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Serum Luteinizing Hormone, Follicle-Stimulating Hormone, and Prolactin Response to Photoperiod in Postpubertal Holstein Heifers and Prepubertal Bulls

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SERUM LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE, AND PROLACTIN RESPONSE TO PHOTOPERIOD IN POSTPUBERTAL HOLSTEIN HEIFERS AND PREPUBERTAL BULLS

By

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ABSTRACT

SERUM LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE, AND PROLACTIN RESPONSE TO PHOTOPERIOD IN POSTPUBERTAL HOLSTEIN HEIFERS AND PREPUBERTAL BULLS

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In a first experiment the effect of photoperiod on reproduction was studied in Holstein heifers. Heifers, 14 to 18 months of age were randomly assigned to be exposed to 8L:16D or to 16L:8D photoperiods from September 21 through January 31. Serum hormone concentrations were determined from blood collected at 2-h intervals during diestrus and estrus during a single estrous cycle in autumn and again in winter.

Neither photoperiod nor season affected time of day when preovulatory surges of LH and FSH occurred. Similarly, duration and amplitude of LH surges were not affected. Peak preovulatory concentrations of LH during autumn and winter were highly correlated within individual animals ($r \simeq .90$). FSH surges during winter in heifers exposed to 8L:16D were 20% greater than in autumn. Neither photoperiod nor season affected FSH surges in heifers exposed to 16L:8D. Between autumn and winter, FSH surges

were similar in duration and amplitude within individual animals. Lengths of estrous cycles were unaffected by photoperiod or season. Prolactin (PRL) in serum was greater during estrus than diestrus in all animals irrespective of photoperiod and season.

In a second experiment, bull calves were used to determine the effects of supplemental high intensity light (hiL) in the presence of continuous low intensity light (loL) on PRL secretion. Six weeks exposure to 16-hiL:8-loL or 8-hiL:16-loL increased serum PRL 2.6 and 1.8 fold, respectively, after initial 10 wk exposure to 8-hiL:16D. Concentrations of PRL remained unchanged after subsequent 6 wk exposure to 8-hiL:16-loL or 16-hiL:8-loL, respectively.

I conclude that neither photoperiod nor season affect the preovulatory surge of LH and FSH or the rise of prolactin associated with estrus in postpubertal Holstein heifers. Furthermore, increased secretion of PRL occurs provided daily light is interrupted by 8 h of darkness or by 8 h of an intensity of light which differs from that during the 16 h interval.

To Mom and Dad - for always being there

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My feelings on the past few years of my life while at M.S.U. are expressed in the following biblical verse.

> There is an appointed time for everything, and a time for every affair under the heavens.
> A time to be born, and a time to die; a time to plant, and a time to uproot the plant.
> A time to kill, and a time to heal; a time to tear down, and a time to build.
> A time to weep, and a time to laugh; a time to mourn, and a time to dance.
> A time to scatter stones, and a time to gather them; a time to embrace, and a time to be far from embraces.
> A time to seek, and a time to lose; a time to keep, and a time to cast away.
> A time to rend, and a time to sew; a time to be silent, and a time to speak.
> A time to love, and a time to hate; a time of war, and a time of peace.[1]

To my major professor, Dr. H. Allen Tucker, I extend a heartfelt thanks for allowing me the time. I also thank Dr. Tucker for his assistance in the completion of this thesis and in particular, for his unending patience throughout my graduate program. I wish to express my gratitude to the other members of my graduate committee, Drs. R. L. Fogwell and R. K. Ringer for their support and guidance throughout my master's program.

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[1]Ecclesiastes 3:1-8

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ABBREVIATIONS

ACTH	adrenocorticotropin hormone
avg	average
C	centigrade
CL	corpus (corpora) luteum (lutea)
cm	centimeter (s)
D	dark
et al.	and others
F	fahrenheit
FSH	follicle-stimulating hormone
GNRH	gonadotropin releasing hormone
h	hour (s)
hi	high
i.e.	that is
kg	kilogram (s)
L	light
LH	luteinizing hormone
lo	low
MCR	metabolic clearance rate
mm	millimeter (s)
PAS	p-aminosalicylic acid
pg	picogram (s)
PRL	prolactin

RH	relative humidity
RIA	radioimmunoassay
TRH	thyrotropin releasing hormone
wk	week (s)

INTRODUCTION

Unlike the nonruminant species of swine and poultry, ruminants are capable of converting low quality, high fiber foodstuffs such as forages, crop residues and feed by-products to the production of meat and milk products which are highly desired by man. Because dairy cattle have the ability to convert fibrous raw materials unsuitable for consumption by humans to products of nutritional value to humans, dairy cattle are considered one of the most efficient of all livestock in converting feed protein and energy to food. As world population increases, there is a greater need to increase the production of these foodstuffs that do not compete with food supplies that can be consumed directly by humans. In addition, these animal products can be produced more efficiently through improved feeding, management, and genetic selection for milking potential in the face of reduced supplies of fossil fuels and increasing energy costs. It is the responsibility of the scientific and agricultural communities to extend the application of current agricultural methods and implement new agricultural methods to meet the food needs of the future.

Growth, reproduction and lactation are under control of the endocrine system. Elucidation of hormonal patterns during these physiological states has led to a greater understanding of factors controlling meat and milk production and reproduction. In the recent past it has been suggested that environmental factors associated with seasonal changes alter hormonal patterns during various physiological periods, and these changes may influence growth, reproduction and lactation in farm animals (Dutt, 1960; Ortavant et al., 1964; Ortavant, 1977; Ringer, 1977). The work of Bourne and Tucker (1975) and Leining et al. (1979) demonstrated that one component of season, light, was capable of altering concentrations of prolactin in serum. Peters et al. (1978) demonstrated that 16 h of daily supplemental light stimulates body growth and milk yield of dairy cattle 10 to 17% when compared with natural length photoperiods of 9 to 12 h.

The use of daily light as a practical management tool to increase meat and milk production has already been demonstrated. The question now becomes: Does altering daily light exposure affect reproduction in dairy cattle as it does in some other species (Ringer, 1977).

The objective of this thesis was to determine if photoperiods of 8L:16D and 16L:8D affected estrous cycles of postpubertal Holstein heifers. Regulation of photoperiod as a method for estrous cycle management, possibly

through synchronization of the preovulatory LH surge, was determined by characterizing changes in serum concentrations of LH, FSH, and prolactin. Because some dairymen expose their cattle to continuous daily light to facilitate nighttime management practices, a second objective was to determine if cattle are capable of responding to supplemental high intensity light in the presence of continuous low intensity light. This was done by characterizing changes in serum concentrations of prolactin.

REVIEW OF LITERATURE

Hormonal Regulation of Estrous Cycles

Estrous Cycle

Domestic cattle are polyestrus and therefore capable of breeding throughout the year (Asdell, 1964). Lengths of estrous cycles vary within and among animals ranging from 18 to 24 days (avg. = 21 days). Overall, lengths of estrous cycles are shorter in heifers compared with cows. Lengths of estrous cycles average 20 days in heifers (Rajakoski, 1960; Asdell, 1964; Desjardins and Hafs, 1968; Morrow, 1969) and 21 days in cows (Asdell, 1964).

The major behavioral event of an estrous cycle is estrus. Heat or estrus comes from the Latin oestrus meaning possessed by the gadfly, insane, in a frenzy (Nalbandov, 1976). It is at this time that the greatest increase in size of the ovulatory follicle occurs. The physiological manifestations of estrus are attributed to the female sex hormone, estrogen, which is produced by developing ovarian follicles. The primary sign of estrus is standing when mounted by other animals. Thus it is only during estrus that females are sexually receptive to the males. Other signs of estrus include restlessness, bellowing, swollen and reddened vulva, vaginal discharge,

and attempting to mount other animals. In some instances a so-called "silent estrus" occurs. A female that has a silent estrus does not exhibit the behavioral signs of estrus or signs of estrus which occur are not detected. Changes in vaginal and uterine histology and glandular secretions, follicular development, and hormonal patterns that correspond with behavioral signs of estrus are also present during silent estrus (Nalbandov, 1976).

Rottensten (1957) established that there are differences among heifers in the degree of expression of estrus. Heifers conceiving on first service had a greater expression of behavioral manifestations of estrus than those that failed to conceive. Generally duration of estrus is slightly shorter for heifers than for cows. Trimberger (1948) reported the average duration of estrus was 15.3 h in heifers and 17.8 h in cows. Hall et al. (1959) suggested that a shortened duration of estrus in heifers and cows of 11.9 h may be associated with high ambient temperatures in subtropical environments.

Estrous cycles may be divided into proestrus, estrus, metestrus, and diestrus stages. Proestrus is 2 to 3 days in length and is a period of rapid follicular growth. There is an increase in blood flow to ovaries and other reproductive organs. This increase in blood supply is responsible for swelling of the vulva and reddening of the vestibule which occurs at the end of proestrus. Estrus,

as described previously, is the time that females are receptive to males. The most rapid follicular growth followed by maturation of the ovulatory follicle also occurs during estrus. Metestrus begins with the end of standing heat and is approximately 3 days in length. Ovulation occurs and luteal development begins during this phase. Ovulation has been reported to occur between 2½ to 22 h after the end of estrus (Nalbandov, 1976). Ovulation occurred an average of 11 to 13 h and 12.3 h after the end of estrus as reported by Salisbury et al. (1978) and Hansel and Trimberger (1952), respectively. Nalbandov and Casida (1942) observed that ovulation occurred 14.2 h after estrus. Metestrus bleeding, due to diapedesis and associated with some destruction of the endometrial epithelium in the intercaruncular areas, occurs within 48 h after the end of heat or 15 to 20 h after ovulation (Nalbandov, 1976). These findings agree with those demonstrated by Hansel and Asdell (1952) and Trimberger (1941). Generally, heifers show signs of metestrus bleeding more often than cows. Metestrus bleeding is a good indication that a heifer was in heat 2 to 3 days earlier. It is during diestrus that the CL becomes fully developed and functional. This phase of a cycle extends over a 12 to 14 day interval and is dominated by secretion of progesterone (luteal phase of estrous cycle). There is a thickening of the endometrium of the

uterus and an increase in endometrial glands in preparation for implantation and pregnancy. If pregnancy does not occur, the CL regresses and a new estrous cycle begins.

Luteinizing Hormone

The development of a specific assay for LH (based on ovarian ascorbic acid depletion) hastened the quantification of LH in the pituitary during estrous cycles (Karg, 1957; Parlow, 1961). Sensitivity of this assay was limited, but it distinguished between the activity of LH and total gonadotropin activity.

The time of rapid degranulation of the PAS-positive delta cells in the pituitary is variable but usually occurs around the onset of estrus (Jubb and McEntee, 1955; Hansel et al., 1968). Degranulation of these cells is coincident with a decrease in content of LH in the pituitary around the time of estrus and a corresponding increase in plasma LH on the day of estrus (Anderson and McShan, 1966). Rakha and Robertson (1965) reported a 61% decrease in the content of LH in the pituitary gland between the onset and end of estrus. In agreement with these findings, Hackett and Hafs (1969) observed a 71% decrease occurring between day 20 and the day of estrus and a further 61% decrease occurring between estrus and day 2.

LH levels in the blood are highest around the time of estrus (Niswender et al., Hansel and Snook, 1970;

Hendricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). A linear increase of 0.1 ng/ml plasma LH from 6 days before peak concentrations to 1.06 ng/ml at 8 h preceding peak concentrations of LH (Chenault et al., 1975) were similar to data previously reported by Swanson and Hafs (1971). Peak preovulatory concentrations of LH are variable ranging from 7 to 60 ng/ml (Niswender et al., 1969; Snook et al., 1971; Swanson and Hafs, 1971; Hansel and Echternkamp, 1972; Chenault et al., 1975). Preovulatory surges of LH remained elevated above baseline values of 1.4 (Swanson and Hafs, 1971) and .85±.27 ng/ml (Chenault et al., 1975) for 8 and 10.5±.8 h, respectively.

Snook et al. (1971) reported a small luteal phase rise in basal concentrations of LH occurred in all of seven heifers on days 9 to 13, with highest concentrations during this period ranging from 2.3 to 8.2 (avg. = 4.6 ng/ml). A subsequent secondary rise in basal concentrations of LH ranging from 3.5 to 7.8 (avg. = 5.3 ng/ml) occurred 4 to 7 days prior to ovulation in five of the same animals. The occurrence of luteal phase rises in LH is in agreement with the previous work of Hansel and Snook (1970) and Shams and Karg (1969) and is associated with the growth of anovulatory midcycle follicles observed by Rajakoski (1960).

Follicle-Stimulating Hormone

Knowledge of endocrine profiles for bovine FSH is limited and reports of hormonal concentrations and patterns during estrous cycles vary depending on method of hormonal analysis. A 27% decrease in the content of FSH in the pituitary between the onset and end of estrus was reported by Rakha and Robertson (1965). Desjardins and Hafs (1968) reported a decrease in content of FSH in the pituitary at this same time. The decrease in the quantities of FSH in the pituitary coincide with high blood levels around estrus (Schams and Schallenberger, 1976). Hackett and Hafs (1969) reported a 49% decrease in levels of FSH in the pituitary between day 18 and 20 with a subsequent 46% reduction occurring by day of estrus.

Decreases in concentrations of FSH in the pituitary have also been observed during the luteal phase of the estrous cycle. Desjardins and Hafs (1968) demonstrated a decrease in levels of FSH in the pituitary (relative to day of estrus) as midcycle (day 6 to 9) approached. FSH concentrations in the pituitary gland were restored by day 10 to 13. A subsequent 27 to 37% decrease in FSH content of the pituitary was observed during the week (day 14 to 21) before next anticipated estrus. The biphasic release of FSH from the pituitary soon after ovulation and during midcycle is associated with the two waves of follicular growth described by Rajakoski (1960).

