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Effect of Blinding and Pinealectomy on Photoperiod and Seasonal Variations in Secretion of Prolactin in Cattle[1] (41726)

D. PETITCLERC,*[,2] R. R. PETERS,* L. T. CHAPIN,* W. D. OXENDER,† K. R. REFSAL,† R. K. BRAUN,† AND H. ALLEN TUCKER*

**Department of Animal Science and †Department of Large Animal Surgery and Medicine, Michigan State University, East Lansing, Michigan 48824*

Abstract. The role of the eyes and pineal gland on photoperiod- and season-induced changes in secretion of prolactin were studied in male cattle. Increasing exposure to light from 8 to 16 hr each day increased basal and thyrotropin-releasing-hormone-induced secretion of prolactin 3.7- and 4.1-fold in three sham-pinealectomized steers. In contrast, basal and thyrotropin-releasing-hormone-induced increases in secretion of prolactin did not change in four blind bulls and were markedly suppressed in three pinealectomized steers when exposure to light was increased from 8 to 16 hr/day. There was no diurnal variation in secretion of prolactin regardless of photoperiod or surgical treatment. However, seasonal changes (averaged 46 ng/ml in June–Aug vs 7 ng/ml in Dec–Feb) in secretion of prolactin persisted in blind and pinealectomized steers previously shown to be nonresponsive to changing photoperiods. Ambient temperature and photoperiod account for most, but not all, of the seasonal variation in secretion of prolactin. We hypothesize there is an endogenous annual rhythm in the secretion of prolactin in cattle.

Secretion of prolactin varies seasonally in cattle (1–3), sheep (4, 5), and goats (6); prolactin secretion is greatest in summer and lowest in winter. Increasing daily light from 8 to 16 hr increases secretion of prolactin several-fold (7–10). The pathway involved in the transmission of photic signals to the anterior pituitary is not known. Although blinding increases secretion of prolactin into blood of rats (11), it is not established if the eyes are essential to mediate photoperiod-induced changes in secretion of prolactin. In the present study, this question was examined in cattle.

Some photic signals impinging upon the retina of the eyes are transferred over a circuitous connection of neurons to the pineal gland (12, 13) involving retinohypothalamic fibers, suprachiasmatic nuclei, and superior cervical ganglia. Light suppresses and darkness increases secretory activity of the pineal gland. In turn, the pineal gland may affect secretion of the anterior pituitary gland. In fact, pinealectomy or superior cervical ganglionectomy of sheep and goats abolishes photoperiod-induced increases in secretion of prolactin (14–17). The role of the pineal gland in photoperiod-induced changes in secretion of prolactin in cattle is addressed in the present study.

Seasonal variation in secretion of prolactin has been attributed to changing photoperiods (5, 18–20). However, ambient temperatures were not controlled in those studies, and temperature markedly affects secretion of prolactin in cattle (21); within hours of changing ambient temperature from 21 to 32°C or from 21 to 4.5°C, concentrations of prolactin increased 113% or decreased 80%, respectively. Furthermore, ambient temperatures at freezing or below inhibit the ability of 16-hr photoperiods to stimulate secretion of prolactin (22, 23). The last objective of the present study was to characterize seasonal variation in secretion of prolactin in blind and pinealectomized cattle.

Materials and Methods. *Management of animals and blood.* In Experiments 1 and 2, animals were housed in temperature- and light-controlled chambers as previously described (10). They were fed *ad libitum* a complete pelleted diet, alfalfa hay, and trace-mineralized salt with free access to water. In ex-

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[2] On leave from Lennoxville Research Station, Agriculture Canada, Lennoxville, Quebec.

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periments 3 and 4, animals were enclosed in adjacent pens outdoors with free access to a covered, three-sided shelter. These animals were fed daily 1 kg of grain concentrate with free access to alfalfa hay, trace-mineralized salt, and water. To minimize danger to human safety, bulls which were scheduled to be used for many months were castrated, a procedure which does not affect basal or photoperiodic-induced release of prolactin in cattle (Stanisiewski, Chapin, and Tucker, unpublished observations). In all cases, castration occurred before puberty which occurs after 9 months of age in bulls.

