Photoperiodic control of prolactin secretion in the goat

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Summary. Effects of photoperiods on prolactin (PRL) secretion were investigated by keeping female Saanen goats under artificial long days (16L8D) or short days (8L16D). Exposure to experimental photoperiods was started in December 1980 (day 0) and continued for about a year. Plasma PRL of the long-day group was elevated to the levels much higher than those of the short-day group. Effects of photoperiods on the diurnal fluctuation of plasma PRL were also evident on selected days when blood samples were taken at short intervals (day 24 and 118): being enhanced under long days and damped under short days. In July and August 1981, a marked elevation of PRL levels was noted in both groups, probably due to high ambient temperature which might have masked the effects of short-days. In the long-day group, goats were anovulatory when PRL levels were high (day 78-148), however, by day 200 they resumed estrous cyclicity spontaneously in association with the decrease in plasma PRL, suggesting that there might be an intrinsic mechanism which gradually increases the critical day lengths.

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Introduction

Prolactin (PRL) secretion in sheep^{1,2)} and goats³⁻⁵⁾ is known to show a marked seasonal fluctuation: peripheral PRL concentrations are high in the spring and summer (corresponding with seasonal anestrus) and low in the autumn and winter (breeding season). Contribution of the daylength to this annual PRL profile has been well established in sheep; artificial reversal of annual change in daylength resulted in an inverted annual pattern of PRL secretion⁶⁾, and repeated exposure to long days and short days resulted in cyclical changes in plasma PRL concentrations according to the photoperiods⁷⁾. In addition to the photoperiod ambient temperature^{5,8)} may affect

seasonal profile of PRL secretion in sheep.

The purpose of the present study was first to evaluate the effect of photoperiod on PRL secretion in female goats. Secondly, since a line of evidence suggests that hyperprolactinemia causes hypogonadism in women^{9,10,11)}, ewes^{12,13)}, the relationship between plasma PRL levels and reproductive activities was investigated in the goats living under different artificial lighting conditions, of which estrous cyclicity has already been reported¹⁴⁾.

Materials and Methods

Animals:

Female Saanen goats which had been recurring normal estrous cycles were divided into long-day group ($n=8$) and short-day group ($n=5$). The animals and experimental conditions were the same as those previously described¹⁴⁾. Exposure of the goats to arti-

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ficial long days (16h light and 8h dark; 16L8D) was commenced on December 4, 1980 (day 0) and continued for about a year. The goats of the short-day group were kept under natural short days until day 63 (Feb. 3) and onwards under the artificial short days (8L16D); except for one animal which remained under natural daylight. Two goats of the long-day group were subjected to the short days after day 55. Changes in outdoor and indoor temperatures were continuously recorded throughout the experimental period.

Collection of blood samples:

In addition to the twice weekly blood sampling throughout the experiment¹⁴⁾, on day 42 and day 118 more intensive sampling was performed at 2h intervals for 24h by puncturing the jugular vein aiming to investigate the diurnal pattern of PRL secretion under respective lighting regimen. On each occasion particular care was taken not to disturb the animals which remained in their normal pens. Local and faint lighting method was used to aid blood sampling during

the dark period of each 24h cycle. Plasma was immediately separated by centrifugation and stored at -20°C until assayed for PRL.

Hormone assay:

Plasma PRL concentrations were assayed for the 24h blood samples and selected biweekly samples by the double-antibody radioimmunoassay for goat PRL established by JOHKE *et al.*⁵⁾ with some modification as described below. Highly purified ovine PRL (Li-sheep PRL) was iodinated with ^{125}I (New England Nuclear Co., USA) by the lactoperoxidase method¹⁵⁾. Specific activity of iodinated PRL was $94.6 \mu\text{Ci}/\mu\text{g}$. Samples of $20 \mu\text{l}$ goat plasma was diluted with $80 \mu\text{l}$ of phosphate buffered saline ($\text{pH} 7.4$, 0.01 M) containing 1% bovine serum albumin (PBS-BSA) and incubated with $100 \mu\text{l}$ of ^{125}I -PRL ($27,000 \text{ dpm}$ in PBS-BSA) and $100 \mu\text{l}$ of anti-bovine PRL guinea pig serum ($1:150,000$ in PBS containing 0.5% normal guinea pig serum and 0.05 M EDTA) which was developed and characterized by JOHKE¹⁶⁾. After 24h incubation at 4°C , $200 \mu\text{l}$ of anti-guinea pig gamma globulin caprine serum developed

Fig. 1. Displacement curves for serial dilutions of pooled goat plasma (■) and a goat pituitary homogenate (▲) demonstrating parallelisms with the displacement curve of standard prolactin (NIH PS-8, ●). Each point represents the mean $\pm$ SD ($n=3$).

