for a neuroanatomic separation of the mechanisms that produce the two responses in the ewe (Pau and Jackson, 1983). The available information is therefore compatible with the view that photoperiod can modulate gonadotropin secretion by means of a number of systems, some utilizing gonadal steroids and others not doing so, and that an interaction among these systems determines the degree of gonadotropic stimulation, and hence seasonal reproductive condition.

ACKNOWLEDGMENTS

We express our gratitude to Mr. Douglas D. Doop for expert assistance in all aspects of the animal experimentation and blood collection, to Ms. Barbara Glover for conducting the radioimmunoassays, to Ms. Ann Betz for excellent secretarial support, and to Mr. Robert W. Jopling for drawing the figures. We also thank Drs. Gordon D. Niswender and Leo E. Richert, Jr. for supplying reagents used for the radioimmuno-

assays and Dr. Morton Brown for help with the statistical analysis.

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