Photoperiodic modification of negative and positive feedback effects of oestradiol on LH secretion in ovariectomized goats

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Summary. Ovariectomized Shiba goats carrying an oestradiol implant (4–10 pg/ml) were kept under a short-day light regimen (10L:14D; Group 1, N = 4) or a long-day regimen (16L:8D; Group 2, N = 4). Plasma LH concentrations were lower ($P < 0.05$) in Group 2 than in Group 1 between Days 40 and 200, suggesting an enhanced negative feedback effect of oestradiol on LH secretion under a long-day regimen.

On Days 30, 60, 100, 149 and 279, an LH surge was induced by i.v. infusion of oestradiol for 48 h; the infusion rate was gradually increased from 0.5 (0 h) to 4.1 (48 h) $\mu\text{g/h}$, thereby mimicking the preovulatory increase of oestradiol secretion. The duration and magnitude of the induced LH surge were indistinguishable between the groups. The latency from the onset of oestradiol infusion to the LH surge was relatively constant in Group 1, 41.1 ± 0.9 h (mean $\pm$ s.e.m., $n = 17$) but was shorter in Group 2 (19.7 ± 3.7 h, $P < 0.05$) on Day 149; less oestradiol was therefore required for induction of the LH surge (27.4 vs 89.7 μg , $P < 0.01$), suggesting an increased sensitivity to the oestradiol positive feedback under a long-day regimen.

These results might be interpreted to indicate that the hypothalamic–pituitary axis of the goat becomes hypersensitive to the positive as well as the negative feedback effect of oestradiol under long-day conditions.

Introduction

The reproductive activity of the goat is known to be under photoperiodic control. Short daylength is stimulatory whereas long daylength has a suppressive effect on female ovulatory cyclicity. The effects of long daylength, however, continue only for 150–200 days after exposure to such a regimen; ovulation recurs despite continuous exposure to long days (Mori *et al.*, 1984; Maeda *et al.*, 1986). Since the length of gonadal quiescence is similar to that of the anoestrous season, the onset of the breeding season might be due to the photorefractoriness of the animal to long daylength as has been suggested in ewes (Robinson *et al.*, 1985). However, the mechanism by which long daylength induces gonadal involution or its reactivation is, as yet, poorly understood.

In sheep the regulation of LH secretion is considered to be of central importance in seasonal breeding; there is an apparent seasonal change in the responsiveness of the hypothalamic–pituitary axis to the negative feedback effects of oestradiol, which is dictated by changing daylength (Legan & Karsch, 1980; Webster & Haresign, 1983). The positive feedback action of oestradiol eliciting the LH surge may also undergo seasonal alternation, as Land *et al.* (1976) have found a reduction in responsiveness in anoestrous ewes, although other authors have failed to do so (Beck & Reeves, 1973; Goodman & Karsch, 1980; Haresign & Friman, 1983). In goats, however, little is known about the endocrinological background of seasonal reproduction.

The present study was undertaken to investigate how photoperiod influences the feedback effects of oestradiol on LH secretion in the Shiba goat, a Japanese native miniature goat (Mori & Kano, 1984) which breeds non-seasonally in its natural habitat but ceases ovulatory cyclicity if it is exposed to a lighting regimen of long days (Mori *et al.*, 1984).

Materials and Methods

Experimental design. The time course of the experimentation is illustrated in Fig. 1. Eight sexually mature females Shiba goats weighing 25–30 kg, which had been having normally recurring oestrous cycles outdoors (36°3'N), were housed in light-controlled rooms in May and maintained under short day conditions (10 h light:14 h darkness (10L:14D), lights on 05:00 h). The room was illuminated by fluorescent bulbs, and the light intensity was 400 lux at the level of the goat's head. Room temperature was kept at $22 \pm 2^\circ\text{C}$ throughout the experimental period. A commercial pelleted diet was supplied once a day (10 g/kg) with free access to hay and fresh water.

