J. Reprod. Fert. (1988) **84,** 283–293

Printed in Great Britain

Photoperiod requirements for puberty differ from those for the onset of the adult breeding season in female sheep*

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Summary. Reproductive responses to photoperiod were directly compared in mature ewes and in their spring-born twin female lambs. All females were ovariectomized and treated with oestradiol implants before transfer into artificial photoperiod; serum LH concentrations and pulsatile LH patterns provided an index of neuroendocrine reproductive activity. Mothers were transferred from natural photoperiod to artificial long days (16 h light:8 h dark) at the summer solstice so that no decrease in photoperiod would be experienced. These ewes began reproductive activity synchronously at the expected time in the autumn. One of each pair of twin lambs was treated exactly as the mothers; to determine the normal timing of puberty the remaining twin was maintained in a photoperiod simulating the natural decrease in daylength. In all 6 control lambs experiencing the simulated natural photoperiod, reproductive activity occurred synchronously at 28 ± 1 weeks of age (2 October ± 7 days). However, in their twin sisters which did not experience a decrease in photoperiod, only 2 of 6 lambs had begun reproductive activity by the end of the experiment at 52 weeks of age (March), and these were both delayed relative to their twin control lambs exposed to decreasing daylength. Therefore, a decrease in photoperiod is necessary for the normal timing of puberty in the spring-born, female sheep, whereas seasonally anoestrous, mature sheep can enter the breeding season at a normal time in the absence of decreasing photoperiod. We suggest that the requirement for a decreasing photoperiod by the spring-born lamb reflects its limited photoperiodic history as compared to the adult.

Keywords: puberty; photoperiod; sheep; LH

Introduction

Onset of the adult breeding season in sheep has been considered as a recurrence of puberty, since both are transitions from an anovulatory state to ovarian cyclicity (Geschwind, 1974). The major environmental cue regulating the timing of these transitions in temperate-zone breeds of sheep is photoperiod. A series of studies suggests that the spring-born female Suffolk lamb requires a decrease in daylength for the normal timing of puberty (Foster, 1981; Yellon & Foster, 1985; Foster *et al.*, 1988). However, adult sheep do not necessarily require a decrease in daylength for the onset of the breeding season. For example, Dorset Horn and Welsh Mountain ewes kept on long days of 16 h light:8 h darkness from early May began reproductive activity at a time similar to that normally observed when sheep of these breeds are maintained in natural conditions (Worthy *et al.*, 1985; Webster & Haresign, 1983), and Suffolk ewes kept in the summer solstice photoperiod from

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mid-June become reproductively active at the same time as controls in a simulated natural photoperiod (Robinson *et al.*, 1985). Taken together, the foregoing observations raise the possibility that there is developmental change in the photoperiod requirements for the onset of reproductive activity. The aim of this study was to test directly this hypothesis by comparing reproductive responses in mature ewes and their spring-born female lambs raised under identical photoperiod treatments. In addition, circulating prolactin concentrations were measured to provide a second comparison of neuroendocrine responses between adult and prepubertal sheep, because changes in daylength modulate prolactin secretion in the sheep (Pelletier, 1973; Forbes *et al.*, 1975). A preliminary report has been published in abstract form (Ebling & Foster, 1987).

Materials and Methods

General. Six mature ewes of predominantly Suffolk breed which gave birth to twin female lambs were selected for this study. Mean (±s.e.m.) birthdate of the lambs was 17 March ± 2 days (range: 9–24 March). Lambs were housed in natural photoperiod with their mothers at a commercial sheep facility (Bresbois, Freeland, MI) until weaning at about 10 weeks of age on 27 May, when they were transported to The Reproductive Endocrinology Program Sheep Research Facility in Ann Arbor, MI. After weaning, the lambs and their mothers were kept in natural photoperiod until 19 June (13 weeks of age), when they were moved into controlled photoperiod rooms. All animals were fed alfalfa hay *ad libitum*; the lambs were also fed a commercial pellet ration (Lamb 18, Kent Feed Inc., Muscatine, IA) supplemented with vitamins and minerals. Body weights were determined at approximately 2-week intervals until 30 weeks of age, and then monthly.