In all experiments, blood was collected from a polyvinyl cannula inserted in a jugular vein. Blood samples were allowed to clot for 2 to 6 hr at room temperature. Then, samples were kept at 4°C for approximately 24 hr before centrifugation at 2000*g* for 20 to 30 min. Sera were decanted and stored at −20°C until assayed for prolactin (24).

Experiment 1. On a single day in March, eyes and a narrow rim of tissue containing the eyelashes of seven prepubertal Holstein bulls (2 to 3 months of age) were removed surgically under general anesthesia induced with sodium thiamylal (Surital, Parke-Davis) and maintained by 2-bromo-2-chloro-1,1,1 trifluoroethane (Halothane, Halocarbon Laboratories, Inc.) and oxygen. Gelfoam (Upjohn Co.) was inserted into the orbital cavities and eyelids were sutured together. Then, animals were exposed for 6 weeks to 8 hr of cool-white fluorescent light daily (lights on at 0700 hr). Between Weeks 7 and 12, one group ($N = 4$) was switched to 16 hr of light daily (lights on at 0300 hr) while the control group was maintained on 8 hr of light daily (lights on at 0700 hr). At the end of Weeks 6 and 12, concentrations of prolactin were determined in serum collected every 30 min for 24 hr. At Week 12, following the 24-hr sampling period, all animals were injected intravenously with thyrotropin releasing hormone (TRH, 33 μg/100 kg body weight), and blood samples were collected at frequent intervals for 60 additional min. Ambient temperature was recorded each time a blood sample was collected.

Experiment 2. Between September and November, Holstein bulls were either pinealectomized ($N = 3$) or sham-pinealectomized ($N = 3$) at 2 to 10 weeks of age as previously described (25). One month before beginning of the experiment, all bulls were castrated. In December, the two groups of animals were exposed to 8L:16D daily for 6 weeks followed by 6 additional weeks (7 through 12) of 16L:8D daily. At the end of Weeks 6 and 12, blood samples were collected at 30-min intervals for 48 hr; then, animals were injected intravenously with 33 μg of TRH/100 kg body weight, and blood samples were collected at frequent intervals for 30 additional min. During sampling, ambient temperature was recorded hourly.

Experiment 3. Four blind bulls were selected randomly from animals used in Experiment 1 and matched with sighted bulls of similar age and weight. Animals were exposed to natural duration photoperiods between the end of Experiment 1 on June 12 and the beginning of Experiment 3 on October 2. Two months before beginning the experiment, all animals were castrated. On a single day in October, December, February, April, June, and August, a jugular vein of all animals was cannulated at 0700 hr. Blood collection started at 1100 hr and continued at 30-min intervals for 4 consecutive hr.

Experiment 4. This experiment was initiated approximately 1 year and 3 months after the animals from Experiment 2 were pinealectomized. On a single day in December, February, April, June, August, and October, a jugular vein of the pinealectomized ($N = 3$) and sham-pinealectomized ($N = 3$) steers of Experiment 2 was cannulated. Blood samples were drawn the subsequent day at 30-min intervals for 6 hr. During sampling, ambient temperature was recorded hourly.

At the end of Experiment 4, pinealectomized steers were killed. Gross inspection of the area of the pineal gland and histological sections of this region revealed that the pineal glands were completely removed.

Statistical analysis. To minimize heterogeneity of variance of PRL means, statistical analyses of serum PRL were conducted on data transformed to natural logarithms. These transformed data were analyzed by least-squares split-plot analysis of variance (26). Logarithmic transformation was only for statistical inferences. Means and standard errors reported are untransformed. In Experiments 3 and 4, linear regression for ambient tem-

perature and photoperiod were introduced as covariates into the statistical model in an attempt to account for monthly variation in secretion of prolactin. The average temperature during sampling was used as the covariate.