A heterologous radioimmunoassay for bovine FSH was developed by Akbar et al. (1974), Schams and Schallenberger (1976) and Dobson (1978a), and more recently a highly sensitive and specific homologous radioimmunoassay for cattle was developed by Cheng (1978). Serum FSH of cows in luteal and follicular phases of the estrous cycle ranged from 26 to 106 (avg. = 64) and 44 to 100 (avg. = 66) ng/ml, respectively. Mean concentrations on the day of estrus, 78 (range 60 to 106) ng/ml, were not significantly different from values measured at the other stages of the estrous cycle (Akbar et al., 1974). In contrast, Cheng (1978) reported a 2 to 3 fold increase in concentrations of FSH at estrus. Concentrations of FSH at estrus averaged 58.4±12.6 ng/ml compared with 25.4±4.7 ng/ml in cows not in estrus. Akbar et al. (1974) did, however, report a significant increase in peak concentrations of FSH coincident with peak concentrations of LH when heifers were sampled at 2 h intervals around estrus. Peak concentrations of FSH coincident with peak concentrations of LH have also been reported by Kaltenbach et al. (1974) and Dobson (1978b). According to Akbar et al. (1974), Schams and Schallenberger (1976), Dobson (1978b) and Roche and Ireland (1981) duration of the FSH surge that occurs around estrus ranges from 8 to 30 h.

In addition to the surge of FSH observed around estrus, Schams and Schallenberger (1976) observed a

rhythmic pattern of elevated concentrations of FSH occurring at 4-day intervals after ovulation. Peak concentrations of FSH 8 days after and 3 days before ovulation were coincident with significant increases in concentrations of LH. Concentrations of FSH 16 to 30 h after the preovulatory peak of LH were increased compared with the period from 4 to 15 h (Dobson, 1978b). Roche and Ireland (1981) observed a similar increase in levels of FSH following the surge of LH. These elevations in concentrations of FSH in the blood occur around the same time as the decrease in pituitary levels of FSH cited earlier and may be involved in follicular growth (Rajakoski, 1960).

Prolactin

The function of prolactin (PRL) in reproduction of cattle has not been established. It does, however, appear that secretion of PRL is greater during estrus than other phases of the estrous cycle in heifers (Swanson and Hafs, 1971; Raud et al., 1971; Swanson et al., 1972) but does not change during different phases of the estrous cycle in cows (Schams and Karg, 1970; Edgerton and Hafs, 1973; Koprowski and Tucker, 1973).

Swanson and Hafs (1971) reported that concentrations of PRL averaged 8±2 ng/ml 4 days prior to peak concentrations of 17±2 ng/ml on the day of estrus and then declined to 13±4 ng/ml 2 days following estrus. Peak concentrations of serum PRL averaged 46 ng/ml at estrus

and decreased to 35 ng/ml during metestrus in Holstein heifers (Swanson et al., 1972). Concentrations of PRL during most of diestrus were not different from those during metestrus and were lowest (20 ng/ml) 3 days prior to estrus. Raud et al. (1971) also observed peak concentrations of PRL occurred during proestrus and estrus in dairy heifers. The decrease in concentrations of PRL in the pituitary gland at estrus through day 2 of the estrous cycle observed by Sinha and Tucker (1969) may reflect increases in blood levels of PRL observed around estrus. Day of the estrous cycle did not affect the response in PRL to thyrotropin releasing hormone (TRH) administration in heifers although the PRL response at days 2 and 4 tended to be less than at other stages of the estrous cycle (Vines et al., 1977).

Edgerton and Hafs (1973) reported no difference between mean concentrations of prolactin ranging from 49±10 ng/ml, on the day of estrus, to 74±10 ng/ml on day 18 of the estrous cycle in lactating Holstein cows. These results are similar to those observed by Koprowski and Tucker (1973). Shams and Karg (1970) also found no changes in PRL during the estrous cycle of lactating and non-lactating cows.

Estrogen

Estrogens are secreted by developing ovarian follicles and are responsible for the behavioral manifestations of estrus. Prior to the discovery of a radioimmunoassay for estrogens, estrogenic activity had been detected in the urine of cycling cows by bioassay and fluorimetry. Mellin and Erb (1966) report two distinct peaks of urinary estrogen excretion. The largest peak occurred during the last three days of an estrous cycle and declined rapidly to pre-peak levels by day 1 of the next estrous cycle. A second smaller peak was observed during days 6 to 11. Varman et al. (1964) also observed a peak in estrogen in urine on days 6 to 8 and 18 to 20 after estrus. The reader is referred to Mellin and Erb (1965) for an extensive review of early literature reports on estrogenic activity during the bovine estrous cycle.

The advent of radioimmunoassays (RIA) for the quantification of estrogens in plasma or serum of blood has led to similar reports of estrogen secretory patterns during estrous cycles in cattle. However, reported concentrations of estradiol in bovine peripheral plasma are varied depending on the method of analyses. Peterson et al. (1975) suggests the differences may be due to interfering substances and that chromatography overcomes this interference.

Baseline concentrations of estrogens remain low throughout the luteal phase of the estrous cycle ranging from 2 to 10 pg/ml (Hendricks et al., 1971; Wettemann et al., 1972; Kanchev et al., 1976). A rise in basal concentrations of estradiol-17β ranging from 4 to 12 pg/ml was observed between days 4 to 8 (avg. = day 6) of an estrous cycle (Peterson et al., 1975; Shemesh et al., 1972; Glencross et al., 1973; Smith et al., 1975). Shemesh et al. (1972) and Kanchev et al. (1976) observed an increase in concentrations of estradiol of 8.1±3.6 and 10.4±3.9 pg/ml on days 11 and 14 of the estrous cycle, respectively. Although early and mid-luteal increases in estrogens have not been observed by all authors, it is believed, but not substantiated, that these luteal phase increases in estrogens may be associated with the development and regression of midcycle follicles as observed by Rajakoski (1960).

It has been reported that peak concentrations of estrogens occur before, during, and after estrus. An increase in blood levels of estradiol occurs 4 to 5 days prior to estrus (Wettemann et al., 1972; Hansel and Echternkemp, 1972; Chenault et al., 1975; Kanchev et al., 1976). Peterson et al. (1975) reported peak concentrations of estradiol-17β of approximately 15 pg/ml occur the day before estrus whereas Glencross et al. (1973) reported peak concentrations of 5 to 7 pg/ml. Wettemann et al.

(1972) reported peak estradiol concentrations of 9.7 pg/ml 0.5 days before estrus. Peak concentrations of estradiol between 11 and 14 pg/ml on the day of estrus were observed by Hansel and Echternkemp (1972), Dobson and Dean (1974) and Kanchev et al. (1976). Concentrations of estradiol decreased 50% within 1 to 3 h after the onset of estrus and returned to a baseline of 2 pg/ml by 14 h post-estrus (Chenault et al., 1975). A rapid decrease in concentrations of estrogens after estrus has been confirmed by Hendricks et al. (1971) and Dobson (1978b). The decline in behavioral manifestations of estrus is coincident with the decrease in estrogen concentrations.

Progesterone

Progesterone was first identified in the bovine CL by Edgar (1953). Since then several other progestational agents, progestins, have been isolated from other tissues and organs. The reader is referred to Gomes and Erb (1965) for an extensive review of progestins in bovine reproduction.

Throughout estrous cycles it is possible to generalize that the weights of the corpora lutea (CL) and content of progesterone, synthesis, and secretion are closely related to the degree of luteinization as determined by histologic studies (Cupps et al., 1959; Mares et al., 1962; Gomes et al., 1963). Mares et al. (1962) reported

concentrations of progesterone and 20β-hydroxy-pregn-4-en-3-one (20β-ol) increased from day 7 to day 15 and then decreased at day 17. Progesterone made up the major portion of progestogen concentrations with 20β-ol being found only on day 13 and 15. Total progestogen content was greatest on day 15. This study is in agreement with results reported by Erb and Stormshak (1961) and Gomes et al. (1963) of peak concentrations of progestins at days 14 to 16 and day 14, respectively. Peak levels of progesterone in peripheral blood were 7.0 to 10 ng/ml on days 14 to 16 of the estrous cycle (Pope et al., 1969; Stabenfeldt et al., 1969).

The CL rapidly regresses in size prior to estrus, coincident with a precipitous decline in progesterone. The time at which this occurs varies among individual animals. Hansel and Snook (1970) reported CL regression to occur as early as day 15 or as late as day 19 in estrous cycles of 20 to 23 days in length. The combined data of Gomes et al. (1963), Hafs and Armstrong (1968), and Pope et al. (1969) report that regression occurs on day 18. Concentrations of progesterone in corpora lutea on day 18 are 30 to 40% of peak values on days 11 to 14 of an estrous cycle (Hafs and Armstrong, 1968; Gomes et al., 1963). Coincident with the decrease in concentrations of progesterone in corpora lutea is a decrease in levels of progesterone in peripheral blood. Concentrations of progesterone in blood are

<0.5 ng/ml or are nondetectable during the period of estrus (Gomes et al., 1963; Hendricks, 1970; Kazama et al., 1970).

Environmental Regulation of Hormones

The evolution of endogenous biological rhythms enable animals to anticipate changes in their natural habitat and thus make the appropriate physiological and/or behavioral modifications necessary to insure their survival (Ringer, 1977). Although ambient temperature, humidity, rainfall, and food availability are important cues to seasonal changes, they vary from year to year and thus are only secondary sources of stimuli used by animals to coordinate endogenous biological rhythms with season. Daily photoperiod is the only environmental factor that remains the same from year to year. It enables animals to anticipate changes in season prior to their occurrence and then synchronize biological processes with environment. For example, horses (Loy, 1968; Sharp and Ginther, 1975; Tucker and Oxender, 1980), sheep (Yeates, 1956; Ducker and Bowman, 1972) and goats (Asdell, 1964; Shelton, 1978) respond to seasonal changes in daily photoperiod for initiation and cessation of reproductive cyclicity. Evidence of a photoperiod effect on reproduction in cattle, however, is scarce (Tucker and Oxender, 1980).

Since environmental factors may stimulate hypothalamic neurons which in turn release neurohormones involved in the synthesis and release of anterior pituitary hormones (Convey, 1973), the following discussion addresses those environmental factors which influence concentrations of hormones in serum. Major emphasis will be on seasonal effects, mainly ambient temperature and photoperiod.

Luteinizing Hormone

Research concerning environmental factors affecting LH secretion in cattle is limited. In dairy heifers, exposure to 18.2C and 55% relative humidity (RH) for two consecutive estrous cycles followed by exposure to 33.5 C and 55% RH for an additional two estrous cycles reduced baseline and peak preovulatory concentrations of LH from 2.15 and 61 ng/ml to 1.25 and 45 ng/ml, respectively (Madan and Johnson, 1973). The decrease in concentrations of LH may be due to an increase in plasma progesterone throughout the third estrous cycle and during the first 8 days of the fourth estrous cycle (Abilay et al., 1975). They did not report whether or not the heifers ovulated. Miller and Alliston (1974) also observed a decrease in baseline and peak preovulatory LH concentrations in beef heifers subjected to heat stress. Although all animals ovulated, failure to detect surges of LH in five of eight heat stressed heifers may be due to the infrequent sampling

times (only at 0800 and 1600 h) on the day of estrus (Miller and Alliston, 1974).

The decrease in LH observed during heat stress may be associated with an increase in adrenal progesterone secretion resulting from increased adrenocorticotropin (ACTH) secretion in response to heat stress (Wagner et al., 1972; Abilay et al., 1975; Johnson and Vanjonack, 1976). The negative feedback effect of progesterone on ovarian activity might be responsible for anovulatory periods of estrus and anestrus in cattle (Plasse et al., 1970; Bond and McDowell, 1972).

Evidence of a seasonal or photoperiodic effect on LH secretion in cattle is sparse. Increased frequency of LH peaks coincident with the longest natural daily photoperiod was observed in Holstein bulls, steers and short scrotum bulls at 4 months of age, however, this evidence of a seasonal effect on LH secretion was confounded with age (McCarthy, 1978; McCarthy et al., 1979). Although average concentration of LH did not differ with time on experiment, or duration, or wavelength of daily light in bull calves, increased frequency of episodic releases was observed but these data were confounded with age (Leining, 1978). Bourne and Tucker (1975) observed that photoperiod had no effect on concentrations of LH in serum of prepubertal bull calves.

In prepubertal Holstein heifers, baseline concentrations of LH did not differ with length of daily light (Goodman, unpublished). However, heifers exposed to 16L:8D showed a 40% greater response to gonadotropin releasing hormone (GnRH) injections than heifers exposed to 8L:16D photoperiod (Goodman, unpublished).

At the present time there is no evidence that season or daily duration of exposure to light affects secretion of LH in prepubertal bull calves or during estrous cycles in cattle.

Progesterone

Evidence of environmental factors regulating progesterone secretion in cattle is also limited primarily to the effects of ambient temperatures. Heat stress in heifers and cows increases adrenal progesterone in response to increased ACTH secretion (Gwazdauskas et al., 1972; Wiersma and Stott, 1969; Wagner et al., 1972).

In Guernsey heifers, exposure to 18.2 C and 55% RH for two consecutive estrous cycles followed by exposure to 33.5 C and 55% RH during a third and fourth estrous cycle increased mean plasma progesterone throughout the third estrous cycle and during the first 8 days of the fourth estrous cycle (Abilay et al., 1975). Plasma progesterone concentrations for the remainder of the fourth estrous cycle were not different from concentrations during the

first two estrous cycles when heifers were exposed to 18.2 C and 55% RH. Gwazdauskas et al. (1973) also observed increases in progesterone in cows exposed to high ambient temperatures during the summer in Florida.

In contrast to the work of previous authors, Rosenberg et al. (1977) observed a reduction in concentrations of plasma progesterone throughout the luteal phase of the estrous cycle in cows exposed to summer ambient temperatures in Israel (average maximum temperature of 29-32 C) as compared with winter ambient temperatures (average maximum temperature of 18-26 C). In contrast, Rhodes and Randel (1980) observed an increase in total progesterone content of the CL in beef heifers in the winter as compared with summer in Texas.