Reproductive condition was assessed by changes in serum LH concentrations and pulsatile LH patterns. All animals were ovariectomized on 17 June (lambs were 13 weeks of age) under pentobarbital anaesthesia, and received a subcutaneous Silastic oestradiol implant (see Karsch *et al.*, 1973, for details) 3 weeks later (7 July) to provide a constant steroid feedback signal. The ewes were implanted with a capsule in which the packed column of oestradiol was 15 mm; the lambs received a 7·5-mm capsule, which was subsequently replaced with a 15-mm capsule on 26 August (23 weeks of age) to compensate for increased body size. These treatments were designed to produce physiological serum oestradiol concentrations (2–4 pg/ml) in both lambs and adults. They were chosen on the basis of several previous studies in which the serum oestradiol concentrations produced by Silastic implants have been measured (Foster & Ryan, 1979; Foster, 1984). For measurement of LH and prolactin, blood samples were collected by jugular venepuncture twice a week at 08:00–10:00 h, beginning on 7 July and continuing until March of the following year. One adult ewe died on 7 October. A second ewe which was in poor condition throughout the study died on 5 March; data from this animal were not used in the analyses.

Photoperiod treatments. Figure 1(a) depicts the design of this study which compared the timing of the onset of reproductive activity in lambs and adults in the absence of a decrease in daylength. On 19 June, near the summer solstice, the mothers were transferred from the natural photoperiod to an artificial photoperiod of 16 h light:8 h dark (16L:8D), lights on at 06:00 h EST, in which they remained for the duration of the experiment. This photoperiod was chosen as an approximation of the duration of light at the summer solstice at Ann Arbor, MI (42°18′N) which is 15 h and 16 min (excluding civil twilight); the additional 44 min in the artificial photoperiod compensates for civil twilight. On the same day (19 June), one of each of the pairs of twin lambs was transferred from natural light to a separate room with photoperiod conditions identical to those for the mothers. The remaining lamb from each pair was housed in a third room in which the natural changes in photoperiod were simulated by timed changes in artificial light. Electronic time clocks (System X-10 Appliance Module, BSR, Miami, FL) were adjusted twice a week from settings calculated from the *Tables of Sunrise and Sunset, Nautical Almanac Office, U.S. Naval Observatory* for Detroit, MI. In all rooms, fluorescent lighting provided ~350 lux for the light phase; a single 15-W red light bulb was on continuously providing <2 lux during the dark phase. The rooms were ventilated through a light baffle, but were not air conditioned or heated artificially; temperature therefore fluctuated with the external temperature.

Detailed analysis of LH patterns. The pulsatile pattern of LH secretion was determined by measurement of the hormone in serial blood samples collected by repeated jugular venepuncture from all three experimental groups (at 12-min intervals for 4 h) when the lambs were 35 weeks of age (16–17 November). This was to determine the effect of the photoperiod treatments on LH pulse frequency in the presence of a constant oestradiol feedback signal. This key time point was chosen as an age at which the control lambs in the natural photoperiod would be expected to be postpubertal had they been ovary-intact. The oestradiol implants were then removed, and all animals were blood sampled 7 days later to determine LH pulse frequency in the absence of ovarian steroid feedback under the different photoperiod treatments. The oestradiol implants were replaced on 2 December when the lambs were 37 weeks of age.

Hormone assays. LH was measured in duplicate 25–200 µl samples of serum using the radioimmunoassay developed by Niswender *et al.* (1969) and modified by Hauger *et al.* (1977). Assay sensitivity, defined as 2 standard deviations from the buffer control, was 0·23 ± 0·12 ng/ml (mean ± s.d.) for 200 µl serum expressed relative to NIH-

LH-S12. The overall mean intra-assay coefficient of variation (CV) for duplicates of three quality control pools running at 82%, 67% and 30% on the standard curve was 5·1%. The overall mean interassay CV for these pools was 12·4% (8 assays).

Prolactin was measured in duplicate 20 µl samples using the radioimmunoassay developed by Davis *et al.* (1971) with the following modifications: the tracer preparation was iodinated with ^{125}I rather than ^{131}I, phosphate-buffered saline containing 1·0% bovine serum albumin (Sigma, St Louis, MO) was used as the assay buffer, and a preprecipitated second antibody was used to separate bound antibody from free ligand (Midgley & Hepburn, 1980). Serial dilution of serum pools collected from female lambs at 4 and 17 weeks of age produced inhibition curves parallel to the standard. Assay sensitivity was 5 ng/ml for 20 µl serum, expressed relative to NIH-P-S8. The overall mean intra-assay CV for duplicates of three serum pools running at 85%, 60% and 32% on the standard curve was 11·5%. The overall mean interassay CV for these pools was 12·6% (3 assays).