In July the goats were ovariectomized and subcutaneously implanted with Silastic tubing (i.d. 3.35 mm, o.d. 4.65 mm, length 30 mm; Dow Corning, Midland, MI, U.S.A.) containing crystalline oestradiol (Sigma Chemical Co., St Louis, MO, U.S.A.) under halothane (in oxygen) anaesthesia. The goats were randomly allocated to one of two groups 1 month after surgery: 4 goats were successively maintained under short-day conditions (Group 1, 10L:14D) and 4 were transferred to a long-day regimen (16L:8D, lights on at 05:00 h; Group 2). The first day of exposure to the long-day regimen was designated as Day 0. Blood samples were collected from the jugular vein into heparinized syringes every 1–2 weeks during the period between Day 0 and Day 260. Plasma was separated by immediate centrifugation and stored at -20°C until assayed for oestradiol and LH concentrations.

A physiological amount of oestradiol was infused intravenously as described below on Days 30, 60, 100, 149 and 279 to examine the sensitivity of the hypothalamic–pituitary axis to the positive feedback effect of oestradiol.

Oestradiol infusion. Each goat was catheterized and tied to a stanchion on the day before each oestradiol infusion. At catheterization, two 70-cm vinyl chloride catheters (i.d. 0.97 mm; Japan Sharwood, Tokyo) were inserted caudally into both lateral jugular veins, one for infusion and the other for blood sampling. The subcutaneous oestradiol implant was removed 2 h before the start of oestradiol infusion and replaced 8 h after the end of infusion. These minor operations were carried out under light anaesthesia with a bolus i.v. injection of ketamine HCl (1.0 mg/kg) and xylazine HCl (0.02 mg/kg) as described previously for goats (Mori *et al.*, 1983).

Figure 2 summarizes the oestradiol infusion used in this experiment. Oestradiol (0.9 µg/ml in sterilized saline (9 g NaCl/l) containing 0.1% ethanol) was intravenously infused with a microtubing pump (SJ-1211, Attoh, Tokyo) for 48 h starting at 12:00 h (0 h). As shown in Fig. 2(a), the infusion rate was gradually raised from 0.5 µg/h (0 h) to 4.1 µg/h (48 h) by automatically increasing the pumping speed. The accumulated amounts of infused oestradiol were recorded hourly; 110.4 ± 0.8 (mean $\pm$ s.e.m., $n = 35$) µg were administered to each animal during the 48 h. In a preliminary experiment using 4 ovariectomized goats, a gradual increase in plasma oestradiol concentration within the physiological range was observed as the infusion rate increased (Fig. 2b). Blood samples (2 ml) were withdrawn via

Fig. 1. Ovariectomized (OVX) goats were implanted with a subcutaneous capsule containing oestradiol during the period represented by the clear bar to examine the negative feedback effect of oestradiol under short-day (10L:14D, Group 1) and long-day (16L:8D, Group 2) conditions. The first day of exposure to the long-day regimen was designated Day 0. On Days 30, 60, 100, 149 and 279, an LH surge was induced in both groups by means of a programmed oestradiol infusion (▼) to examine the positive feedback effect.

Fig. 2. Summary of the oestradiol infusion. (a) The infusion rate was linearly increased from 0.5 to 4.1 $\mu\text{g/h}$ and the accumulated amount of infused oestradiol during the 48-h period averaged 110.4 μg . (b) Plasma oestradiol concentration ($N = 4$, mean $\pm$ s.e.m.) showed a gradual rise within the physiological range as the infusion rate increased. (c) Plasma LH profiles during the oestradiol infusion in Group 1 (○) and Group 2 (●) examined on Day 30. Each point represents the mean value ($\pm$ s.e.m.) for 4 goats.

the sampling catheter at 2-h intervals between -2 and 54 h. Plasma was separated and stored at -20°C until assayed for LH concentration.

Hormone assays. Plasma concentrations of oestradiol and LH were measured by specific radioimmunoassays as described previously (Mori & Kano, 1984). All the weekly samples were analysed in the same assay. Assay sensitivities were 3 pg oestradiol/ml and 0.8 ng LH (NIH-S-19)/ml. The intra- and inter-assay coefficients of variation were all less than 12.0%.

Statistical analysis. Results were expressed as means $\pm$ s.e.m. and Student's t test, Cochran-Cox test or Duncan's new multiple range test (Steel & Torrie, 1960) were adopted for detection of statistical differences between mean values.