The work of Trimberger and Hansel (1955) indicates that high daily progesterone concentrations administered from midcycle onward are capable of delaying both estrus and ovulation in cattle. Thatcher (1974) suggests that elevated plasma progestins may cause an inbalance between progesterone and estrogen and that secretion of LH may be altered during heat stress (Madan and Johnson, 1973; Miller and Alliston, 1974). Indeed, these alterations in response to heat stress may be responsible for shortening the duration of estrus (Hall et al., 1959; Gangwar et al., 1965), reducing intensity of estrus (Gangwar et al., 1965), and increasing incidence of silent estrus (Labhsetwar

et al., 1963; Plasse et al., 1970) and anestrus (Bond and McDowell, 1972).

Prolactin

Concentrations of serum PRL are highest during summer and lowest during winter in cattle (Shams, 1972; Koprowski and Tucker, 1973), sheep (Ravault, 1976) and goats (Buttle, 1974; Hart, 1975). Dular and LaBella (1977) observed that concentrations of PRL in bovine pituitary tissue slices, in addition to release of PRL from pituitary tissue cultured in vitro varied seasonally and was parallel with seasonal variations observed in vivo.

Average concentrations of PRL during the month in which birth occurred (blood was collected within 3 days of birth and thrice weekly thereafter for two years) were greater in calves born in May or June in comparison with calves born in October and November in Germany (Schams and Reinhardt, 1974). Lacroix et al. (1977) report no difference in concentrations of plasma PRL at birth (blood was collected once per week from one week after birth through week 52) for bull calves whatever the month of birth in France. However, they did observe a decrease in concentrations of PRL in winter-born calves and an increase in concentrations of PRL in spring-born calves by the time they reached one month of age. These data suggest that the mechanism whereby PRL secretion responds to seasonal

changes in temperature is established shortly after birth in calves. Baseline concentrations of PRL in serum of bulls ranging from 10 to 160 months of age were more than two fold greater in serum collected in July as compared with serum collected in January, and concentrations of PRL were not affected by age (Tucker et al., 1974).

Although serum PRL was higher during April to September (74 ng/ml) than during October to March (35 ng/ml) in lactating Holstein cows, PRL release in response to milking was lower (19 ng/ml) in the summer than in the winter months (33 ng/ml) and paralleled changes in milk yield (Koprowski and Tucker, 1973). These observations are supported by data from Peters et al. (1981) who demonstrated that the milking-induced release of PRL was not correlated with ambient temperatures from October to March in Holstein cows exposed to various photoperiods and that cows in early lactation release 2.4 times more PRL after milking than those in late lactation. PRL release in response to TRH was 6 to 16 times greater in summer than in spring or fall in pregnant heifers and 3 times greater in spring, summer, and fall than during winter in lactating cows (Vines et al., 1977). Although concentrations of PRL tended to be greater with advancing pregnancy in heifers, but not in cows, there appears to be a seasonal trend in release of PRL in response to TRH for all animals. These data suggest that season of the

year affects capacity of the anterior pituitary to release PRL and is in agreement with the *in vitro* work of Dular and LaBella (1977). Dular and LaBella suggest that seasonal factors affect primarily the synthesis and storage of PRL and that the seasonal pattern in blood levels of PRL are directly related to pituitary and secretory granule content of PRL.

Ambient temperature varies throughout the year and appears to be involved in the seasonality of PRL secretion in cattle. Serum PRL in heifers maintained on 12L:12D photoperiod increased from 8 to 22 ng/ml during a 3-h interval when ambient temperature was increased from 21 to 27 C, whereas serum PRL decreased from 13 to 4 ng/ml during a 4-h interval when ambient temperature was reduced from 21 to 10 C (Wettemann and Tucker, 1974). Chronic exposure to 10 C for 5 days reduced serum PRL 38% as compared with a control period at 21 C whereas 5 days of exposure to 27 C increased serum PRL 200% above the control period of 21 C. When heifers were injected with TRH, serum PRL increased from 20 to 140 ng/ml when ambient temperatures were 27 C. PRL increased from 8 to 70 ng/ml after TRH when heifers were at 10 C. A reduction in metabolic clearance rate (MCR) as well as an increase in the secretion rate of PRL was observed in steers when ambient temperature was increased from 10 to 30 C (Smith *et al.*, 1977).

Serum PRL averaged 2.6, 13.0 and 27.7 ng/ml in heifers exposed to 4.5, 21 or 32 C, respectively (Tucker and Wettemann, 1976). Serum PRL decreased linearly (.6 ng/ml/C) as temperature was reduced from 21 to 4.5 C. Conversely, a linear increase (1.17 ng/ml/C) was observed when ambient temperature was increased from 21 to 32 C. Following TRH a 5.4-fold increase in serum PRL was observed at 32 C and a 4-fold increase reported at 21 C. However, heifers exposed to 4.5 C did not respond to TRH injections. Similarly, Peters and Tucker (1978) and Peters et al. (1980) reported that photoperiod had no effect on concentrations of PRL in serum of heifers when ambient temperatures were below 0 C.

Duration of daily light, photoperiod, is a primary component of season capable of altering secretion of PRL in sheep (Pelletier, 1973; Forbes et al., 1975; Lincoln et al., 1978) and cattle (Bourne and Tucker, 1975; Peters and Tucker, 1978; Leining et al., 1979). Bull calves subjected to a decreasing daily illumination from 16 to 8 h reduced serum PRL from 57 to 8 ng/ml (Bourne and Tucker, 1975). Conversely, calves subjected to increasing daily photoperiod from 8 to 16 h increased PRL from 25 to 100 ng/ml.

In a series of experiments, Peters and Tucker (1978) investigated the effects of increased daily photoperiod during different seasons of the year. Serum PRL in

Holstein heifers exposed to 16L:8D from November 11 to March 9 averaged 42 ng/ml as compared with 11 ng/ml in heifers exposed to a natural length photoperiod of 10 to 11.6 h during the same period. Heifers exposed to 16L:8D photoperiod between April 30 and August 13 averaged 78 ng/ml in comparison with 48 ng/ml for heifers exposed to natural length photoperiods ranging from 13.6 to 15.3 h.

Exposure to 8L:16D for two weeks followed by a 0.38 h daily increase in photoperiod until continuous illumination (24L:0D) was attained increased serum PRL 3-fold in bull calves (Leining et al., 1979). However, serum PRL decreased within 1 week of exposure to 24L:0D and subsequently was not different than concentrations reported after exposure to 8L:16D. In another experiment, Leining et al. (1979) determined that 16 and 20 h of daily light are equally effective in increasing basal PRL over concentrations found during 8L:16D photoperiods.

Bunning (1960) hypothesized the existence of a circadian rhythm of photosensitivity with two distinct periods each day. During the first period of the circadian rhythm the animal is insensitive to light while during the second period the animal becomes sensitive to the light and somehow responds. Whether or not the animal responds depends on the position of the light relative to the circadian rhythm of photosensitivity. Thus, it should be possible to elicit an increase in PRL secretion if animals

are exposed to light during the photosensitive period. Indeed, Ravault and Ortavant (1977) demonstrated that a photoperiod of 7L:9D:1L:7D stimulated prolactin secretion in rams whereas insertion of the 1 h burst of light at 11, 14 and 20 h after dawn did not. More recently, Petitclerc et al. (1980) demonstrated a photosensitive phase in cattle. Specifically, two groups of bull calves were exposed to 8L:16D for 6 weeks followed by 16L:8D or 6L:8D:2L:8D for an additional 6 weeks. The 2-h pulse of light between 14 and 16 h after subjective dawn (subjective dawn is represented by the beginning of each light interval, i.e. 8-, 16-, and 6-h, respectively) in the 6L:8D:2L:8D photoperiod was hypothesized to be the photosensitive phase. In this experiment, serum PRL averaged 8.3 and 8.5 ng/ml after exposure to 8L:16D and increased to 42.0 and 37.3 ng/ml after exposure to subsequent photoperiods of 16L:8D or 6L:8D:2L:8D, respectively.

Further evidence of a photosensitive phase for PRL secretion between 20 and 22 h after subjective dawn was investigated in a second experiment. Bull calves were exposed to 8L:16D for 6 weeks followed by 6L:8D:2L:8D or 6L:14D:2L:2D photoperiods through week 12. Serum PRL averaged 13.0 and 11.4 ng/ml after exposure to 8L:16D. Serum PRL increased to 49.0 and 22.5 ng/ml after exposure to 6 weeks of 6L:8D:2L:8D or 6L:14D:2L:2D, respectively. Thus, it appears that a photosensitive period for the

secretion of prolactin in cattle exists, and maximum response occurs 14-16 h after dawn.

Little is known about the effect of wavelength and intensity of light on the secretion of PRL in cattle. Leining et al. (1979) determined that exposure of bull calves to 8 h of cool-white fluorescent light plus 8 h of blue or red fluorescent light was as effective in stimulating PRL secretion as providing 16 h of fluorescent light. It has also been determined that intensities of light ranging from 102 to 600 lux are sufficient to alter concentrations of PRL in serum of cattle (Bourne and Tucker, 1975; Peters and Tucker, 1978; Peters et al., 1980).

Serum PRL increased 15-fold in prepubertal bull calves exposed to 16L:8D of high intensity (540 lux) light after initial exposure to 16L:8D of low intensity (22 lux) light whereas concentrations of PRL in bulls exposed to the alternate sequence of light intensities remained the same (Leining, 1978). Intensity of light did not influence secretion of PRL in response to TRH injections. Leining suggests that the sequence of exposure to different intensities of light may be important in terms of pituitary responsiveness and PRL secretion because bulls exposed to 540 lux of light after initial exposure to 22 lux of light had a greater PRL response to TRH than bulls receiving the alternate sequence of intensities.

A 2.4-fold increase in concentrations of serum PRL was observed in two groups of Holstein heifers exposed to either 8L:16D of 22 lux of light followed by 16L:8D of 540 lux of light or to the alternate sequence of light intensities (Goodman, unpublished). Evidence suggests that light intensities as low as 22 lux stimulate PRL secretion in cattle similar to light intensities of 540 lux.

Other Hormones

At present, literature on the effects of environmental factors on secretion of FSH or estradiol in cattle are not available. One might speculate that factors affecting secretion of progesterone and LH may affect the secretion of FSH and/or estradiol. Increased secretion of progesterone during hot weather may reduce or block secretion of FSH and estradiol which in turn may shorten the duration and/or intensity of estrus. This has yet to be determined. Obviously more research is needed before we can clearly understand the effects of environment on reproduction in cattle.

Environmental Regulation of Estrous Cycles

Although cattle are polyestrus there appear to be environmental factors (photoperiod, climate, nutrition and season) that may directly or indirectly affect the

periodicity of bovine estrous cycles (Dutt, 1960; Hafez, 1965, 1967).

Anderson (1936) observed that mean duration of estrus was shorter in Zebu cattle grazing pastures of poor nutritive value in Kenya as compared with cattle receiving an energy and protein supplement, but lengths of estrous cycles were not affected. Anderson (1944) also observed a decrease in duration of estrus and lengths of estrous cycles when ambient temperature and duration of daily sunshine increased and vice versa. Duration of estrus was shorter in April (10.6 h) and May (10.8 h) than in September (13.9 h) and December (13.7 h) in cattle exposed to subtropical climatic conditions in Louisiana, however, lengths of estrous cycles were unchanged (Hall et al., 1959).

Research indicates that high ambient temperatures and relative humidity increases the length of estrous cycles and decreases duration of estrus in cattle. Holstein heifers exposed to cool climatic conditions (62 to 65 F) for 60 days and then to controlled, cycled hot temperatures (animals were subjected to 4, 6, 6 and 8 h of 85, 95, 85 and 75 F temperatures, respectively, in a 24 h period) for an additional 60 days had an average estrous cycle length and mean duration of estrus of 20 and 25 days and 20 and 11 h, respectively (Gangwar et al., 1965). Abilay et al. (1975) reported similar results for Guernsey heifers

subjected to 33.5 C and 55% RH for a duration of two estrous cycles after initial exposure to 18.2 C and 55% RH. Because lactating cows produce more body heat than non-lactating cows their ability to dissipate increased body heat associated with high ambient temperatures is reduced. Therefore, lactating cows are more susceptible to the effects of heat stress (Thatcher, 1974). Wolf and Montry (1974) reported a decrease in duration of estrus in lactating cows exposed to ambient temperatures (range from 34 to 41 C) during the summer in Arizona but not in non-lactating cows.

Five of six Hereford heifers became anestrus within 34 days of exposure to 32 C and 60% RH after initial exposure to ambient temperatures (avg. = 1.2 C) during the winter in Maryland; however, animals became acclimated to high temperatures and normal estrous cycles were reestablished after 112 days (Bond and McDowell, 1972). These data suggest reduced production and/or release of gonadotrophic hormones after exposure to chronic heat stress but reestablishment of normal function occurs after an adaptation period. In another group of animals, five of six Angus heifers exposed to 32 C and 60% RH after previous exposure to summer ambient temperatures (avg. = 24.4 C) continued to have estrous cycles of normal length. These data suggest that the effects of heat stress on lengths of estrous cycles in cattle conditioned to high

ambient temperatures are not as severe as compared with animals conditioned to cold ambient temperatures. However, in a subsequent experiment the following year, exposure to 38 C and 60% RH caused anestrus in five of six Angus heifers previously conditioned to ambient temperatures during the summer in Maryland (Bond and McDowell, 1972). In contrast to the effects of high ambient temperatures on lengths of estrous cycles, heat tolerant Brahman heifers have a greater incidence of quiet ovulations during the winter than summer in Florida (Plasse et al., 1970).

The increase in plasma progesterone (Abilay et al., 1975) and decrease in plasma LH (Madan and Johnson, 1973) during the estrous cycle in heat-stressed cattle suggest an imbalance in normal hypothalamic-pituitary ovarian activity. Bond and McDowell (1972) reported inactive ovaries (based on rectal palpation of ovarian follicles and corpora lutea) in heifers that became anestrus after exposure to high ambient temperatures. Of the total number of estrous cycles during the summer in Brahman heifers, there was a significant increase (5.4%) in anovulatory periods of estrus during summer as compared with winter (Plasse et al., 1970). Serial sectioning of ovaries at the time of slaughter indicated that total number of follicles ($\geq$ 1 mm in diameter) varied with season with the lowest mean total of 73.1 follicles per heifer during the autumn and 118.5

and 116.5 follicles per heifer during the winter and spring, respectively (Rajakoski, 1960).