in our laboratory (1:30 in PBS) was added. Following second incubation for 24 h at 4°C the mixture was centrifuged for 30 min at 4°C and the radioactivity of precipitate was counted. The specificity of the assay was validated by comparing displacement curves for serial dilutions of pooled goat plasma and a goat pituitary homogenate with the displacement curve of the standard ovine prolactin, NIH P-S8; the 3 curves did not depart from parallelism (2×3 points design, $P > 0.05$) as shown in Fig. 1, and the interference by endogenous PRL in caprine second antiserum was negligible. To ascertain the accuracy of the assay various amounts of PRL were added to a pooled goat plasma. They were assayed in triplicate by the radioimmunoassay as described above, and a satisfactory correlation ($r = 0.998$, $P < 0.01$, $n = 12$) was obtained between added (x) and recovered PRL (y) ($y = 1.07x + 0.07$). All samples from one animal were assayed in a single assay. The limit of detection (95% confidence limits of buffer control) averaged 3.9 ng/ml for 20 μ l plasma. The intra- and inter-assay coefficients of variation were 7.0% ($n = 6$) and 12.5% ($n = 5$), respectively.

Assessment of estrous cyclicity:

Percentages of the goats recurring estrous cycles under either long days or short days were calculated by dividing the observed number of ovulation by the expected number of ovulation (one ovulation per animal per three weeks); the occurrence of ovulation was estimated from plasma progesterone profiles of these goats which have already been reported¹⁴⁾.

Statistical analysis:

Results were expressed as mean $\pm$ SEM and Student's t test or Cochran-Cox test was used

to determine the significance of difference between the means.

Results

Plasma PRL concentrations of the long-day goats tended to stay in higher levels than those of short-day group as shown in Fig. 2, and were significantly higher on days between 78 and 148; besides this period a significant difference was obtained only occasionally on day 247 and day 320. Remarkable elevations in plasma PRL with similar amplitude (> 80 ng/ml) and pattern in both groups were observed during the summer period between day 218 (Jul. 10) and day 261 (Aug. 22) when ambient temperature exceeded 27°C at the time of blood sampling.

Figure 3 shows diurnal patterns of plasma PRL on day 42 (Jan. 15) and day 118 (Mar. 31). On day 42 the long-day goats exhibited high plasma PRL levels with marked nocturnal elevation (Fig. 3 A; mean PRL level on that day was 47.0 ± 6.7 ng/ml), while the

Fig. 2. Plasma prolactin concentrations (mean $\pm$ SEM) in female Saanen goats which were subjected to artificial long days of 16L8D (closed circle, $n = 5$) and short days of 8L16D (open circle, $n = 4$). Exposure to 16L8D was commenced on day 0. Changes in ambient temperature recorded at the time of blood sampling (around 10:00 h) are presented as the broken line.

Fig. 3. Diurnal patterns of plasma prolactin concentrations (mean $\pm$ SEM, or $\pm$ range (C)) in the goats staying under different photoperiodic conditions. Blood sampling at 2 h intervals for 24 h was performed on day 42 (left panels) and day 118 (right panels). Shaded area shows the dark phase and the horizontal broken line indicates the mean prolactin level for the group on that day. A: 42nd day under 16L8D, B: 118th day under 16L8D. C: 63rd day after transferred from 16L8D to 8L16D. D, E: under natural photoperiod on Jan. 15 and Mar. 31. F: 63rd day after transferred from outdoor to 8L16D.

goats under natural short days showed low PRL secretion (D; 6.7 ± 0.5 ng/ml). On day 118 plasma PRL were relatively high in the long-day goats (B; 22.3 ± 2.7 ng/ml), while PRL levels were very low in the goats which had been transferred from long days to short days (C; 5.7 ± 0.8 ng/ml) as in the goats continuously exposed to short days (F; 6.7 ± 1.0 ng/ml). The goat staying outdoor showed higher PRL levels on day 118 (E; 16.4 ± 1.3 ng/ml) than did on day 42 (D).

Figure 4 shows effects of long days on estrous

Fig. 4. Effects of photoperiod on estrous cyclicity and prolactin secretion in the goat. A) Percentages of the goats recurring estrous cycles under long days (16L8D, $n=5$) or short days (8L16D, $n=4$). On day 0 the exposure to long days was commenced. B) Differences in the means of plasma prolactin (Δ PRL) between the long-day group and short-day group; the mean prolactin value for the short-day group was subtracted from that for the long-day group on each occasion. * $P < 0.05$, ** $P < 0.01$.

cyclicity and PRL secretion in the goat. In order to extract the effect of photoperiod on PRL secretion the difference in the mean plasma PRL concentration between the two groups was calculated on each occasion by subtracting the mean PRL value for the short-day group from that for the long-day group and was presented as a function of the time course of long-day treatment. The difference in PRL level between the two groups gradually increased and was significant on days 78–148. On the other hand, the number of goats recurring estrous cycles under long days gradually declined and the estrous cyclicity of this group was completely abolished by day 100, while the goats kept under short days continued to cycle. In the long-day goats the acyclic period synchronized with the time when plasma levels of PRL

were significantly higher than the short-day group. The difference in mean PRL levels between the two groups diminished by day 200 when the long-day goats spontaneously resumed estrous cyclicity.