Results

Negative feedback

Constant release of oestradiol from the implant maintained plasma oestradiol concentrations at 4–10 pg/ml, and this did not differ between groups (Table 1). Plasma LH values, however, were lower ($P < 0.05$) in Group 2 than in Group 1 during the period between Days 40 and 200.

Table 1. Plasma concentrations of LH and oestradiol in ovariectomized oestradiol-implanted goats kept under a 10L:14D (Group 1, N = 4) or 16L:8D (Group 2, N = 4) regimen

Day†	LH (ng/ml)		Oestradiol (pg/ml)	
	Group 1	Group 2	Group 1	Group 2
0–40	4.9 ± 0.3	5.5 ± 0.6	10.0 ± 1.8	8.5 ± 2.3
41–80	5.1 ± 0.5	3.2 ± 0.6*	7.0 ± 1.8	5.0 ± 1.6
81–120	3.7 ± 0.5	1.8 ± 0.3**	4.0 ± 1.0	5.3 ± 1.8
121–160	3.5 ± 0.7	1.4 ± 0.2*	6.0 ± 0.2	8.0 ± 2.0
160–200	3.1 ± 0.6	1.3 ± 0.1*	8.0 ± 2.5	9.8 ± 2.1
201–260	3.7 ± 0.4	3.3 ± 0.8	4.5 ± 1.0	7.0 ± 1.8

Values are mean ± s.e.m. of 12–20 pooled observations.

†Group 2 goats were transferred from 10L:14D to 16L:8D on Day 0.

* $P < 0.05$, ** $P < 0.01$ compared with Group 1.

Table 2. LH surges induced by oestradiol infusion in ovariectomized goats kept under a 10L:14D (Group 1) or 16L:8D (Group 2) regimen

Day†		Timing (h)‡	Duration (h)§	Peak LH (ng/ml)
30	Group 1 (4)	41.0 ± 1.6	8.0 ± 0.6	110.8 ± 13.8
	Group 2 (4)	42.5 ± 1.5	8.5 ± 1.0	152.2 ± 48.5
60	Group 1 (4)	42.0 ± 1.3	8.0 ± 0.8	82.3 ± 24.0
	Group 2 (4)	45.5 ± 2.8	8.0 ± 1.0	113.0 ± 55.8
100	Group 1 (3)	36.3 ± 0.7	9.3 ± 1.2	165.1 ± 6.3
	Group 2 (3)	31.7 ± 11.4	8.7 ± 0.6	178.3 ± 60.7
149	Group 1 (3)	41.7 ± 1.3	8.7 ± 0.6	156.8 ± 31.9
	Group 2 (3)	19.7 ± 3.7*	10.0 ± 1.2	111.5 ± 27.6
279	Group 1 (3)	45.3 ± 2.4	10.0 ± 3.5	156.0 ± 49.5
	Group 2 (4)	43.0 ± 2.2	9.0 ± 1.3	116.3 ± 38.0

Values are mean ± s.e.m. for the no. of animals in parentheses.

†Group 2 goats were transferred from 10L:14D to 16L:8D on Day 0.

‡Interval between the start of oestradiol infusion and the onset of LH surge.

§Period when plasma LH exceeded 10 ng/ml.

*Significantly different ($P < 0.05$) from Group 1.

Positive feedback

The oestradiol infusion produced a gradual rise in plasma oestradiol and induced a surge release of LH which was comparable in magnitude and duration to the preovulatory LH surge (Fig. 2c). The duration of the LH surge (defined as the period when the plasma LH concentrations exceeded 10 ng/ml) and its magnitude (peak LH concentration) did not differ significantly between groups throughout the experimental period (Table 2). Due to the failure of catheterization, oestradiol infusion was not performed in 2 goats on Day 100 and 149 and in 1 animal on Day 279.

Timings of the induced LH surge oestradiol infusion are presented individually in Fig. 3. The latency from the start of oestradiol infusion to the onset of the surge averaged 41.1 ± 0.9 h ($n = 17$) in Group 1, with no significant differences between trial days. Contrary to this relatively consistent latency in Group 1, the onset of the LH surge was advanced in 1 goat on Day 100 and then in all 3 animals examined on Day 149. There was a significant between-group difference ($P < 0.05$) in the latency to onset of the LH surge (19.7 vs 41.7 h) or to the LH peak (24.7 vs 46.0 h) on Day 149.