MATERIALS AND METHODS

Animals

Eighteen postpubertal Holstein heifers, ranging from 14 to 18 months of age and from 271 to 445 kg body weight at the beginning of the experiment were used in a first experiment. Heifers were paired for line of selection for genetic differences in milk yield and randomly assigned to one of two pens in an enclosed barn and maintained under loose housing conditions. Each pen of heifers was fed as a group and on the average each heifer was fed 9 kg of a protein-energy concentrate, and 5 kg of alfalfa hay per day. Trace mineralized salt and water were available ad libitum. Heifers were fitted with K-Mar heat detection patches (Kamar Inc., Colo.), housed with androgenized cows and observed for signs of estrus thrice daily.

In a second experiment, eight Holstein bulls, ranging from 2 to 5 days of age at the beginning of the experiment, were used. Bulls were housed individually within a room in which daily lighting and ventilation were controlled. Bulls were fed milk, calf starter concentrate, and alfalfa hay until weaned at 44 days of age. At the time of weaning, bulls were paired by body weight and

randomly assigned within pairs to one of two rooms of an environmental chamber (Leining, 1978). There were four bulls assigned per room. Bulls were fed a complete pelleted ration, hay, trace mineralized salt, and water ad libitum.

Blood Sampling Procedures

Heifers and bulls were bled from a jugular vein through indwelling polyvinyl cannulas (Ico-Rally Corp., Palo Alto, Calif.). Animals' necks were wrapped with 7.62 cm Wide Elastoplast elastic adhesive tape (Beiersdorf Inc., South Norwalk, CT) and secured with safety pins to protect indwelling cannulas between intervals of blood sampling. To prevent blood coagulation, cannulas were filled with sodium citrate (3.5% in sterile water) during intervals between blood sampling. Blood was allowed to clot for 4 to 6 h at room temperature (18 to 21 C) then stored at 4 C for 24 h before centrifugation at 3000 x g for 15 minutes. Sera were decanted and stored at -20 C until assayed for hormone concentrations. Details of blood sampling procedures which were specific to individual experiments will be discussed in Objectives and Experimental Design.

In experiment 1, serum concentrations of LH during the preovulatory surge of LH were determined from blood samples collected around the time of estrus. Concentrations

of FSH in serum were determined from blood collected 10 h before through 10 h after peak preovulatory concentrations of LH. Concentrations of serum PRL during late diestrus were determined from selected blood samples collected for 24 h, beginning 98±4 h prior to the preovulatory peak concentration of LH, and having a mean progesterone concentration > 1 ng/ml. Concentrations of serum PRL during the estrus phase of the estrous cycle were determined from selected blood samples collected 10 h before through 10 h after peak preovulatory concentrations of LH and containing < 1 ng/ml of progesterone.

Hormone Assays

Protein hormones were quantified by double antibody radioimmunoassays (RIA) as previously described for luteinizing hormone (Convey et al., 1976), prolactin (Tucker, 1971), follicle stimulating hormone (Carruthers, 1979; Carruthers et al., 1980), and progesterone was quantified by RIA as described by Louis et al. (1973).

Objectives and Experimental Design

Experiment 1
Effect of 8L:16D and 16L:8D Photoperiods on Serum LH, FSH, and PRL at Different Stages of the Estrous Cycle During Autumn and Winter in Postpubertal Holstein Heifers

This experiment was designed to study effects of photoperiod on concentrations of LH, FSH, and PRL in sera from postpubertal Holstein heifers during late diestrus,

and estrus. The objectives were to determine: 1) if photoperiods of 8L:16D and 16L:8D affected the time of day when the preovulatory surge of LH and FSH occurred; and 2) if photoperiods affected concentrations of LH, FSH, and PRL during diestrus and estrus.

Duration of the experiment was 19 weeks beginning on September 21 and extending to January 31. Prior to the start of the experiment heifers had been exposed to a natural photoperiod which was 12.2 h (based on sunrise to sunset intervals) on September 21 when heifers were initially housed in the barn. Nine heifers were exposed to 8 h of cool white fluorescent lighting (General Electric Co.) and 16 h dark (D), whereas the second group was given 16 h fluorescent light (L) daily (16L:8D). Median light intensities at eye level of heifers exposed to 8L:16D and 16L:8D photoperiods ranged from 43 to 484 (mean = 152) and 43 to 861 (mean = 170) lux, respectively.

Blood samples were collected during diestrus and estrus of a single estrous cycle at the beginning of the experiment (September and October) and again during a single estrous cycle in January. Blood sampling was to begin on day 17 (day 0 = estrus) of the first estrous cycle after exposure to their respective photoperiod in September. Estrus data collected during the four months preceding the experiment was used to predict day 17 of the estrous cycle. Rectal palpation of the reproductive tract was performed

at this time to confirm the predicted day of next anticipated estrus. Jugular cannulation was also performed on day 17 of the estrous cycle. Blood was collected at 2-h intervals and continued through 24 h after the onset of standing heat. Animals were haltered and tied for the first 12 to 24 h of blood sampling to facilitate blood collection and familiarize animals to blood sampling procedures. Heifers were then released and allowed to move freely in the pen. All animals were released prior to the onset of estrus. This was necessary to prevent animal injury from repeated mountings by other animals. Ambient temperatures were recorded at each sampling of blood. Animals which were in estrus at the onset of the experiment and those which were missed during the first estrous cycle were bled during the second subsequent estrous cycle. Animals which showed initial signs of estrus behavior prior to day 17 were cannulated immediately and blood sampling ensued.

Collection of blood in autumn began on September 23. Total duration of the sampling period in autumn extended over 28 and 32 consecutive days for heifers exposed to 8L:16D and 16L:8D photoperiods, respectively. Approximately 11 weeks lapsed between the end of blood collection in October and the beginning of blood collection on January 5. Blood collection in January required 20 and 27 consecutive

days for heifers exposed to 8L:16D and 16L:8D photoperiods, respectively.

Experiment 2
Effect of Continuous Low Intensity Light Supplemented with High Intensity Light on Concentrations of PRL in Serum of Prepubertal Bulls

This experiment was designed to study the effects of continuous low intensity light (loL) supplemented with high intensity light (hiL) on concentrations of PRL in serum. The objective was to determine: 1) if 8 or 16 h of supplemental high intensity light in the presence of continuous low intensity light affected concentrations of PRL in serum of prepubertal bull calves.

Duration of the experiment was 22 weeks beginning on August 14. Prior to the start of this experiment, the bull calves, 3 to 5 days of age, had been exposed to a natural photoperiod which was 14L:10D on August 14 when bulls were initially housed in the barn. All bulls were exposed to 8 h of cool white fluorescent lighting and 16D (8-hiL:16D) each day for 68 days. Subsequently, one group of bulls received continuous low intensity light supplemented with 16 h of high intensity light (16-hiL:8-loL) for an additional 46 days, whereas a second group of bulls received continuous low intensity light supplemented with 8 h of high intensity light (8-hiL:16-loL). Forty-six days later, treatments were reversed for each group of

bulls for an additional 43 days. Median light intensities at eye level of bulls exposed to 16-hiL:8-loL were 665 and 8 lux of light for high and low light intensities, whereas bulls exposed to 8-hiL:16-loL received 493 and 9 lux of light for high and low light intensities.

On the penultimate day of exposure to 8-hiL:16D, 16-hiL:8-loL, and 8-hiL:16-loL photoperiods, cannulations were performed. On the last day of these photoperiods animals were restrained with halters and blood was collected at 30-min intervals for 8 hours. Blood collected during the first 2 h was discarded. This was done to condition animals to sampling procedures. Ambient temperature was recorded at the time of collection of each blood sample. Temperatures ranged from 18 to 21 C (mean = 20) during all three blood collection periods. Animals had access to feed and water throughout the blood collection period.

Statistical Methods

Because of the normal distribution of data and homogeneity of variance, statistical analyses of serum LH, FSH, and PRL in experiment 1 were calculated using untransformed data. In experiment 1, comparison between autumn and winter concentrations of LH and FSH within photoperiod were made by a paired-t test (Gill, 1978) or a Welch-like approximate-t test (Lin, 1973) for testing the differences of means with incomplete data. Animals

that had LH and FSH surges during the time when blood samples were collected are represented in Figures 1 and 2, and 5, respectively. Comparisons between diestrus and estrus concentrations of PRL in October for animals exposed to 8L:16D was made by a paired-t test while all other comparisons of concentrations of PRL were made using a Welch-like approximate-t test. Concentrations of PRL in serum of heifers 10 h before through 10 h after peak preovulatory concentrations of LH and containing < 1.0 ng/ml of progesterone (estrus) and for 24 h during diestrus and having a mean progesterone concentration > 1.0 ng/ml are represented in Figure 8.

In experiment 2 a sequence of three different photoperiods made up a treatment. Thus, bulls in treatment 1 received a sequence of light exposures that went from 8-hiL:16D to 16-hiL:8-loL followed by 8-hiL:16-loL while bulls in treatment 2 were exposed to 8L:16D followed by 8-hiL:16-loL and then 16-hiL:8-loL photoperiods. Statistical analyses of serum PRL in experiment 2 were conducted using natural logarithm (ln) transformed data to minimize heterogeneity of variance. Within each treatment, Bonferroni contrasts were made to analyze transformed data among photoperiods using Bonferroni-t tests (Gill, 1978).

RESULTS

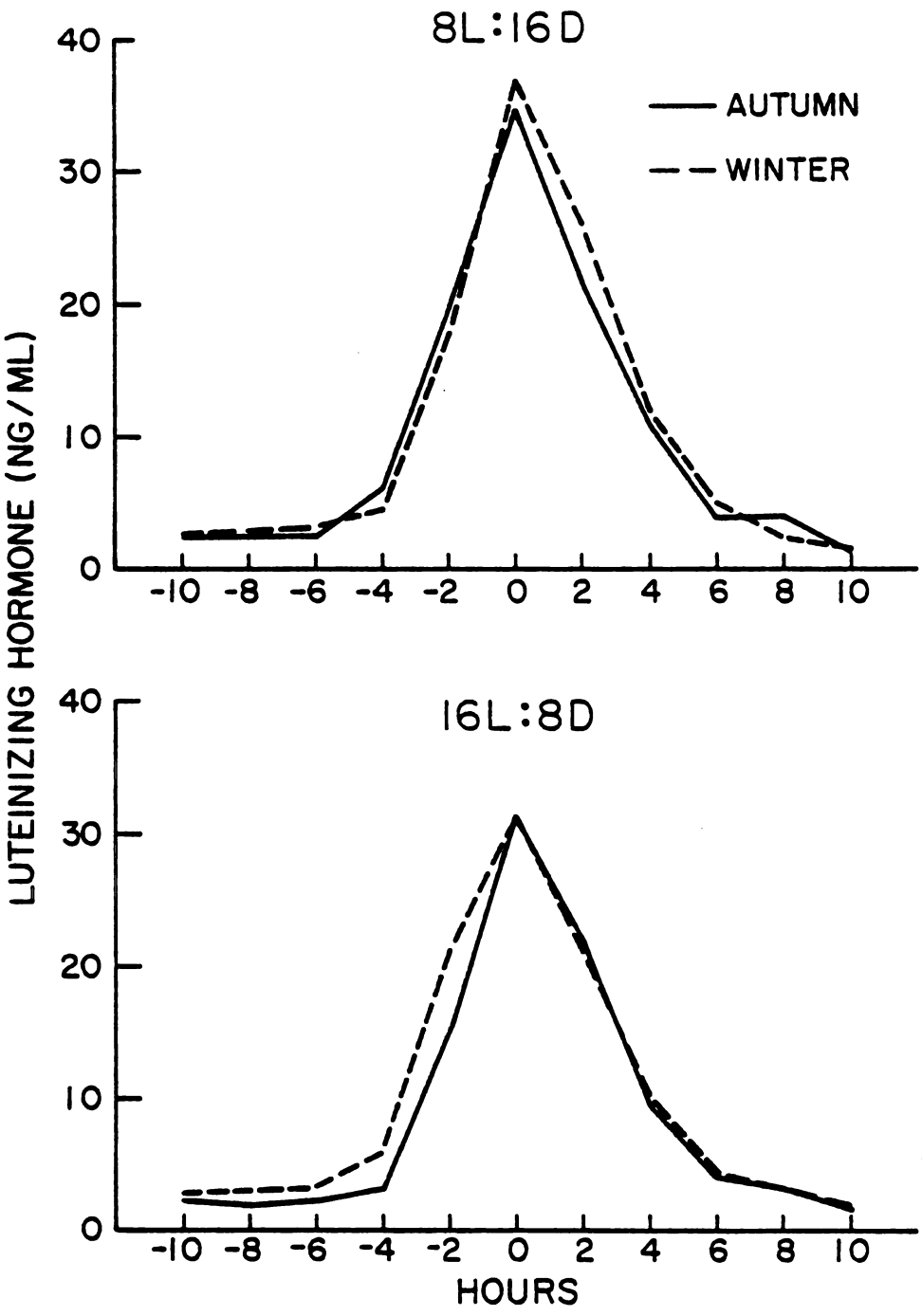
Experiment 1
Effect of 8L:16D and 16L:8D Photoperiods on Serum LH, FSH, and PRL at Different Stages of the Estrous Cycle During Autumn and Winter in Postpubertal Holstein Heifers

Luteinizing Hormone

Baseline concentrations of serum LH averaged 2.7 ng/ml from 10 through 6 h preceding peak concentrations of LH (peak concentrations of LH = 0 h) during the LH surge in heifers exposed to 8L:16D in autumn and winter (Figure 1). A slight increase at -4 h (5.4 ng/ml) was followed by a precipitious increase at -2 h (18.5 ng/ml). Peak concentrations of LH averaged 35.7 ng/ml at time 0 and decreased linearly to 4.4 ng/ml at 6 h, followed by a further decline to 1.5 ng/ml at 10 h. Concentrations of LH and pattern of LH secretion in heifers exposed to 16L:8D are not different from hormone concentrations in heifers exposed to 8L:16D (Figure 1). Peak concentrations of LH ranged from 12.6 to 63.7 ng/ml in heifers.