Data analysis. Onset of reproductive activity in all animals was defined as a sustained increase (6 consecutive values) of serum LH concentrations above 1 ng/ml. These criteria were chosen because previous studies in both the lamb and adult ewe have shown a good correlation between mean LH concentrations rising above 0·25 ng/ml or 1 ng/ml in oestradiol-implanted ovariectomized sheep and mean onset of oestrous cycles in ovary-intact sheep (lambs: Foster & Ryan, 1979; adult ewes: Legan *et al.*, 1977). For the purpose of statistical analysis, in those lambs which did not start reproductive activity during the time course of the experiment, the onset of reproductive activity was defined as the last day of the experiment. The end of reproductive activity was similarly defined as LH values consistently below 1 ng/ml, since previous studies of lambs and ewes have shown a good correlation between a fall in mean serum LH concentrations in oestradiol-implanted ovariectomized sheep and the onset of anoestrus in ovary-intact sheep (Foster & Ryan, 1981; Robinson & Karsch, 1984). A one-factor analysis of variance (ANOVA) using Statview (Brain Power, Inc., Calabasas, CA) software run on an Apple Macintosh Plus microcomputer was used to compare the onsets of reproductive activity in the three groups, followed by Bonferroni *t* tests (Miller, 1966). Prolactin concentrations were log-transformed, then smoothed by grouping them into time periods of 5 weeks. The values for each time period were subject to ANOVA with repeated measures (BMDP Statistical Software, Inc., Los Angeles, CA) followed by Tukey's test to determine significant differences between particular time periods (Miller, 1966).

The criteria established by Goodman & Karsch (1980) were adopted for the identification of LH pulses in the samples collected at 12-min intervals for 4 h. The three criteria are that (1) the peak concentration must occur within 24 min of the preceding nadir, (2) the amplitude (peak minus nadir) must exceed the sensitivity of the assay, and (3) the peak concentration must be 2 standard deviations above the preceding and subsequent nadirs. LH pulse frequencies, amplitudes and mean concentrations between the groups were compared using the Kruskal–Wallis one-way ANOVA by ranks (Statview: Brain Power, Inc.). Significant differences within groups attributable to the presence or absence of oestradiol were determined by the Wilcoxon signed-ranks test. In all analyses $P < 0·05$ was considered significant.

Results

Average changes in body weights for both groups of growing lambs are illustrated in Fig. 1(b) in relation to the range of adult body weights, and to the photoperiod treatments (Fig. 1a). There was no significant effect of the photoperiod treatments on growth rate. The lambs attained 30 kg by about 17 weeks of age, a weight conservatively considered to be the minimum at which reproductive cycles can begin in Suffolk lambs at our facility (Foster & Ryan, 1979; Foster & Olster, 1985).

Reproductive condition, as assessed by serum LH concentrations in blood samples collected twice weekly, is shown in Fig. 2. There was a significant ($P < 0·001$) variation between groups in the onset of increased LH concentration. Initiation of reproductive activity occurred almost synchronously in all control lambs in the simulated natural photoperiod (Fig. 2a), at a mean ($\pm$s.e.m.) age of 28 $\pm$ 1 weeks (2 October $\pm$ 7 days). Onset of reproductive activity was significantly ($P < 0·01$) later in the lambs in the long-day photoperiod compared to their twin sisters (Fig. 2b). Only 2 of 6 lambs had an increase in LH secretion by the time the experiment was concluded at 52 weeks of age, and the timing of both of these rises was later than those in all the twins in the control group (Fig. 2b). Onset of reproductive activity occurred in 4 of 5 mothers in the constant long-day photoperiod (Fig. 2c). The mother which failed to become reproductively active was in poor condition throughout the study, and subsequently died; her data were excluded from analyses. Mean ($\pm$s.e.m.) date for onset of reproductive activity in mothers was 3 October $\pm$ 5 days, significantly ($P < 0·01$) earlier than that for daughters in constant long days. This onset is