Results. *Experiment 1.* Prolactin in serum of blind animals (Fig. 1) averaged 54 and 47 ng/ml ($P > 0.10$) in each group after 6 weeks of 8L:16D (Week 6). Following 6 additional weeks of either 8L:16D or 16L:8D (Week 12), serum prolactin averaged 63 and 59 ng/ml, respectively ($P > 0.10$). There was a significant increase ($P < 0.05$) in prolactin concentration for both groups between Weeks 6 and 12. Ambient temperatures increased from an average of 18.2 and 18.6°C in the two chambers at Week 6 to 20.2 and 20.6°C at Week 12. Blind bulls exposed to 8L:16D or 16L:8D released similar quantities of prolactin into serum following administration of TRH at Week 12 (Fig. 2). There was substantial variation in concentrations of prolactin among samples collected within animal at each week, but there was no obvious diurnal rhythm (Fig. 1).

Experiment 2. Prolactin in serum of sham-pinealectomized and pinealectomized steers averaged 14 and 16 ng/ml ($P > 0.10$) after 6 weeks exposure to 8L:16D (Fig. 3). Following 6 weeks of 16L:8D (Week 12), prolactin increased to 52 ng/ml ($P < 0.01$) in sham-pinealectomized steers and 30 ng/ml ($P < 0.05$) in pinealectomized steers. However, at Week 12, basal concentration of prolactin in pinealectomized steers was less ($P < 0.01$) than basal concentration in sham-pinealectomized steers. Ambient temperatures averaged 19.1 and 18.6°C in the chambers containing sham-

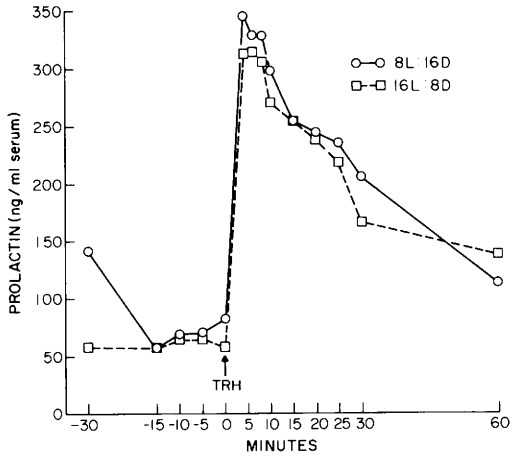

FIG. 2. Prolactin in blind bulls after iv injection of TRH (33 μg/100 kg body wt) at time 0 during the 12th week of exposure to 8L:16D ($N = 3$) or 6th week of exposure to 16L:8D ($N = 4$). Pooled SEM were 20.7 and 15.1 ng/ml, respectively.

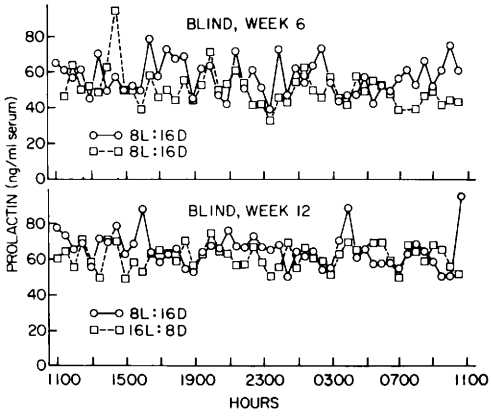

FIG. 1. Prolactin in blind bulls ($N = 7$). All bulls were exposed to 8L:16D for 6 weeks. At Week 7, one group ($N = 4$) was switched abruptly to 16L:8D for 6 weeks, and the other ($N = 3$) was maintained on 8L:16D. Blood was collected during a 24-hr interval at the end of Weeks 6 and 12. Pooled SEM were 4.5 and 5.3 ng/ml at Weeks 6 and 12, respectively.