Discussion

Photoperiod had a profound effect on PRL secretion from the pituitary of female goats; their plasma PRL concentrations showed high level under long days, but remained low under short days. The results agreed with those reported in ewes¹⁷⁾. As a marked elevation in plasma PRL occurred during the summer period, high ambient temperature was another environmental factor responsible for PRL release, because both in the magnitude and the pattern PRL rise in the summer was indistinguishable between two groups receiving different photoperiodic treatments. On hot days ($>27^{\circ}\text{C}$ at the time of blood sampling) very high levels of plasma PRL were recorded invariably. TUCKER *et al.*⁹⁾ reported that, in prepubertal heifers, serum PRL changed rapidly (within minutes) with the changes in ambient temperature, while the PRL response to the changes in photoperiod was slow, requiring at least a week. In goats and heifers highly significant correlation of serum PRL concentration with ambient temperature ($r=0.806-0.850$) has been demonstrated⁵⁾. Changes in ambient temperature therefore would contribute considerably to the annual pattern of PRL secretion in the goats. The high PRL concentrations found in the summer might be concerned with salt retention since a restorative action of PRL on saluretic effect of aldosterone has been shown¹⁸⁾.

The diurnal pattern of PRL secretion was

strongly linked with photoperiod; high mean values for 24 h PRL levels was observed under the longer daylength. A nocturnal rise in PRL levels similar to that seen in the goat was reported in rams staying under long days¹⁹⁾.

Exposure of goats to long days resulted in an abolition of estrous cyclicity and a simultaneous increase in PRL secretion. Although the reason is unclear, plasma PRL levels declined by day 200 despite of continuous exposure to long days and the goats resumed estrous cyclicity¹⁴⁾. Similar results have been reported in sheep kept outdoor that serum levels of PRL are high throughout seasonal anestrus and fell before the onset of estrous cyclicity in the autumn¹⁾.

High concentrations of plasma PRL have been implicated as the cause of lactational anestrus in the ewe¹²⁾, and amenorrhea generally occurs in women with pathological^{9,11)} or lactational¹⁰⁾ hyperprolactinemia. Elevated plasma levels of PRL, induced by TRH injection, resulted in suppression of estradiol secretion in the ewe²⁰⁾. Ovulation in perfused rabbit ovary *in vitro* could be inhibited by PRL²¹⁾, suggesting a direct effect of PRL on the ovaries. The pituitary response to LHRH is reduced by PRL in rats²²⁾, and the positive feedback effects of estradiol on LH release was abolished or greatly impaired by high PRL levels in women⁹⁾ and ewes¹³⁾. The hypothalamo-pituitary axis therefore seems another plausible site of PRL action.

While the antigonadotropic effects of PRL have been consistently demonstrated, LAND *et al.*²³⁾ reported that suppression of PRL secretion with bromocriptine was unable to restore estrous cycles to ewes in seasonal

anestrus. Whether accelerated PRL secretion by long days is directly responsible for the arrested ovarian cyclicity of the goat remains to be solved.

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ヤギのプロラクチン (PRL) 分泌に対する日長の影響

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ヤギでは血中 PRL 濃度に著明な季節変動が認められる。本実験では PRL 分泌に対する光周期の影響を調べる目的でザーネン種雌ヤギに人為的長日 (16L8D) あるいは短日 (8L16D) 条件を負荷し血中 PRL 動態を比較検討した。日長制御は内因性 PRL 分泌の最も低下する 12 月に開始し約 1 年間継続した。

長日条件下では PRL 分泌は促進され、長日開始 42 日後には暗期にピークを示す明らかな日周パターンが認められた。一方、短日条件下では PRL 分泌は抑制され血中 PRL は低レベルに保たれた。7~8 月にかけて長短日の両群で PRL 分泌の著明な亢進が観察されたが、PRL 濃度上昇の程度・パターンのいずれについても両群間に差が認められないことから夏季高温に起因するもの

と推察された。長日により PRL の血中レベルが上昇し長日・短日群間の差が有意となる時期 (長日開始後 78~148 日) は、長日群において性周期が抑制される時期に一致した。また、長日開始後 200 日までには PRL 分泌に対する促進効果は減衰し短日群の血中レベルとの差は消失したが、時を同じくして生殖機能に対する年日の抑制効果も解除され、その結果ヤギは性周期を再開した。

以上の成績から、ヤギの PRL 分泌に対して長日は促進的に、短日は抑制的にそれぞれ影響することが示された。また PRL 分泌の促進と性周期の抑制が長日条件下において同時期に観察され、両現象間に何らかの関連の存在する可能性が示唆された。