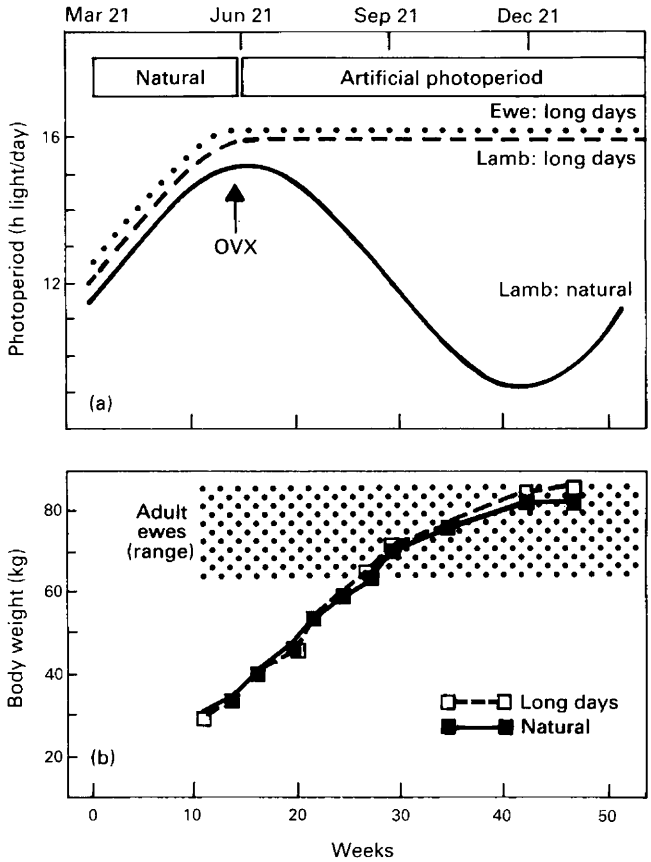

Fig. 1. Photoperiod treatments and body weight. (a) Experimental design. Spring-born twin female lambs (N = 12) were reared with their mothers (N = 6) on pasture until 19 June, then maintained indoors in the photoperiods indicated. (b) Growth curves for these lambs maintained in long days of 16 h light:8 h dark (broken line; N = 6) or in a simulated natural photoperiod (solid line; N = 6). The range of body weights for their mothers is indicated by the shaded area.

consistent with the rise in LH observed (28 September ± 5 days) in a previous study of mature oestradiol-implanted ovariectomized ewes which were moved from natural photoperiod to constant long days of 16·25L:7·75D at the summer solstice (Robinson *et al.* 1985). The rise is slightly later than that in oestradiol-implanted ovariectomized ewes (18 September ± 5 days) that were maintained on pasture with rams in a concurrent study at The Reproductive Endocrinology Program Sheep Research Facility (N. L. Wayne & F. J. Karsch, unpublished results). The duration of the period when LH values were greater than 1 ng/ml did not differ significantly between the lambs in a simulated natural photoperiod and their constant long-day mothers (Student's *t* test).

Figure 3 (upper panels) shows representative LH profiles from a pair of twin lambs at 35 weeks of age kept in a simulated natural photoperiod (left) and constant long days (middle) and from their mother in constant long days (right); all were ovariectomized and chronically treated with an oestradiol implant. The lower panels depict the LH profiles 1 week later following removal of the implant. Data for all the females are summarized in Table 1. No aspects of LH secretion differed significantly between the two groups of reproductively active females, the oestradiol-implanted