Peak concentrations of LH in serum were not different ($P<.01$) between autumn (37.0 ng/ml) and winter (34.5 ng/ml) in heifers exposed to 8L:16D (Figure 1). Similarly, there was no difference ($P<.01$) in peak concentrations of LH in serum between autumn (31.0 ng/ml)

Figure 1.--Effect of photoperiod on preovulatory surges of luteinizing hormone (LH) in heifers in autumn and winter. There are 9 and 8 animals represented in autumn and winter surges of LH, respectively, in heifers exposed to 8L:16D and 7 and 9 animals represented in autumn and winter surges of LH, respectively, in heifers exposed to 16L:8D. Blood was collected at 2-h intervals.


8L:16D
AUTUMN
WINTER
40
30
20
10
0
-10 -8 -6 -4 -2 0 2 4 6 8 10
16L:8D
LUTEINIZING HORMONE (NG/ML)
HOURS

and winter (30.8 ng/ml) in heifers exposed to 16L:8D. Although 4 months lapsed between autumn and winter samplings, LH surges were remarkably similar in amplitude and duration both within and between photoperiods. In fact, peak preovulatory concentrations of LH during autumn were highly correlated with peak preovulatory concentrations during winter within heifers given either 8L:16D or 16L:8D photoperiods ($r = .88$ and $.92$, respectively). Likewise, there is a striking similarity in the magnitude and duration of preovulatory LH surges within individual animals in autumn and winter (Figures 2 and 3).

In autumn, 11 of 18 heifers, and in winter, 16 of 18 heifers were observed in estrus during the preovulatory surge of LH. There was no detectable association between time of day and occurrence of preovulatory LH surges in heifers exposed to 8L:16D or 16L:8D photoperiods (Figure 4). Similarly, there was no detectable association between the occurrence of a LH surge during the light or dark period of the day in autumn and the occurrence of a LH surge during the light or dark period of the day in winter within an individual animal. Even though a 4 month period lapsed between estrous cycles during autumn and winter, heifers remained randomly distributed as to time of day at which peak concentrations of LH occurred during winter.

Figure 2.--Effect of 8L:16D photoperiod on preovulatory surges of luteinizing hormone (LH) in two individual heifers in autumn and winter. Blood was collected at 2-h intervals.

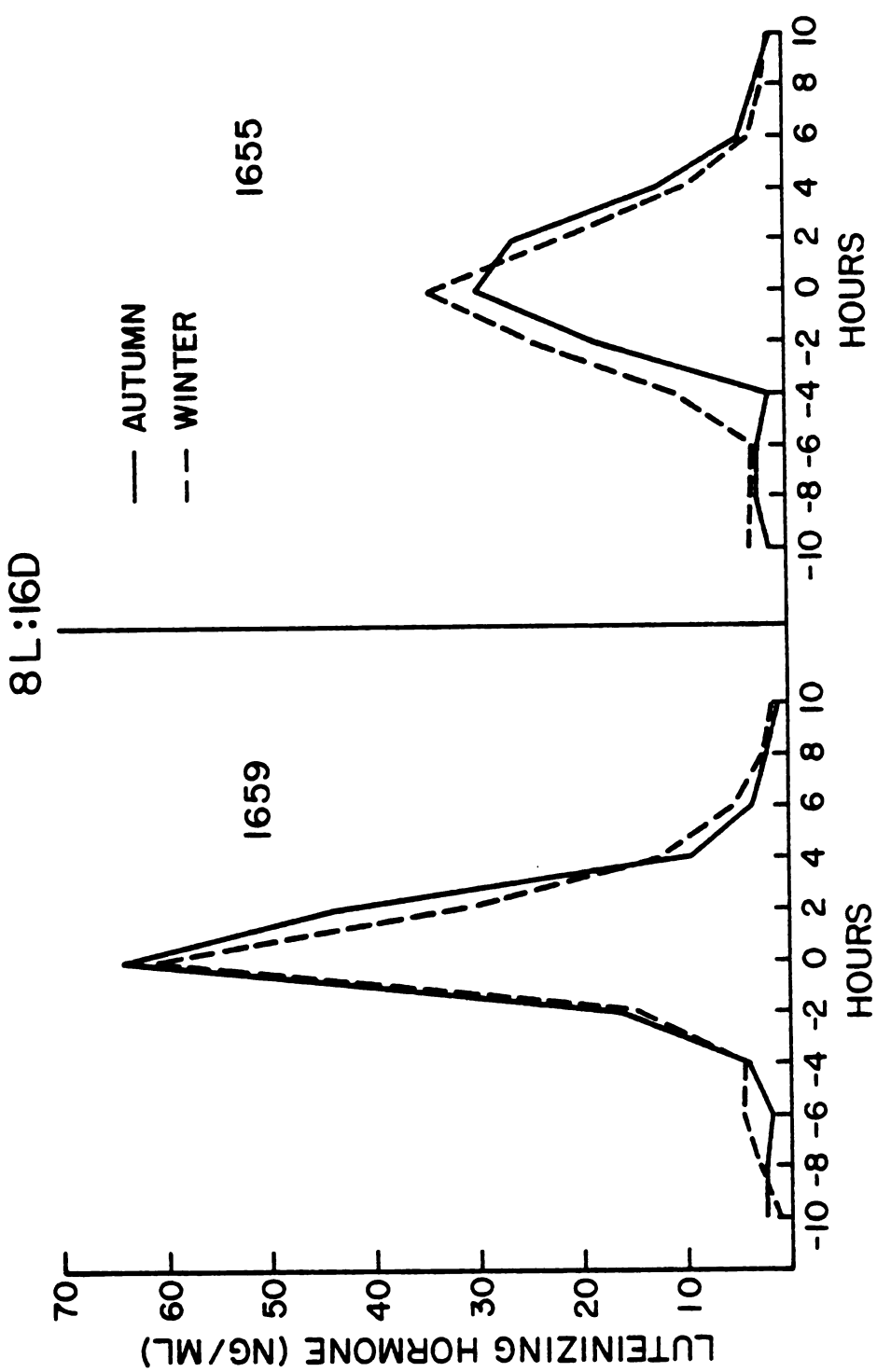

LUTEINIZING HORMONE (NG/ML)
70
60
50
40
30
20
10
8L:16D
1659
1655
AUTUMN
WINTER
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS

Figure 3.--Effect of 16L:8D photoperiod on preovulatory surges of luteinizing hormone (LH) in two individual heifers in autumn and winter. Blood was collected at 2-h intervals.

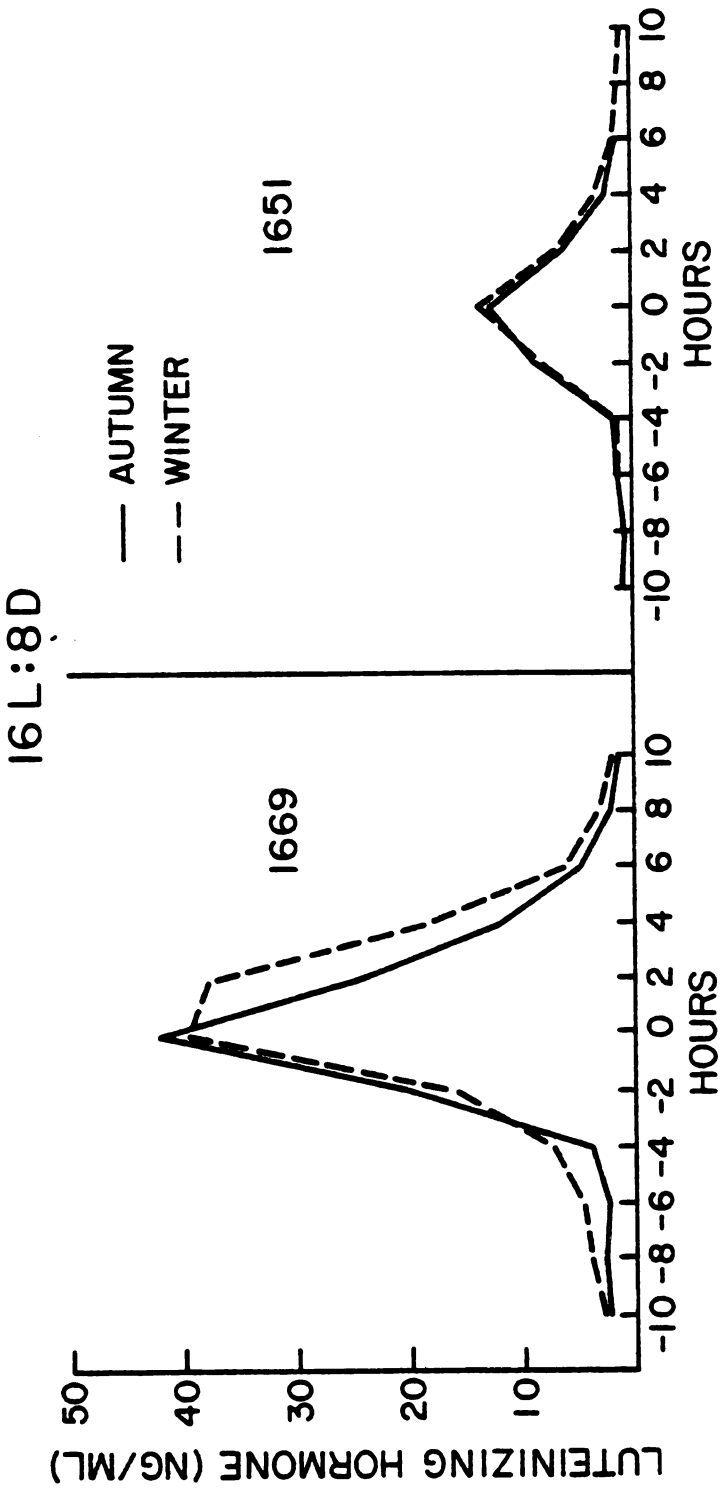
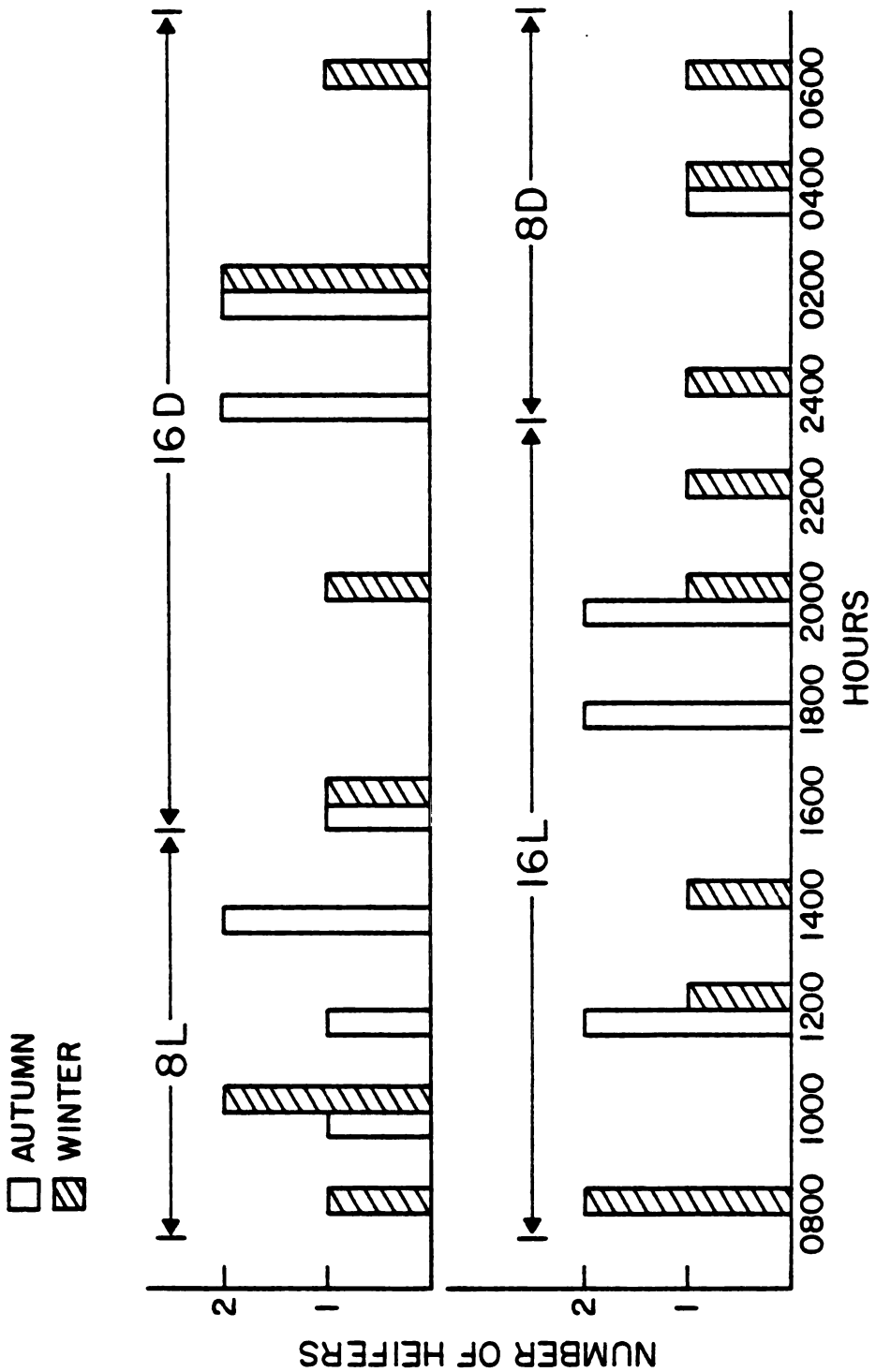

16L:8D
AUTUMN
WINTER
1669
1651
LUTEINIZING HORMONE (NG/ML)
50
40
30
20
10
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS

Figure 4.--Time of day at which peak preovulatory concentrations of luteinizing hormone (LH) occurred in Holstein heifers exposed to 8L:16D or 16L:8D in autumn and winter.