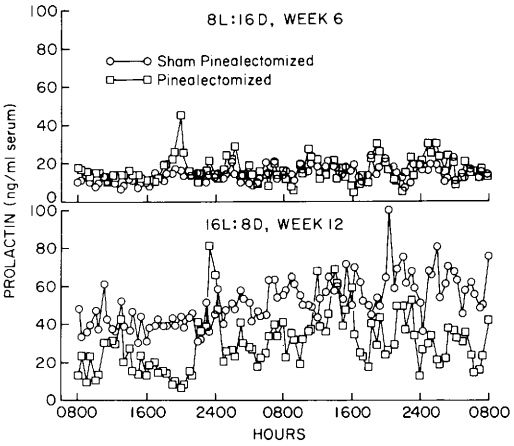

FIG. 3. Prolactin in sham-pinealectomized ($N = 3$) and pinealectomized ($N = 3$) steers during a 48-hr interval after 6 weeks exposure to 8L:16D followed by 6 weeks of 16L:8D. Pooled SEM were 0.4 and 0.7 ng/ml for each group at week 6 and 1.2 and 1.3 ng/ml at Week 12.

pinealectomized and pinealectomized animals at Week 6 and 19.8 and 19.7°C at Week 12, respectively.

TRH-induced release of prolactin after 6 weeks of 8L:16D was similar in sham-pinealectomized and pinealectomized steers (Fig. 4). For example, peak heights 6 min after TRH averaged 106 and 90 ng/ml. After 6 weeks of 16L:8D, peak height (6 min) of prolactin released after TRH was ($P < 0.05$) 435 ng/ml of serum in sham-pinealectomized steers (Fig. 5). In contrast, the peak height of TRH-induced release of prolactin (6 min) in pinealectomized steers given 16L:8D averaged 149 ng/ml. This was less ($P < 0.05$) than the peak height of sham controls, but not different ($P > 0.10$) from the quantity released during exposure to 8L:16D (Fig. 4).

Experiment 3. At every month tested, concentrations of prolactin were virtually identical in blind and sighted steers averaging overall 27 and 28 ng/ml of serum, respectively (Fig. 6; $P > 0.10$). However, prolactin in sera of blind and sighted steers varied among months ($P < 0.01$) from minimums of 11 and 9 ng/ml in December to maximums of 77 and 96 ng/ml in August, respectively. Split-plot analysis of seasonal variation in concentration of prolactin in serum of blind and sighted steers is shown in Table I. Introduction of ambient temperature and photoperiod as covariates into the model reduced the variance associated with month by 84%; nonetheless, variance associated with month remained significant ($P < 0.01$).

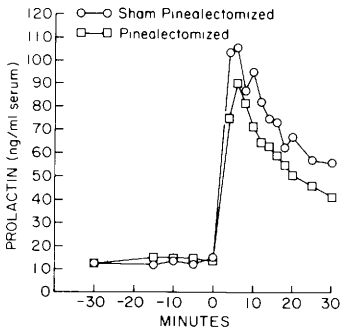

FIG. 4. Prolactin in sham-pinealectomized ($N = 3$) and pinealectomized ($N = 3$) steers after iv injection of TRH (33 μg/100 kg body wt) at time 0 during the 6th week of exposure to 8L:16D. Pooled SEM were 5.3 and 4.7 ng/ml for each group.

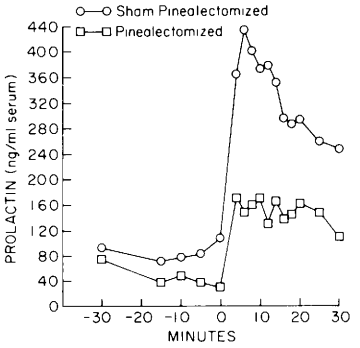

FIG. 5. Prolactin in sham-pinealectomized ($N = 3$) and pinealectomized ($N = 3$) steers after iv injection of TRH (33 μg/100 kg body wt) at time 0 during the 6th week of exposure to 16L:8D. Pooled SEM were 20.2 and 12.3 ng/ml for each group.