Fig. 3. Timings of the induced LH surge (shaded area) in individual ovariectomized goats kept under short-day (Group 1) or long-day (Group 2) regimens. Mean ($\pm$ s.e.m.) time of maximum LH concentration for the two groups is presented at the top of each panel. * $P < 0.05$ between groups on the same trial day.

When the amounts of oestradiol administered before the onset of the LH surge were compared there was also a significant difference between Groups 1 and 2 (27.4 ± 8.07 vs 89.7 ± 4.86 μ g, $P < 0.05$) on Day 149, while no difference was found on any other trial days (e.g. 95.5 ± 5.3 vs 87.5 ± 5.97 μ g on Day 30).

Discussion

The results of this study showed that the negative feedback effect of oestradiol on LH secretion in the goat was affected by the photoperiod. Basal LH secretion in ovariectomized goats was more suppressed under a long-day regimen than under a short-day regimen by the oestradiol released from the chronic implant, although circulating oestradiol concentrations did not differ between groups throughout the experimental period. This is considered to indicate an enhanced negative feedback effect of oestradiol on LH secretion induced by exposure to a long-day regimen. The time when the oestradiol negative feedback was potentiated under long daylength, i.e. Days 40–200, coincided with the anovulatory period induced by long daylength in intact goats (Mori *et al.*, 1984; Maeda *et al.*, 1986). The synchrony of these two reproductive indexes suggests that gonadal

involution and reactivation under a long-day regime may be associated with changes in the sensitivity of the hypothalamic–pituitary axis to the oestradiol negative feedback, and this agrees well with previous observations in ewes (Legan *et al.*, 1977; Webster & Haresign, 1983).

The positive feedback effect of oestradiol was also influenced by photoperiod. Simulation of the preovulatory increase in circulating oestradiol by means of a programmed infusion induced a secretory profile of LH similar to that seen during the follicular phase in the Shiba goat (Mori & Kano, 1984). The interval of about 40 h between the initiation of oestradiol infusion and the onset of the LH surge was similar to the interval between the luteal regression, after which plasma oestradiol started to rise, and the LH surge during the periovulatory period (Mori & Kano, 1984). The latency to the surge, however, was shortened under a long-day regimen on Day 149; a smaller amount of oestradiol had therefore been infused by the onset of the LH surge. In other words, less oestradiol was required to elicit the surge release of LH under long-day conditions. This would seem to suggest an increased sensitivity of the hypothalamic–pituitary axis to the positive feedback effect of oestradiol in goats exposed to a long-day regimen for 100–150 days.

This finding, however, is inconsistent with previous observations in sheep. Goodman & Karsch (1980) demonstrated a lack of seasonal change in the positive feedback ability of oestradiol by giving various dosages of oestradiol implant to progesterone-primed ovariectomized ewes. Land *et al.* (1976) observed a decreased sensitivity to exogenous oestradiol given as a bolus injection in anoestrous sheep. It is uncertain whether this inconsistency should be ascribed to species difference or to differences in experimental conditions.

McNeilly *et al.* (1982) induced ovulation in anoestrous ewes with pulsed injections of LH; although the data indicate that ovarian secretion of oestradiol during the induced follicular development was much smaller in comparison with the spontaneous ovulation occurring in the breeding season, the minute amount of oestradiol secreted was nevertheless sufficient to elicit a normal LH surge. If this were due to altered sensitivity of the central nervous system to oestradiol, the results of McNeilly *et al.* (1982) might be interpreted as indicating that the positive feedback effect of oestradiol is strengthened under long-day conditions in the anoestrous season, lending support to the present observation.

In previous studies we have observed that goats develop photorefractoriness to the effects of long daylength on gonadal function and prolactin secretion after prolonged exposure to a long-day regimen (Mori *et al.*, 1985; Maeda *et al.*, 1986). The present results suggest that the LH secretory system becomes tolerant to the effect of long daylength around the time of appearance of photorefractoriness.

The present results might be interpreted to indicate that the hypothalamic–pituitary axis of the goat becomes hypersensitive not only to the effect of negative feedback but also to that of positive feedback of oestradiol on LH secretion under long-day conditions.

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