AUTUMN
WINTER
8L
16D
16L
8D
0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600
HOURS
NUMBER OF HEIFERS
1
2
1
2

Follicle-Stimulating Hormone

Baseline concentrations of serum FSH, mean = 96.8 ng/ml at 10 and 8 h prior to peak concentrations, gradually increased to 105.5 ng/ml at -4 h then sharply increased to peak levels of 308.5 ng/ml at 0 h in heifers. FSH rapidly decreased over the next 6 h to 99.5 ng/ml and slowly declined thereafter to 65.8 ng/ml at 10 h. Peak concentrations of FSH in serum ranged from 153.0 to 432.4 ng/ml in heifers. Invariably, peak concentrations of FSH coincided with peak concentrations of LH in heifers exposed to 8L:16D in autumn and winter. Peak concentrations in 12 of 16 FSH surges coincided with peak concentrations of LH in heifers exposed to 16L:8D in autumn and winter. The four which did not coincide, did not deviate more than 2 h from peak concentrations of LH.

Peak concentrations of FSH were 286.6 and 337.3 ng/ml in autumn and winter, respectively, in heifers exposed to 8L:16D ($P<.01$). Area under surges of FSH in winter were 20% larger ($P<.01$) than surges of FSH in autumn (Figure 5). In contrast, peak concentrations of FSH were not different ($P>.10$) between autumn and winter in heifers exposed to 16L:8D and averaged 342.6 and 308.2 ng/ml, respectively. Similarly, area under the FSH surges were not different between autumn and winter. Between autumn and winter magnitude and duration of FSH surges were similar within individual animals (Figures 6 and 7). Peak concentrations

Figure 5.--Effect of photoperiod on surges of follicle-stimulating hormone (FSH) in Holstein heifers in autumn and winter. There are 7 animals represented in autumn and in winter surges of FSH in heifers exposed to 8L:16D and 6 and 9 animals represented in autumn and winter surges of FSH, respectively, in heifers exposed to 16L:8D. Blood was collected at 2-h intervals.

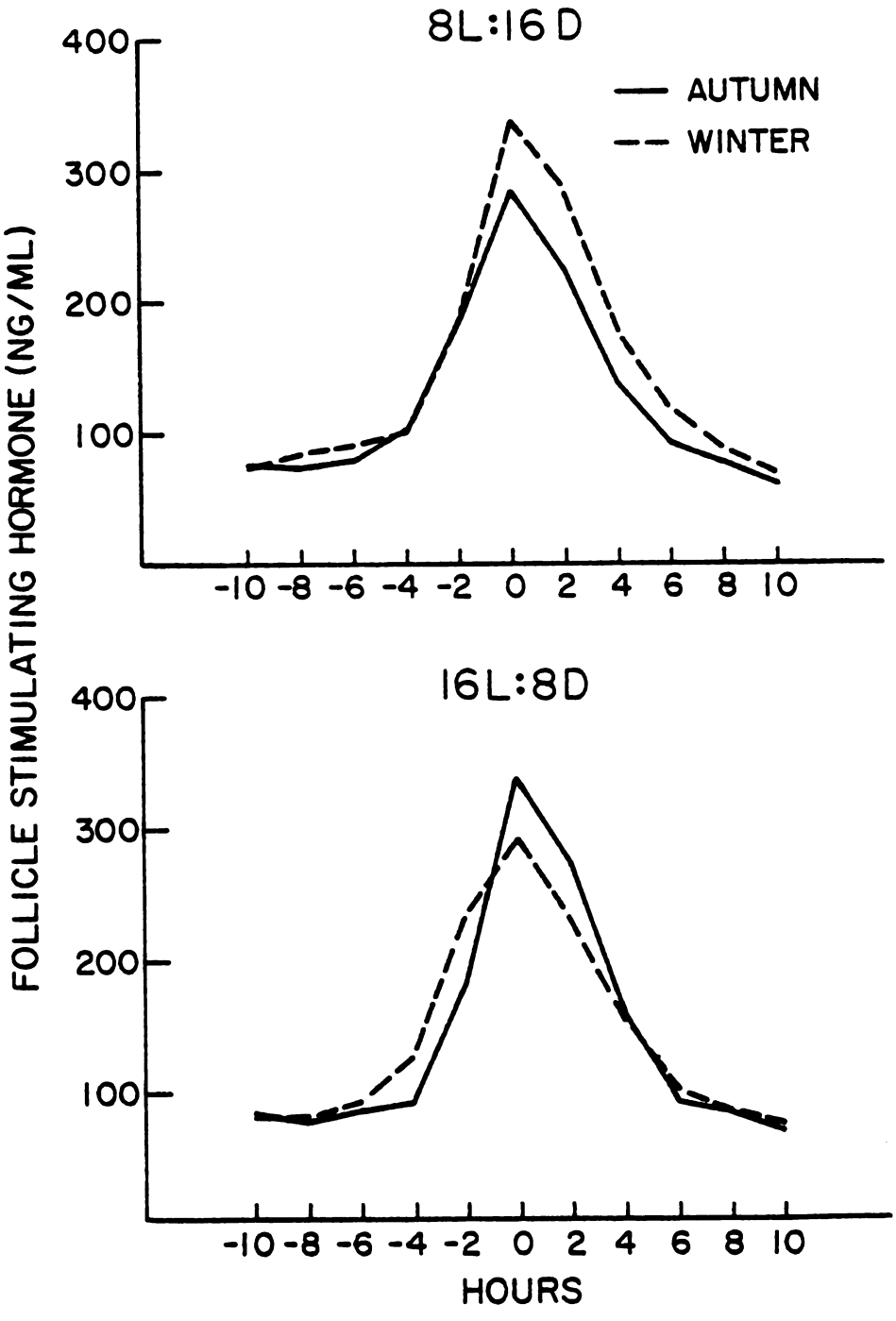

8L:16D
AUTUMN
WINTER
400
300
200
100
-10 -8 -6 -4 -2 0 2 4 6 8 10
16L:8D
400
300
200
100
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS
FOLLICLE STIMULATING HORMONE (NG/ML)

Figure 6.--Effect of 8L:16D on follicle-stimulating hormone (FSH) surges in two individual heifers in autumn and winter. Blood was collected at 2-h intervals.

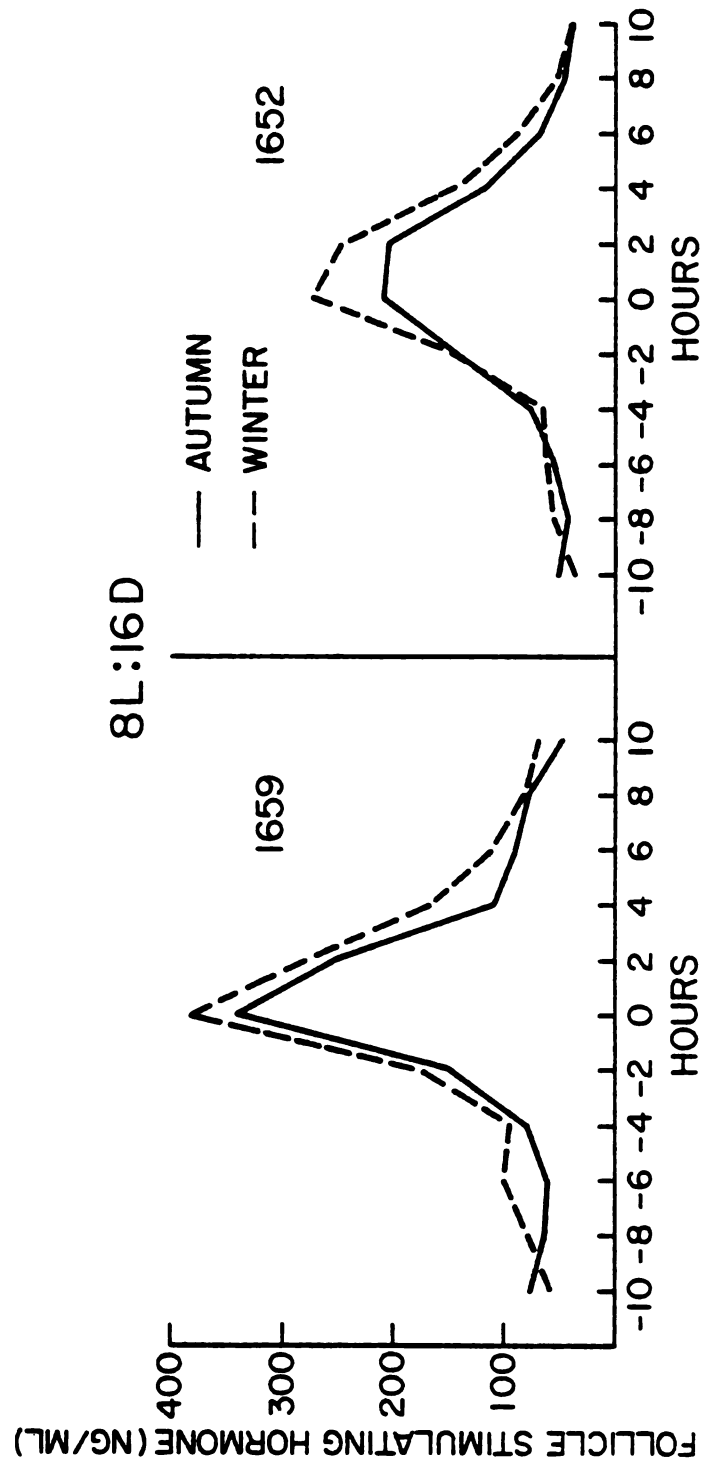
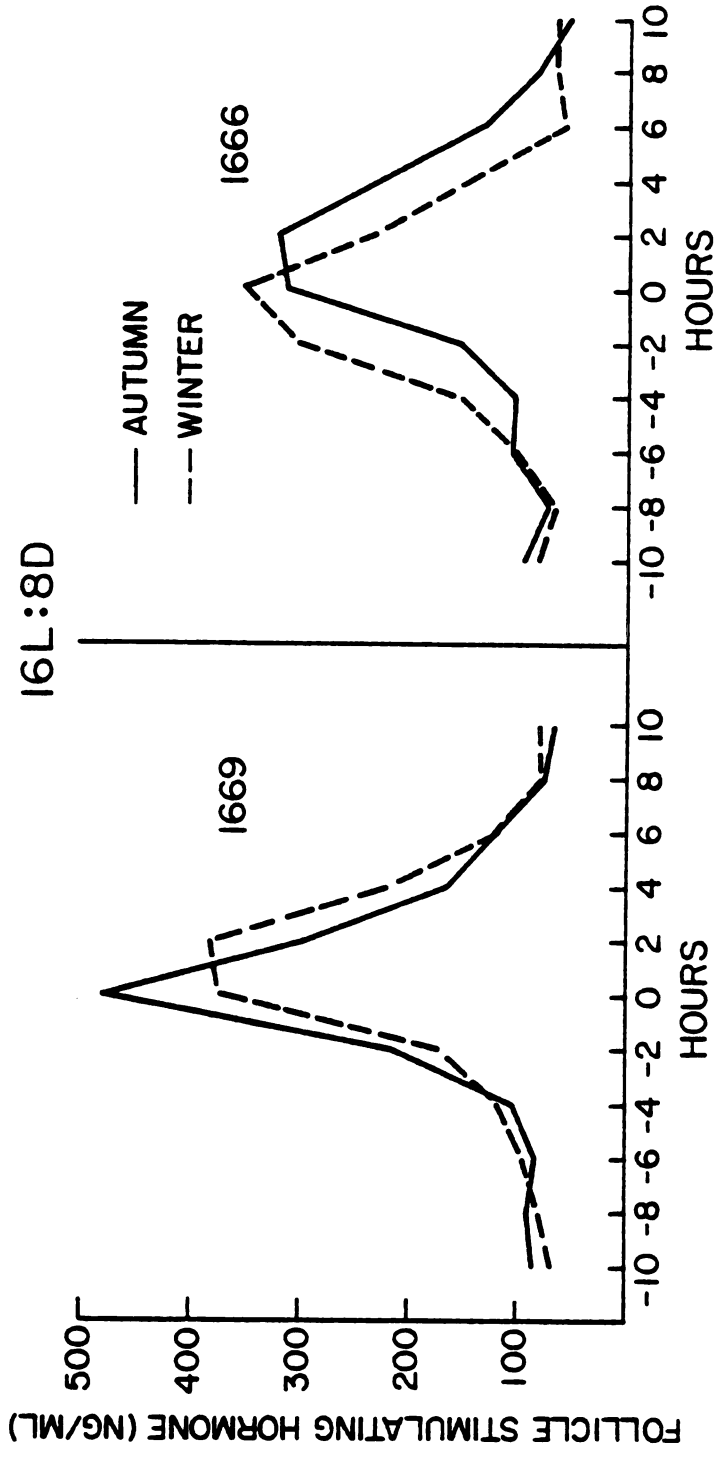

FOLLICLE STIMULATING HORMONE (NG/ML)
400
300
200
100
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS
1659
8L:16D
— AUTUMN
-- WINTER
1652
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS

Figure 7.--Effect of 16L:8D on follicle-stimulating hormone (FSH) surges in two individual heifers in autumn and winter. Blood was collected at 2-h intervals.


FOLLICLE STIMULATING HORMONE (NG/ML)
500
400
300
200
100
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS
1669
16L:8D
AUTUMN
WINTER
1666
-10 -8 -6 -4 -2 0 2 4 6 8 10
HOURS

of FSH during autumn were correlated with peak concentrations during winter within heifers given either 8L:16D or 16L:8D photoperiods (r = .68 and .40, respectively).