Experiment 4. At each month tested, concentrations of prolactin were similar in sham- and pinealectomized steers (Fig. 7); between groups, the overall averages across months were 16 and 18 ng/ml ($P > 0.10$). Prolactin in sera of sham-pinealectomized and pinealectomized steers fluctuated among months ($P < 0.01$) from minimums of 3 and 2 ng/ml in February to maximums of 45 and 54 ng/ml in June, respectively. Insertion of ambient temperature and photoperiod as covariates into the statistical model reduced variance associated with month by 98% (Table II); despite

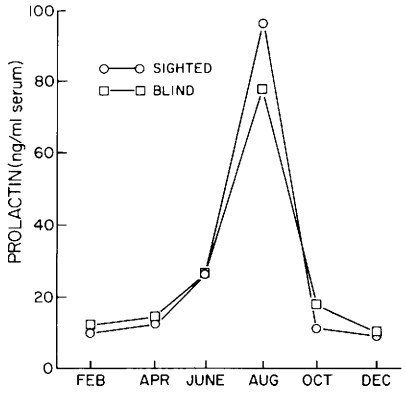

FIG. 6. Seasonal variation of prolactin in sighted ($N = 4$) and blind ($N = 4$) steers. Each point represents the mean of 36 samples. Ambient temperatures (°C) averaged −2 (Feb), 5 (April), 15 (June), 30 (Aug), 16 (Oct), and 10 (Dec) at the time of sampling.

TABLE I. SPLIT-PLOT ANALYSIS OF SEASONAL VARIATION IN CONCENTRATIONS OF PROLACTIN IN SERA OF BLIND AND SIGHTED STEERS

Source[a]	*df*	MS	*F*
Blind vs sighted (trt)	1	0.304	2.11
Error a	6	0.144	
Month	5	4.823	56.67**
Photoperiod	1	2.498	28.58**
Remainder of month	4	0.975	11.16**
Month × trt	5	0.075	0.89
Error b	30	0.085	
Temperature[b]	1	7.060	80.78**
Remainder of error b	29	0.087	

[a] Indented rows indicated further partitioning for analysis with covariates.
[b] Due to missing value, temperature was partitioned from error b instead of month.
** $P < 0.01$.

TABLE II. SPLIT-PLOT ANALYSIS OF SEASONAL VARIATION IN CONCENTRATIONS OF PROLACTIN IN SERA OF SHAM-PINEALECTOMIZED (SHAM-PX) AND PINEALECTOMIZED (PX) STEERS

Source[a]	*df*	MS	*F*
Sham-PX vs PX (trt)	1	0.034	0.09
Error a	4	0.359	
Month	5	8.368	92.27**
Temperature	1	12.672	139.71**
Photoperiod	1	4.572	50.41**
Remainder of month	3	0.303	3.34*
Month × trt	5	0.032	0.36
Error b	20	0.091	

[a] Indented rows indicate further partitioning for analysis with covariates.
* $P < 0.05$.
** $P < 0.01$.

this adjustment, variation associated with month remained significant ($P < 0.05$).

Discussion. Increasing daily light from 8 to 16 hr markedly increases basal as well as TRH-induced secretion of prolactin in sighted cattle (7, 10) and in the sham-pinealectomized steers of the present study. However, as shown in the current experiments, blinding abolishes (Figs. 1 and 2) and pinealectomy substantially reduces (Fig. 3) or abolishes (Figs. 4 and 5) the expected changes in basal or TRH-induced release of prolactin. The effect of pinealectomy in cattle supports similar observations made in pinealectomized sheep (15–17) and superior-cervical-ganglionectomized goats (14).