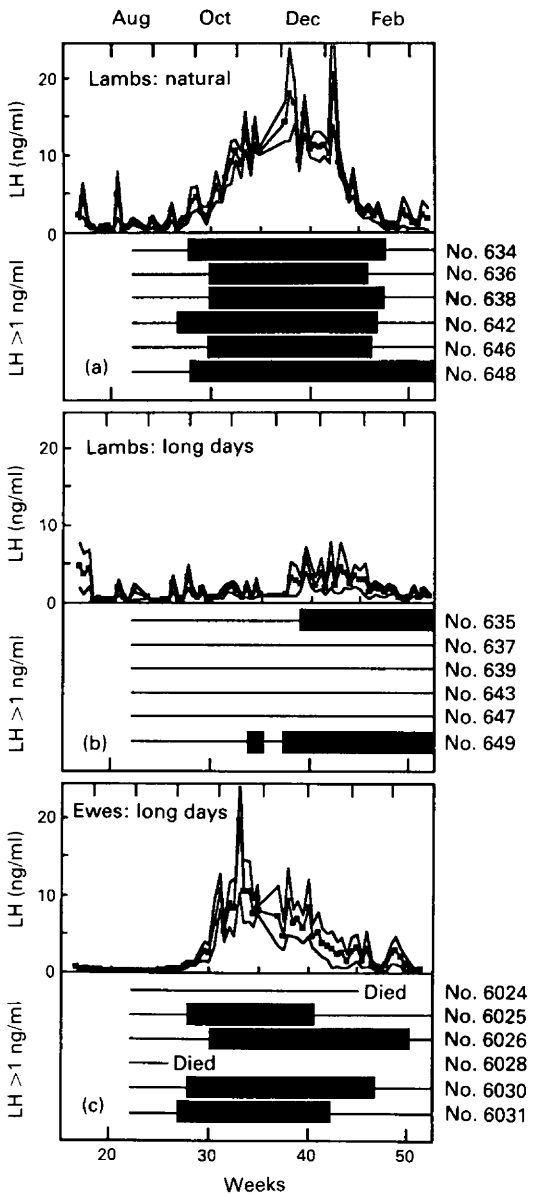

Fig. 2. Reproductive activity in oestradiol-implanted ovariectomized female lambs in a simulated natural photoperiod (**a**, N = 6), their ovariectomized oestradiol-treated twin sisters exposed to constant long days of 16 h light:8 h dark from 13 weeks of age (**b**, N = 6), and their ovariectomized oestradiol-treated mothers also exposed to constant long days (**c**, N = 6, Nos 6028 and 6024 excluded from the mean). For each group, the upper half of the panel shows the mean (±s.e.m.) serum LH concentrations and the lower half shows periods when serum LH was < 1 ng/ml (solid line) and > 1 ng/ml (solid box) for each individual.

ovariectomized lambs in the simulated natural photoperiod and their oestradiol-implanted ovariectomized mothers on constant long days (Table 1). LH pulse frequency and mean LH concentrations were significantly lower in the oestradiol-implanted ovariectomized lambs in constant long

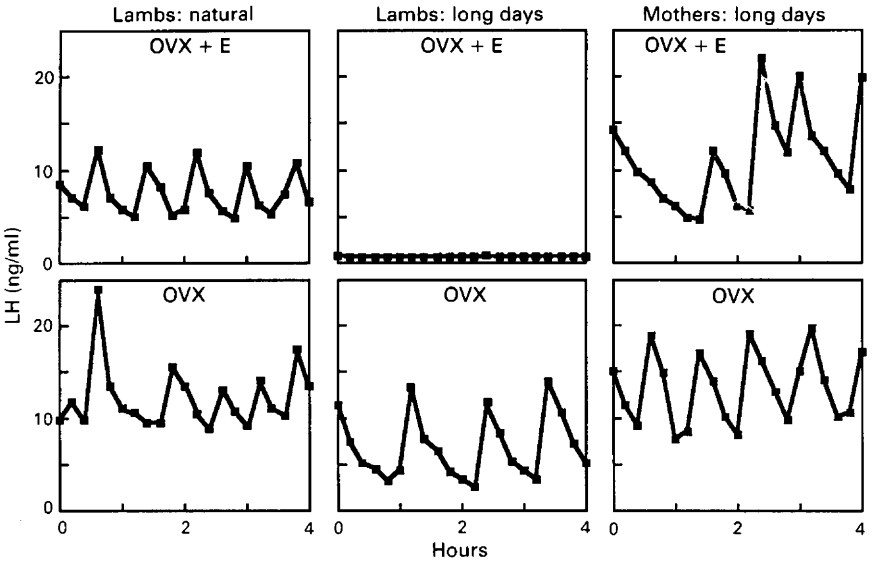

Fig. 3. Representative LH profiles in a 35-week-old oestradiol-implanted ovariectomized (Ovx + E) lamb in a simulated natural photoperiod (left), her Ovx + E twin sister in constant long days (middle), and their Ovx + E mother also in constant long days (right). LH patterns in the same animals 7 days after removal of the 15-mm oestradiol implant are shown below.

Table 1. Characteristics of LH secretion in 35-week-old ovariectomized lambs and their mothers implanted with oestradiol (Ovx + E), and 1 week after removal of the implant (Ovx)

		Lambs		Mothers
		Natural	Long days	Long days
Ovx + E	LH pulse frequency (pulses/4 h)	5·5 ± 0·4	1·8 ± 0·7[a]	4·3 ± 0·9
	LH pulse amplitude (ng/ml)	5·8 ± 0·7	3·6 ± 1·1	9·2 ± 2·3
	Mean LH (ng/ml)	9·4 ± 0·7	2·0 ± 0·7[b]	10·5 ± 2·2
Ovx*	LH pulse frequency (pulses/4 h)	6·3 ± 0·2	3·5 ± 0·2[c,d]	4·3 ± 0·5[c]
	LH pulse amplitude (ng/ml)	8·3 ± 1·0	10·3 ± 0·9	19·6 ± 5·9[e]
	Mean LH (ng/ml)	14·6 ± 0·9[d]	8·9 ± 0·9[a,d]	23·4 ± 5·2

Values are mean ± s.e.m., N = 6 for each group of lambs, N = 4 for the mothers. See text for explanation of photoperiod treatments.