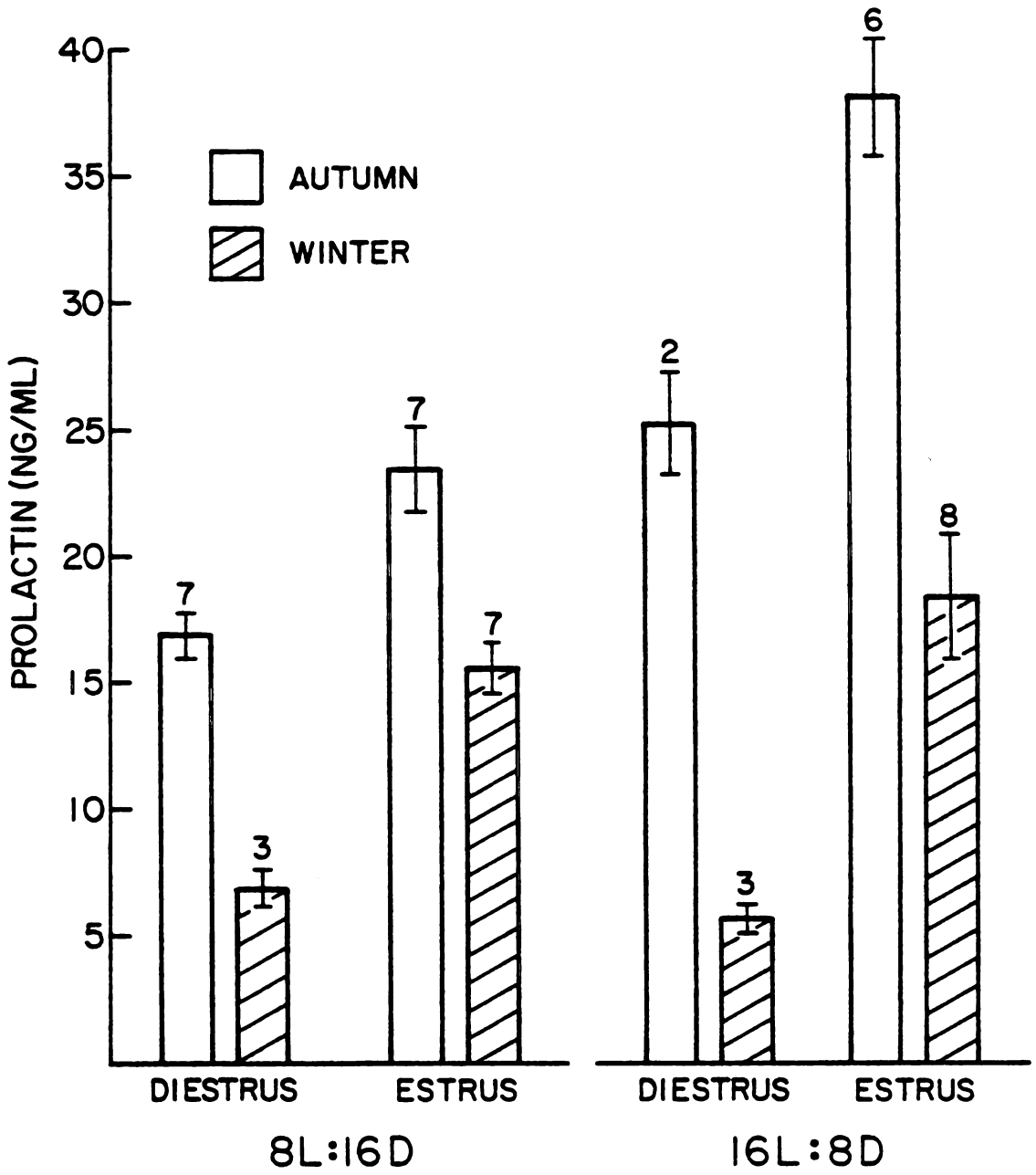
Prolactin

Concentrations of PRL in serum of heifers exposed to 8L:16D increased ($P<.02$) from 16.2 ng/ml during late diestrus to 22.4 ng/ml during estrus in September-October (Figure 8). When animals given 8L:16D were sampled in January, serum PRL increased ($P<.03$) from 6.9 ng/ml at diestrus to 14.7 ng/ml at estrus. Serum PRL in heifers exposed to 16L:8D daily increased ($P<.05$) from 25.3 ng/ml at diestrus to 38.2 ng/ml at estrus in autumn. In the winter, serum PRL averaged 5.7 and 18.4 ng/ml ($P<.02$) during diestrus and estrus, respectively, for heifers exposed to 16L:8D.

Concentrations of PRL in serum averaged 16.2 and 25.3 ng/ml ($P>.20$) during diestrus in heifers exposed to 8L:16D and 16L:8D photoperiods, respectively, in autumn. During estrus, however, concentrations of PRL were greater ($P<.01$) in two heifers exposed to 16L:8D in autumn than in seven heifers subjected to 8L:16D during estrus.

Overall, concentrations of PRL in serum averaged 20.0 ng/ml in autumn and 12.1 ng/ml in winter in heifers exposed to 8L:16D ($P>.20$). In heifers exposed to 16L:8D,

Figure 8.--Serum prolactin (PRL) during diestrus and estrus in autumn and winter in postpubertal Holstein heifers exposed to 8L:16D and 16L:8D photoperiods. Concentrations of PRL during diestrus are from blood collected at 2-h intervals during a 24 h period during late diestrus whereas blood collected at 2-h intervals 10 h before through 10 h after peak preovulatory concentrations of luteinizing hormone (LH) represent serum PRL during estrus. Number of animals are given at top of each bar.


40
35
30
25
20
15
10
5
PROLACTIN (NG/ML)
AUTUMN
WINTER
7
3
7
7
2
3
6
8
DIESTRUS
ESTRUS
DIESTRUS
ESTRUS
8L:16D
16L:8D

serum PRL was greater ($P<.01$) in autumn than in winter and averaged 34.2 and 15.6 ng/ml, respectively.

Estrous Cycles

Photoperiod had no effect on lengths of estrous cycles in heifers exposed to either 8L:16D or 16L:8D photoperiods (Table 1).

Experiment 2
Effect of Continuous Low Intensity Light Supplemented with High Intensity Light on Concentrations of PRL in Serum of Prepubertal Bulls

Concentrations of PRL in sera collected at the end of 68 days exposure to 8-hiL:16D of high intensity light averaged 20.9±.7 ng/ml in treatment 1 bulls (Table 2). Subsequent exposure for 46 days to continuous low intensity light (8 lux) supplemented with 16 h of high intensity (665 lux) daily light (16-hiL:8-loL) increased ($P<.01$) serum PRL to 53.3±2.3 ng/ml. After exposure to an additional 43 days of continuous low intensity light supplemented with 8 h of high intensity light (8-hiL:16-loL) concentrations of PRL remained unchanged ($P>.10$) and averaged 40.7±2.5 ng/ml. Thus, concentrations of PRL after exposure to 8-hiL:16-loL were two fold greater than during exposure to 8-hiL:16D ($P<.01$).

In the second treatment, serum PRL of bulls averaged 28.5±1.6 ng/ml after an initial 68 days of exposure to 8-hiL:16D (Table 2). Subsequent exposure

TABLE 1.--Effect of photoperiod on lengths of estrous cycles in 18 Holstein heifers from September 21 through January 31.

Heifer number	Estrous cycle 1	2	3	4	5	6	Mean	SE
	----------8L:16D----------							
1643	22	20	20	20	22		20.8	1.1
1648	20	22	----------85[b]----------				21.0	1.4
1649	20	21	20	23	21		21.0	1.2
1650	20	22	21	20	21		20.8	0.8
1652	21	21	20	19	21		20.4	0.9
1655	23	23	20	21	23		22.0	1.4
1657	22	20	21	20	19		20.4	1.1
1659	21	22	19	22	23		21.4	1.5
1660	24	27	27	23			25.3	2.1
Mean	21.4±1.4	22.0±2.1	21.0±2.5	21.0±1.5	21.4±1.4	Overall $\bar{x}$	21.4	1.8
	----------16L:8D----------							
1644	19	20	20	19	20		19.6	0.6
1645	25	18	-----39[b]-----		19	20	20.5	3.1
1651	21	-----45[b]-----		22	31		24.7	5.5
1653	24	19	21	20	22	20	21.0	1.8
1654	20	20	19	21	21	18	19.8	1.2
1663	22	24	23	24	22		23.0	1.0
1666	22	20	21	21	19		20.6	1.1
1668	22	25	21	22	23		22.6	1.5
1669	20	21	19	19	20	20	19.8	0.8
Mean	21.7±1.9	20.9±2.4	20.6±1.4	21.0±1.7	21.9±3.7	19.5±1.0 Overall $\bar{x}$	21.1	2.3

[a]Estrus data based on standing heat, intense riding activity and activated K-Mar heat detection patches. Estrus = day 0.

[b]Mean estrous cycle length of individual heifers does not include estrous cycles greater than 31 days in length.

TABLE 2.--Average concentrations of prolactin (PRL) in serum of two groups of prepubertal Holstein bulls exposed to various photoperiods[a].

Date	Photoperiod sequence	Prolactin[b] (ng/ml) Overall $\bar{x}$	Pooled SE
	Treatment I		
October 19	8-hiL:16D	20.9	0.7
December 4	16-hiL:8-loL	53.3[c]	2.3
January 17	8-hiL:16-loL	40.7[c]	2.5
	Treatment II		
October 19	8-hiL:16D	28.5	1.6
December 4	8-hiL:16-loL	51.8[c]	2.2
January 17	16-hiL:8-loL	47.8[d]	2.3

[a]hiL ranged from 194 to 807 lux (mean = 579); loL ranged from 2 to 22 lux (mean = 9).

[b]Mean concentration collected at .5 h intervals for 6 h. There were four bulls per treatment.

[c]Means within treatments are significantly different from 8-hiL:16D on October 19 ($P<.01$).

[d]Significantly greater than mean for bulls given 8-hiL:16D on October 19 ($P<.05$).

to continuous low intensity (9 lux) light supplemented with 8 h of high intensity (493 lux) light through week 16 increased ($P<.01$) serum PRL to 51.8±2.2 ng/ml. Serum PRL averaged 47.8±2.3 ng/ml and remained unchanged ($P>.20$) after an additional 6 weeks exposure to 16-hiL:8-loL.

DISCUSSION

Although there appears to be environmental factors (photoperiod, climate, nutrition, and season) that may affect periodicity of bovine estrous cycles (Dutt, 1960; Hafez, 1965, 1967), evidence of a photoperiodic effect on reproduction in cattle is lacking. The data presented in this thesis indicate that photoperiod does not affect preovulatory concentrations of LH and FSH, nor time of day at which surges of LH and FSH occur, nor lengths of estrous cycles in postpubertal Holstein heifers. Furthermore, these results support that duration of daily light alters secretion of PRL in cattle (Bourne and Tucker, 1975; Peters and Tucker, 1978; Leining et al., 1979).

McCarthy (1978) and McCarthy et al. (1979) reported that the frequency of episodic releases of LH in bulls and steers were increased as natural daily light increased, but these data were confounded with age. Similarly, Leining (1978) reported an increase in frequency of episodic releases of LH in bull calves exposed to 16L:8D as compared with 8L:16D, however, these data were also confounded with age. Photoperiods of 8L:16D and 16L:8D had no effect on concentrations of LH in prepubertal bull calves (Bourne and Tucker, 1975) or in prepubertal heifers

(Goodman, unpublished). However, there is an increase in concentrations of LH in rams (Pelletier and Ortavant, 1975; Lincoln et al., 1977) and ewes (Scaramuzzi and Baird, 1977; Legan and Karsch, 1979) exposed to short-day photoperiods as compared with long-day photoperiods. Since sheep are seasonal breeders these hormonal patterns likely reflect periods of sexual activity and inactivity, respectively. In the present study, neither photoperiod nor season affected concentrations of LH during preovulatory surges investigated in heifers. These findings support previous observations in prepubertal bulls (Bourne and Tucker, 1975) and heifers (Goodman, unpublished) that serum concentrations of LH are not affected by photoperiod.

Peak concentrations of LH ranged from 12.6 to 63.7 ng/ml, similar to reports of Niswender et al. (1969), Snook et al. (1971) and Hansel and Echternkamp (1972). Duration of LH surges were of similar length as those reported by Hansel and Echternkamp (1972), Chenault et al. (1975) and Rahe et al. (1980). Amplitude and duration of preovulatory surges of LH were remarkably similar within individual heifers between autumn and winter. It appears that the concentration of LH necessary to cause ovulation varies greatly from animal to animal but is consistent within individual animals.

The light-dark cycle determines the time of day of the preovulatory surge of LH in rats (Hoffmann, 1969;

Sridaran and McCormack, 1979). At present, there is no evidence that photoperiod affects the timing of the preovulatory surge of LH in cattle. However, there are numerous reports of diurnal variations on the onset of estrus in cattle (Trimberger, 1948; Hall et al., 1959; Plasse et al., 1970; Williamson et al., 1972; Esslemont, 1974; Hurnik et al., 1975) and the preovulatory surge of LH occurs around the time of estrus (Niswender et al., 1969; Hansel and Snook, 1970; Hendricks et al., 1972; Edgerton and Hafs, 1973; Chenault et al., 1975). Williamson et al. (1972), Esslemont (1974), and Hurnik et al. (1975) observed a circadian rhythm in the behavioral manifestations of estrus in cattle with the highest frequency of mounting behavior occurring during the nocturnal period. Esslemont (1974) observed that 65.5% of the mounts occurred from 1900 to 0700 hours. This is in agreement with Hurnik et al. (1975) that the greatest number of estrus periods occur from 2100 to 0900 hours. Hall et al. (1959) found that 54.8% of estrous periods began during the night, whereas 45.2% began during the day in cows and heifers exposed to subtropical temperatures in Louisiana. In contrast, Plasse et al. (1970) reported that the majority of estrous periods began during the daylight hours in heifers exposed to subtropical temperatures in Florida. Trimberger (1948) reported that more cows and heifers were first observed in estrus in the

morning than in the afternoon and evening in Nebraska. Whether or not photoperiod is responsible for diurnal variations in onset of estrus has yet to be determined. High ambient temperatures during mid-afternoon may affect expression of estrous behavior and duration of estrus (Hall et al., 1959; Gangwar et al., 1965; Abilay et al., 1975). Esslemont (1974) and Hurnik et al. (1975) suggest that the occurrence of management practices during daylight hours might also influence the circadian rhythm in behavioral manifestations of estrus. In this study, 11 of 18 heifers in autumn and 16 of 18 heifers in winter were observed in estrus during the preovulatory surge of LH. The remaining heifers were either not observed in estrus or did not have surges of LH. We observed no affect of photoperiod on time of day at which peak preovulatory concentrations of LH occurred. Heifers were randomly distributed as to time of day at which peak concentrations of LH occurred in autumn and winter. Esslemont (1974) and Hurnik et al. (1975) suggest that management routine affects the time at which estrus occurs. In this study, the frequent presence of humans (at 2-h intervals) during collection of blood may, in fact, be responsible for the absence of a circadian pattern in the occurrence of surges of LH. In view of these data, it does not appear that photoperiod is a major source of diurnal variation in onset of estrus.