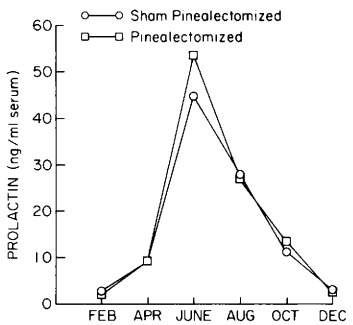

FIG. 7. Seasonal variation of prolactin in sham-pinealectomized ($N = 3$) and pinealectomized ($N = 3$) steers. Each point represents the mean of 39 samples. Ambient temperatures (°C) averaged −6 (Feb), 5 (April), 21 (June), 23 (Aug), 23 (Oct), and −3 (Dec) at the time of sampling.

Thus, blinding and pinealectomy render cattle essentially nonphotoperiodic, at least in terms of prolactin secretion.

Several authors have suggested that photoperiod is the primary climatic variable that regulates seasonal variation in secretion of prolactin in sheep and goats (5, 9, 18–20). In the present studies, however, seasonal rhythms in secretion of prolactin persisted in nonphotoperiodic blind or pinealectomized steers. Furthermore, the seasonal variations in concentrations of prolactin were not different from those of their respective controls. Similar seasonal rhythmic patterns in prolactin secretion were observed in sheep rendered nonphotoperiodic by pinealectomy or removal of sympathetic innervation of the pineal gland (14, 15, 20). In those studies, animals were pinealectomized and shortly thereafter placed on experment. However, the pineal gland seems to have the capability of programming a system for up to a year. A good example of a delayed response to pinealectomy is the ferret. Pinealectomy in October did not alter estrous onset the following spring nor the fall onset of anestrous but during the second subsequent spring or fall, these parameters were markedly affected (27). Our seasonal study with pinealectomized steers started approximately 1 year and 3 months after the pineal gland was removed. Thus, removal of the pineal gland failed to influence seasonal pattern of prolactin even in the second year after pinealectomy.

Persistency of seasonal pattern in secretion of prolactin in cattle and sheep rendered nonphotoperiodic may reflect a response to another climatic variable associated with changing seasons. Since increasing ambient temperatures increase secretion of prolactin (21) and decreasing ambient temperatures reduced the ability of 16L:8D to stimulate secretion of prolactin (22, 23), this variable would be the most obvious candidate. Indeed, in the present study, ambient temperature accounted for a large amount of the seasonal variation in secretion of prolactin (Tables I and II). However, when the data were adjusted by covariance for variation in temperature and photoperiod, significant variation in monthly secretion of prolactin persisted. We believe this constitutes evidence for the existence of an endogenous annual rhythm in the secretion of prolactin in cattle. Alternatively, persistency of seasonal variation in prolactin secretion may represent a response to an environmental variable other than ambient temperature or photoperiod. In addition, social cues from the control groups could have entrained these seasonal cycles in the blind or pinealectomized steers. For example, it is known that sighted rams will entrain blind ewes in their seasonal reproductive cycles (28).

Previously, Koprowski *et al.* (29) observed diurnal variations in secretion of prolactin in cattle with greatest concentrations at 1600 hr and lowest occurring between 0400 and 1000 hr. However, in that experiment, lighting was continuous, and ambient temperatures were not controlled. In the present studies (Experiments 1 and 2), when ambient temperatures were restricted to ±1°C on the day of blood sampling, there was no diurnal pattern in secretion of prolactin regardless of the photoperiod. This contrasts with the sharp increases noted in serum prolactin in sighted sheep when lights are initially turned off (9, 15, 16).

We conclude that blinding abolishes and pinealectomy markedly reduces the effects of photoperiod on secretion of prolactin in cattle. Ambient temperature and photoperiod account for most but not all of the seasonal variation in secretion of prolactin in steers. We hypothesize there is an endogenous annual rhythm in secretion of prolactin in cattle.

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