*7 days after oestradiol implant removed (36 weeks of age).

[a] $P < 0{\cdot}01$ compared with lambs in simulated natural photoperiod, and $P < 0{\cdot}05$ compared with long-day ewes.

[b] $P < 0{\cdot}01$ compared with lambs in simulated natural photoperiod and long-day ewes.

[c] $P < 0{\cdot}01$ compared with lambs in simulated natural photoperiod.

[d] $P < 0{\cdot}05$ compared with value for same group when oestradiol implant present (Ovx + E).

[e] $P < 0{\cdot}05$ compared with lambs in simulated natural photoperiod.

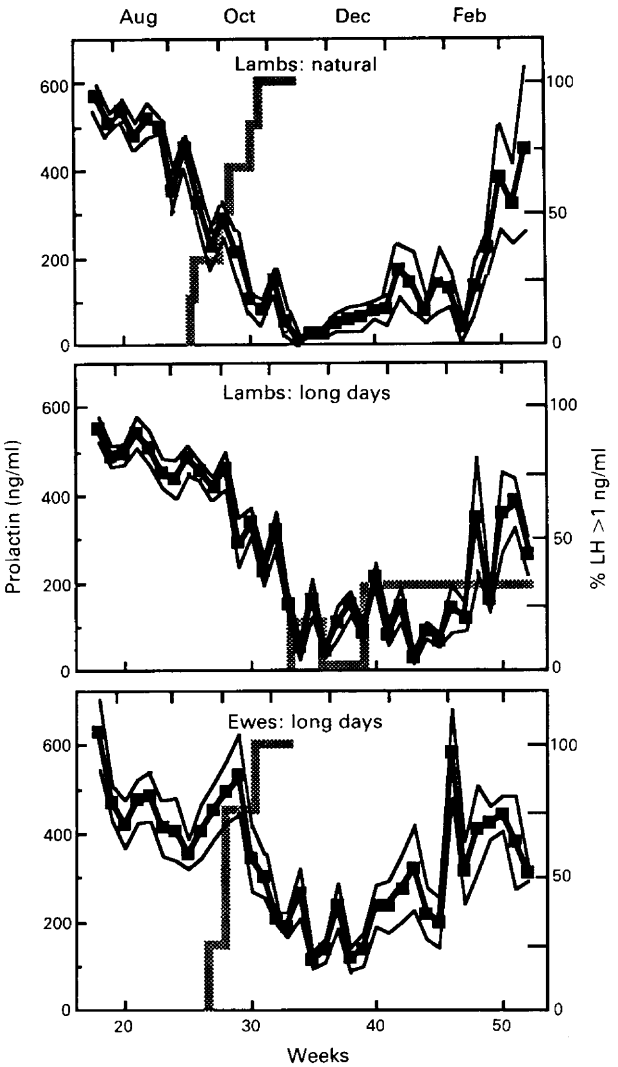

Fig. 4. Mean (±s.e.m.) serum prolactin concentrations in oestradiol-implanted, ovariectomized (Ovx + E) female lambs maintained in a simulated natural photoperiod (top, N = 6), in their Ovx + E twin sisters maintained in constant long days of 16 h light:8 h dark (middle, N = 6), and in their Ovx + E mothers also maintained in constant long-days (bottom, N = 4). The hatched line indicates onset of reproductive activity, expressed as the proportion of females in each group with increased (> 1 ng/ml) serum LH concentrations.

days than in either of the other two groups (Table 1). Removal of the oestradiol implant resulted in a significant increase in the LH pulse frequency and mean LH concentrations in the constant long-day lambs but, at 7 days after implant removal, these values were still significantly lower than the corresponding values in their ovariectomized sisters in the simulated natural photoperiod (Table 1). LH pulse frequency was significantly lower in the ovariectomized mothers following implant removal than in the ovariectomized lambs in the simulated natural photoperiod, but LH pulse amplitude was significantly higher.

Serum prolactin concentrations for each group are illustrated in Fig. 4. The patterns were broadly similar in all three groups, circulating prolactin values being initially high, then falling

from August to December, and finally increasing until the end of the experiment in March. Relative to the initial concentrations, prolactin had fallen significantly ($P < 0{\cdot}05$) by 27 weeks of age in lambs in the simulated natural photoperiod, but not until Week 32 in their twin sisters or in their mothers in constant long-day photoperiods. At the nadir of these prolactin profiles (Weeks 32–42), serum concentrations were significantly higher in the constant long-day mothers than in the lambs in natural photoperiod; concentrations in the constant long-day lambs were intermediate.