Literature on the effects of photoperiod on concentrations of FSH in sera of cattle are not available. In my study, peak concentrations of FSH surges and area under surges of FSH were greater (15 and 20%, respectively) during the winter than in autumn in heifers exposed to 8L:16D. The cause of this increase is unexplained. Peak concentrations of FSH were 10% greater in autumn than in winter in heifers exposed to 16L:8D but this difference did not approach significance. Although a photoperiodic effect on gonadotropin secretion in cattle has not been established, an increase in FSH and LH is associated with short length photoperiods and seasonal reproduction in sheep (Lincoln et al., 1977). These data suggest that photoperiod affects secretion of FSH in cattle but whether these changes are of physiological importance in reproduction in cattle has not been determined.

It has been well documented that peak concentrations of FSH are coincident with peak concentrations of LH in cattle (Akbar et al., 1974; Kaltenbach et al., 1974; Dobson, 1978b). In the present study, peak concentrations in 27 of 31 surges of FSH coincided with peak concentrations of LH in heifers. Peak concentrations of FSH which did not coincide occurred within 2 h of peak concentrations of LH. Because we used the same homologous FSH radioimmunoassay as Roche and Ireland (1981), concentrations of FSH reported for FSH surges are similar. However,

concentrations of FSH reported in this thesis are approximately 2-fold greater than those reported by Dobson (1978b) using a heterologous RIA.

There is no evidence that photoperiod affects lengths of estrous cycles in cattle. Data from our study would indicate that exposure to either 8L:16D or 16L:8D photoperiods for 4 months does not affect lengths of estrous cycles in postpubertal Holstein heifers.

In rats, secretion of PRL increases between metestrus-diestrus and proestrus-estrus (Koch et al., 1971; Butcher et al., 1974). Likewise concentrations of PRL are greater during proestrus and estrus phases of the estrous cycle in sheep compared with metestrus-diestrus (Reeves et al., 1970; Bryant et al., 1971). A similar increase in concentrations of PRL was reported by Raud et al. (1971) and Swanson and Hafs (1971) during proestrus and estrus in heifers. These increases in concentrations of PRL in blood of heifers coincide with a decrease in concentrations of PRL in the pituitary gland between estrus and 2 days post-estrus (Sinha and Tucker, 1969). We also observed a significant increase in serum concentrations of PRL during estrus as compared with diestrus in heifers. Concentrations of PRL in serum were 1.4 and 1.5-fold greater during estrus than proestrus in heifers exposed to 8L:16D and 16L:8D, respectively, in autumn whereas a 2.1 and 3.2-fold increase, respectively,

occurred in winter. Absence of a photoperiod effect on concentrations of PRL when ambient temperatures are less than 0 C (Peters et al., 1980) may allow for a greater expression of the increase in concentrations of PRL observed during estrus in the winter when ambient temperatures ranged from -14 to 3.5 C (mean = -3).

The increase in PRL at proestrus and estrus is associated with increased estrogen secretion in rats (Koch et al., 1971; Butcher et al., 1974), sheep (Reeves et al., 1970; Bryant et al., 1971; Cumming et al., 1972) and cattle (Raud et al., 1971; Swanson and Hafs, 1971; Swanson et al., 1972). Even though we did not measure serum concentrations of estrogen, Raud et al. (1971), Swanson and Hafs (1971), and Swanson et al. (1972) suggests that PRL secretion is greater during estrus than during other phases of the estrous cycle. The increase in PRL that occurs around estrus may be associated with increased secretion of estrogen at this same time. The fact that administration of exogenous estrogen increased PRL release in cattle (Schams and Karg, 1972; Schams et al., 1974; Williams and Ray, 1980) is further evidence that estrogen secretion may be associated with release of PRL. This has also been observed in rats (Chen and Meites, 1970) and sheep (Fell et al., 1972). In addition, Padmanabhan and Convey (1979) demonstrated that estradiol-17β increases secretion of PRL in bovine pituitary cell cultures. Also,

a precipitous increase in concentrations of PRL preceding parturition in cows (Edgerton and Hafs, 1973) and heifers (Ingalls et al., 1973) coincides with a marked increase in estrogen at this time (Smith et al., 1973). Indeed, the endogenous rise in blood estrogen levels around the time of estrus induces an increase in PRL by acting either directly at the pituitary or indirectly via the hypothalamus.

Stage of the estrous cycle had no effect on blood concentrations of PRL in lactating and nonlactating cows (Schams and Karg, 1970; Edgerton and Hafs, 1973; Koprowski and Tucker, 1973). The difference between secretion of PRL in heifers and cows at the time of estrus is not understood. An increase in physical activity is associated with estrus in many species of animals. Also, serum PRL can be affected by various stimuli (Raud et al., 1971; Tucker, 1971). Beck et al. (1976) suggested that the increased secretion of PRL around the time of estrus might be due to increased physical activity and excitement associated with behavioral manifestations of estrus in cattle. No increase in PRL secretion at estrus was observed in lactating cows restricted in stanchions (Edgerton and Hafs, 1973; Koprowski and Tucker, 1973). In contrast, secretion of PRL was increased at estrus in heifers that were restricted in stanchions (Swanson and Hafs, 1971; Raud et al., 1971) or group housed (Swanson

et al., 1972). However, Vines et al. (1977) reported that TRH induced release of PRL was unchanged throughout the estrous cycle in heifers restricted in tie stalls, but, blood collection on the day of estrus was made only during a 30-minute interval which may not have coincided with increased endogenous concentrations of estrogen during the estrus period. In the present study, an increase in secretion of PRL at estrus was observed in heifers that were group housed.

PRL is one of several hormones involved in mammary growth, lactogenesis and maintenance of lactation (Cowie, 1969). In addition, postpartum reproduction (i.e., onset of a normal periodicity of estrous cycles) in the cow is greatly affected by the milking stimulus, lactational intensity, nutrition, and periparturient problems associated with parturition. The possibility that changes in metabolism of PRL during lactation and changes in the hormonal milieu during the postpartum period mask the response of PRL to increased estrogen at the time of estrus cannot be excluded.

In ruminants, secretion of PRL varies in response to seasonal changes in temperature and photoperiod (Schams, 1972; Koprowski and Tucker, 1973; Wettemann and Tucker, 1974; Buttle, 1974; Hart, 1975; Ravault, 1976). That is, concentrations of PRL are greatest in summer when ambient temperatures and daily lighting are maximal, and lowest

during winter when temperatures and daily lighting are minimal. In these studies during the first experiment, 16 h of light per day increased serum concentrations of PRL approximately 1.7-fold as compared with heifers exposed to 8L:16D in autumn. Leining et al. (1979) also demonstrated that secretion of PRL in cattle changes within several days to weeks after length of light exposure is increased (from 8 to 16 h daily) or decreased (from 16 to 8 h daily). In this experiment, photoperiod had no effect on serum PRL in winter when ambient temperatures averaged -3 C. These results are in agreement with Peters and Tucker (1978) and Peters et al. (1980) who observed no effect of photoperiod in heifers when ambient temperatures were below 0 C. Likewise, we observed that serum concentrations of PRL in the autumn were 1.7 and 2.2-fold greater than in winter in heifers exposed to 8L:16D and 16L:8D photoperiods, respectively.

It has been determined that 16 and 20 h of daily light are equally effective in increasing basal concentrations of PRL over concentrations found during 8L:16D (Leining et al., 1979). However, serum concentrations of PRL in bull calves exposed to 24 h of high intensity (116 lux) light were not different than concentrations reported after exposure to 8L:16D. In addition, Peters et al. (1980) determined that exposure to 24L:0D or natural photoperiods of 9.3 to 11.6 h of light per day were not

effective in initiating a growth response to photoperiod as observed in heifers receiving 16L:8D. Light intensities as low as 22 lux of light are as effective as 540 lux of light in stimulating a response in PRL to photoperiod in cattle (Leining, 1978; Goodman, unpublished). In a second study, we determined that exposure of bull calves to continuous low intensity light supplemented daily with either 8 or 16 h of high intensity light was equally effective in increasing serum concentrations of PRL after exposure to 8-hiL:16D. The similarity in PRL response to 8-hiL:16-loL and 16-hiL:8-loL may be explained in part by the hypothesis of Bunning (1960). The 16 h of high or low intensity light may overlap the circadian rhythm of photo-sensitivity in bulls and thus PRL secretion is increased in response to a long day photoperiod. It also must mean that 8 h of high or 8 h of low intensity of light are recognized the same as 8 h of darkness.

Cattle alter secretion of PRL in response to changes in duration of daily light (Bourne and Tucker, 1975; Peters and Tucker, 1978; Leining et al., 1979). Secretion of PRL in bull calves that received 24 h of continuous daily light was not different from that after exposure to 8L:16D. These data suggest that cattle use the switch from a light period to a dark period, or vice versa, as a "cue" to initiate a response in secretion of PRL to changes in photoperiod. In addition, our data

suggests that prepubertal bull calves are also capable of altering secretion of PRL by responding to changes in different intensities of daily light.

SUMMARY AND CONCLUSIONS

These experiments were designed to study the effects of photoperiod on reproduction in dairy heifers and to determine whether cattle are capable of responding to supplemental high intensity light in the presence of continuous low intensity light.

The first experiment was designed to determine if photoperiods of 8L:16D and 16L:8D affect the time of day when the preovulatory surge of LH and FSH occurs and if photoperiods affect diestrus and estrus concentrations of LH, FSH and PRL. Eighteen postpubertal Holstein heifers were assigned nine each to separate pens in an enclosed barn and received either 8L:16D or 16L:8D from September 21 through January 31. Serum hormone concentrations were determined from blood collected at 2-h intervals during diestrus and estrus phases of the estrous cycle during a single estrous cycle in autumn and again 4 months later, during a single estrous cycle in winter.

Neither photoperiod nor season affected time of day at which LH and FSH surges occurred in heifers. LH surges were remarkably similar in amplitude and duration within and between photoperiods. In addition, LH surges were similar within individual animals. In fact, peak

concentrations of LH during autumn was highly correlated with peak concentration of LH during winter in heifers exposed to 8L:16D and 16L:8D photoperiods (r = .88 and .92, respectively).

Neither photoperiod nor season affected time of day at which peak preovulatory concentrations of FSH occurred in heifers. In fact, peak concentrations in 28 of 32 surges of FSH coincided with peak concentrations of LH. Peak concentrations of FSH were greater ($P<.01$) during the winter (337.3 ng/ml) than during autumn (286.6 ng/ml) in heifers exposed to 8L:16D but were not different between autumn (342.6 ng/ml) and winter (308.2 ng/ml) in heifers exposed to 16L:8D. Between autumn and winter surges of FSH were similar in magnitude and duration within individual animals, and peak concentrations of FSH during autumn were correlated with peak concentrations during winter within heifers given either 8L:16D or 16L:8D photoperiods (r = .68 and .40, respectively). We observed no effect of photoperiod nor season on lengths of estrous cycles.

In autumn, concentrations of PRL in sera increased ($P<.02$) from 16.2 ng/ml during diestrus to 22.4 ng/ml during estrus in heifers exposed to 8L:16D and increased ($P<.03$) from 6.9 to 14.7 ng/ml from diestrus to estrus, respectively, in winter. Similarly, concentrations of PRL in sera from heifers exposed to 16L:8D increased from

25.3 ng/ml during diestrus to 38.2 ng/ml during estrus in autumn ($P<.05$) and from 5.7 ng/ml during diestrus to 18.4 ng/ml during estrus in winter ($P<.02$).

Overall, concentrations of PRL increased 1.7-fold in sera from heifers which received 16L:8D as compared with heifers exposed to 8L:16D in autumn. Photoperiod had no effect on concentrations of PRL during the winter. During autumn, concentrations of PRL were 1.7 and 2.2-fold greater than in winter in heifers exposed to 8L:16D and 16L:8D, respectively.

In a second experiment, Holstein bull calves were used to determine effects of supplemental high (hi) intensity (665 or 493 lux) light in the presence of continuous low (lo) intensity (8 or 9 lux) light on secretion of PRL. Bulls were housed in one of two rooms of an environmental chamber in which daily lighting, temperature, and ventilation were controlled. In the first group of bulls, concentrations of serum PRL averaged 20.9±0.7 ng/ml during 6 h on the last day of 10 wk exposure to 8-hiL:16D and increased ($P<.01$) to 53.3±2.3 ng/ml after exposure for 6 weeks to 16-hiL:8-loL. Subsequent exposure for 6 weeks to 8-hiL:16-loL did not change concentrations of PRL (avg. = 40.7±2.5 ng/ml). In the second group of bulls, serum PRL increased ($P<.01$) from 28.5±1.6 ng/ml after initial exposure for 10 weeks to 8-hiL:16D to 51.8±2.2 ng/ml after 6 weeks exposure to 8-hiL:16-loL.

Concentrations of PRL remained unchanged following 6 weeks exposure to 16-hiL:8-loL (47.8±2.3 ng/ml).

In conclusion, neither photoperiod nor season affect the preovulatory surge of LH and FSH or lengths of estrous cycles in postpubertal Holstein heifers. In addition, photoperiod and season did not affect the rise in PRL associated with estrus in heifers. Thus, it appears that short and long day photoperiods (8 and 16 h, respectively) do not affect reproduction in postpubertal heifers. Furthermore, increased secretion of PRL occurs provided daily light is interrupted by 8 h of darkness or by 8 h of an intensity of light which differs from that during the 16 h interval. Therefore, the economical benefits associated with increased growth rate and milk production in cattle exposed to 16 h of light per day can still be realized by dairymen that expose their cattle to continuous daily light (to facilitate nighttime management practices) provided cattle receive 8 h of an intensity of light that differs from that during the 16 h interval.

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