Discussion

This study confirms that mature Suffolk ewes do not necessarily require a decrease in daylength for normal timing of the breeding season (Robinson *et al.*, 1985). In marked contrast, their spring-born daughters do. When such lambs were subjected to the same constant long-day regimen, they did not show a synchronized onset of reproductive activity. In fact, 4 out of 6 remained hypersensitive to the inhibitory feedback effects of the oestradiol treatments until the conclusion of the experiment at 1 year of age. In their twin sisters experiencing a simulated natural photoperiod, the increase in serum LH was relatively synchronous. The ages (26–31 weeks) of the start of the rise are in agreement with previous studies on intact and oestradiol-implanted ovariectomized Suffolk lambs maintained in a natural photoperiod (Yellon & Foster, 1985). We conclude that the spring-born lamb requires a decrease in photoperiod for the normal timing of puberty in the autumn.

The increases in mean serum LH concentrations, which provide the index of reproductive activity in ovariectomized, oestradiol-treated sheep, are attributable to an increase in the frequency of episodic LH secretion rather than an increase in the amplitude of the pulses (Table 1). This is consistent with our current hypothesis (Foster *et al.*, 1986) and recent observations (Huffman *et al.*, 1987) that an increase in LH pulse frequency is the critical event inducing follicular development, and thus puberty, in the female sheep. The failure of the lambs in constant long days to begin reproductive activity is partly a consequence of this photoperiod treatment maintaining high sensitivity to oestradiol negative feedback. The lambs in constant long days are capable of producing rapid LH pulses because removal of the oestradiol implant causes an increase in LH pulse frequency. However, LH pulse frequency is lower in the long-day lambs than in their twin sisters in simulated natural photoperiod in the absence of oestradiol negative feedback. This raises the possibility that the failure to reduce sensitivity to oestradiol negative feedback in the lamb under constant long days might reflect a low level of 'direct drive', that is, a low activity of the LH pulse-generating system in the absence of ovarian steroid feedback. On the other hand, in the adult females kept in constant long days, the markedly reduced sensitivity to oestradiol negative feedback is associated with a lower LH pulse frequency in the absence of ovarian steroid feedback than that seen in the lambs in the simulated natural photoperiod in the absence of oestradiol feedback. Therefore, no simple relationship exists between LH pulse frequency in the absence of the ovaries and in the presence of oestradiol negative feedback.

The important question which this study raises is whether the different response to photoperiod in prepubertal and adult sheep reflects a developmental change in the photoperiodic mechanism, or simply a differential photoperiodic history that the adult sheep and spring-born lamb would have. The lamb clearly responds to photoperiod early in life. The ability to generate a serum melatonin pattern which reflects ambient photoperiod is present from the first few days after birth (L. E. Claypool, R. I. Wood, F. J. P. Ebling & D. L. Foster, unpublished results) or possibly *in utero* (Yellon & Longo, 1987). Provided certain growth requirements have been fulfilled (Foster *et al.*, 1986, for review), the lamb can use this information to modulate prolactin secretion in the early postnatal period (Ebling *et al.*, 1988) and the subsequent timing of puberty.

A more attractive explanation for the difference in the photoperiod requirements for initiation of reproductive activity in spring-born lambs and mature ewes relates to their differing photoperiod histories, and the current thought that the role of changes in photoperiod in seasonality is to

entrain endogenous changes in reproductive activity, rather than to drive directly changes in reproductive function (Robinson *et al.*, 1985). This hypothesis, in turn, derives from the observations that sheep exhibit long-term changes in reproductive activity when exposed to a constant photoperiod (Ducker *et al.*, 1973; Howles *et al.*, 1982; Almeida & Lincoln, 1984; Karsch *et al.*, 1987). What is not clear is whether these long-term reproductive changes in sheep are the expression of an endogenous circannual oscillator, perhaps analogous to endogenous circadian rhythms. Alternatively, the periods of reproductive activity and quiescence may themselves be the 'clock mechanism', and so the seasonal reproductive rhythm results from repetition of these 'interval timers' (Enright, 1970). Unfortunately, observations of long-term reproductive changes under fixed photoperiod do not discriminate between these two hypotheses (Enright, 1970). Regardless of the mechanism underlying long-term reproductive oscillations, it is clear that reproductive maturation in the female sheep is an innate process (Ducker *et al.*, 1973), because alterations in ambient photoperiod or the pathways by which light cues are transduced can modify the timing of puberty, but not delay it indefinitely (Kennaway *et al.*, 1985; Yellon & Foster, 1985, 1986). Perhaps the failure of the spring-born lamb to initiate reproductive activity at the normal time when maintained in constant long days is simply because, by virtue only of being born during the increasing daylengths of spring, it has not generated an adequate seasonal photoperiod history to entrain its innate reproductive maturation. Adult ewes maintained in constant long days from the summer solstice are able to begin reproductive activity at the appropriate time because they have previously experienced changes in photoperiod which have entrained their endogenous changes in reproductive activity and quiescence. We suggest that the initial role of decreasing photoperiod in the autumn is to provide a powerful entrainment signal to the lamb. It is the direction of change in photoperiod that is critical. Short days are not in themselves a sufficient entrainment cue since exposure of lambs from birth to constant short days results in delayed puberty (Yellon & Foster, 1985). In support of this 'entrainment' hypothesis, we have observed that if lambs first experience short days (birth to 17 weeks of age), then synchronous puberty will occur in long days during the first year, albeit at a much older age than normal (Foster *et al.*, 1988). Thus, any marked change in photoperiod early in life may serve as an entrainment cue, and the direction of the change would set the phase angle between the onset of reproductive activity and the change in daylength.

Serum prolactin concentrations were monitored in the current study to provide a second comparison of neuroendocrine response to photoperiod in the immature and adult sheep. The overall similarities in the prolactin profiles between the adults and lambs in constant long days and the lambs in the natural simulated photoperiod may reflect the development of refractoriness to the stimulatory effects of long days (Howles *et al.*, 1982; Almeida & Lincoln, 1984). The extended observations by both of these groups that prolactin concentrations continued to show periodic rises and falls support the view that the phenomenon of refractoriness reflects the expression of endogenous changes in prolactin secretion. Again it is unclear as to whether such changes reflect the activity of an endogenous oscillator, or simply alternating periods of low and high prolactin secretion which occur obligatorily, and therefore appear to constitute an endogenous rhythm. A complicating, major consideration is of the role of temperature in modulating prolactin secretion, because this variable was not controlled in the current studies. Annual changes in ambient temperature may modulate prolactin secretion in sheep in which transduction of photoperiod information is interrupted by pinealectomy (Munro *et al.*, 1980; Karsch *et al.*, 1987). It seems likely that in the absence of changing photoperiod, the decreasing ambient temperature from September onwards would decrease prolactin secretion. A final important observation from our study is that reproductive activity does not necessarily occur when circulating prolactin concentrations fall, for example at the start of October in lambs in long days. Conversely, reproductive activity can occur in the presence of high prolactin concentrations, for example in the adult ewes in October. This supports the conclusion of Worthy *et al.* (1985) that, in the sheep at least, prolactin does not inhibit neuroendocrine reproductive function.

In conclusion, the photoperiod requirements for puberty in the spring-born lamb differ from those for the adult sheep. The former must experience a decrease in daylength for the normal timing of puberty, whereas synchronous changes in reproductive activity can occur in the adult in the absence of a change in daylength. We propose that the photoperiod mechanism is fundamentally the same in the lamb and the adult, and so the differences in reproductive response to a fixed photoperiod result from the limited photoperiod history of the lamb.

We thank Mr L. H. Breasbois (Freeland, MI) for providing high quality lambs for experimentation; Mr K. Van Natter for conscientious animal care; Mr D. D. Doop and Mrs T. E. Adel for expert technical assistance; Ms C. Woodfill for help in establishing the prolactin radioimmunoassay; Dr M. Brown for help with the statistical analyses; Mr M. R. Muha for microcomputer support; Mrs D. E. Belleba and Mrs E. C. Lewis for secretarial support; the Reproductive Endocrinology Program Standards and Reagents Core Facility for standardization of hormone radioimmunoassay reagents; and Dr L. E. Reichert Jr, Albany Medical College of Union University, and Dr G. D. Niswender, Colorado State University, for reagents for hormone radioimmunoassay. This work was supported by grants from the NIH (HD-18258, HD-18394 and Biomedical support to the Vice President for Research at The University of Michigan) and a Fulbright Travel Award and Fogarty Fellowship (TWO3766 to F.J.P.E.).

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Received 28 January 1988