LUTEINIZING HORMONE, PROGESTERONE, ESTRADIOL, GLUCOCORTICOIDS AND CHANGES IN ENERGY BALANCE BEFORE FIRST OVULATION IN POSTPARTUM DAIRY COWS

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ABSTRACT

LUTEINIZING HORMONE, PROGESTERONE, ESTRADIOL,
GLUCOCORTICOIDS AND CHANGES IN ENERGY BALANCE
BEFORE FIRST OVULATION IN POSTPARTUM DAIRY COWS

By

Jeffrey Smith Stevenson

In the postpartum cow, factors involved in the initiation of ovarian activity and normal estrous cycles are not well understood. The objectives of this study were to describe relationships among serum concentrations of estradiol, progesterone, glucocorticoids and LH and changes in body weight and energy balance from parturition until first postpartum ovulation.

Ovaries of 28 Holstein cows were palpated per rectum twice weekly to monitor changes in ovarian structures following collection of blood to measure changes in serum progesterone and estradiol concentrations. For comparison, cows were divided into three interval groups according to the time of first postpartum ovulation. Cows which ovulated before day 15, between days 15 and 20, and after day 20 postpartum were assigned to interval groups I, II and III, respectively.

Interval from parturition to first ovulation averaged 17.6 days and ranged from 9 to 34 days. Primiparous cows tended to have shorter ($P<.10$) intervals to first ovulation, while the interval to first estrus was not

different ($P>.10$) among parity groups. Progesterone remained less than 0.2 ng/ml following parturition until concentrations increased ($P<.01$) above pre-ovulation levels 2 to 3 days following the first postpartum ovulation. The first estrus cycle was shorter ($P<.05$) in duration compared to the second ovarian cycle. This may have been due to decreased ($P<.05$) corpus luteum diameter and reduced ($P<.01$) peak progesterone concentrations of primiparous cows compared to older cows.

Changes in serum estradiol concentrations from parturition until first ovulation fluctuated in some cows, while in others concentrations remained low until 2 to 3 days before estrus and then increased. Number of follicles $\geq$ 10 mm was greater ($P<.05$) during the second week in cows which ovulated before day 21 postpartum compared to animals which ovulated during Interval III. Follicular growth may have been reflected in variable estradiol concentrations in observed blood collected from most cows prior to first ovulation.

The interval to first ovulation was extended due to increased milk yield. Cows in interval III produced more ($P<.05$) milk than cows in intervals I and II, while cows which returned to estrus before day 15 postpartum produced less ($P<.05$) milk than cows in intervals II and III. Changes in body weight and energy balance were not different among interval groups, while greatest cumulative or percent loss in body weight occurred during the first two weeks postpartum.

Blood was collected at frequent intervals for 4 hours on day 7 and day 14 postpartum to study change in LH and glucocorticoid concentrations. Glucocorticoids alone, appeared to have no effect on the interval to first ovulation while the interaction between factors involved

in energy balance and glucocorticoids was significant ($P<.05$) in accounting for more than 35% of the variation in days to first ovulation.

LH concentrations were increased ($P<.01$) in cows which ovulated before day 15 postpartum as were the number of episodic LH peaks ($P<.05$) and magnitude ($P<.05$) of the largest LH peak. While LH concentrations were not different among cows ovulating in intervals II and III, cows in interval II had more ($P<.05$) episodic LH peaks compared to animals which ovulated after day 20 postpartum. LH concentrations were higher ($P<.001$) on day 14 than on day 7 postpartum, and LH was negatively related to days to first ovulation ($r = -.40$, $P<.05$).

Both estradiol and the interaction between LH and estradiol were significant ($P<.05$) in accounting for variation in days to first ovulation when added stepwise to a model including the glucocorticoid and energy balance cross-product interaction. These results indicated that pituitary-ovarian relationships may be important in re-establishing ovarian activity. Timing and onset of episodic LH peaks and increased LH concentrations may stimulate ovulation, while estradiol secretion may control LH production in the absence of preovulation progesterone. Glucocorticoids appeared to respond to energy demands and may have been involved in regulation of basal metabolism controlling the negative effects of milk yield on resumption of postpartum ovarian activity.

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Jeffrey Smith Stevenson

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INTRODUCTION

In order to increase efficiency of milk and meat production, methods are needed to reduce time from parturition to conception, thereby decreasing calving intervals in dairy and beef cattle. To develop these methods, it is necessary to understand endocrine factors affecting duration of postpartum anestrus in cattle. Endocrine changes during estrous cycles, pregnancy and parturition in the bovine are well documented. However, information on endocrine changes which occur from parturition until resumption of normal estrous cycles is incomplete.

Various hormonal treatments have been employed to initiate early ovarian activity in cattle. Administration of gonadotropins, steriods, prostaglandins and other hormones alone or in combination has resulted in some successes, however processes whereby these treatments initiated ovulation are not well understood. Thus, numerous gaps in knowledge of postpartum reproductive physiology remain to be filled. This study was undertaken to identify some factors affecting duration of postpartum anestrus.

The primary purpose of the present study was to characterize some endocrine and nonendocrine factors responsible for initiating or delaying postpartum ovarian activity. Specifically, the objectives were 1) to describe changes in blood concentrations of LH, progesterone, estradiol and glucocorticoids from parturition until first ovulation in lactating dairy cows; 2) to examine the effect of early or late return to cyclic

ovarian activity on subsequent postpartum estrous cycles; and 3) to investigate the relationships between hormone concentrations and net energy balance at different postpartum periods.

REVIEW OF LITERATURE

General Overview and Terminology

An increasing interest in controlling animal reproduction has led to the study of mechanisms involved in control of reproductive processes in the postpartum female. Many factors are involved in regulating the interval from parturition to the initiation of postpartum ovarian activity. Principal among these factors is the relationship among endocrine glands including the hypothalamus, pituitary, thyroid, adrenal and ovary. Other factors, whose effects are probably mediated through endocrine pathways, include such parameters as milk yield, intensity and frequency of milking or suckling, energy balance, nutrition, parity, season, genetics, and changes in body weight or condition. These factors will be reviewed and discussed in brevity as they related to the initiation of postpartum ovarian activity in the bovine with special emphasis on the dairy cow.

Postpartum Reproductive Events

Interval to First Ovulation and Estrus

Postpartum intervals to first ovulation and first estrus in dairy cows are apparently much shorter than in beef cows. Casida (1971) surveyed the literature and reported that the interval to first estrus in dairy cows averaged 30 to 72 days, while for beef cows the average was 46 to 104 days. An updated summary of the literature including the

interval from parturition to first ovulation and first estrus, percent of cows in estrus at first ovulation, and effects of frequency of milking (M) or suckling (S) in dairy cows is shown in Table 1.

Table 1. -- Days to first ovulation, first estrus and percent in estrus at first ovulation for milked (M) or suckled (S) cows.

Days to Ovulation	First Estrus	% Estrus at 1st Ov.	Milked or Suckled	Reference Cited
--	46	--	2X	Clapp, 1937
--	50-90	--	2X	Herman and Edmondson, 1950
--	46-104	32	2X	Casida and Wisnicky, 1950
--	32	--	2X	Olds and Seath, 1953
--	33	--	2X	Buch et al., 1955
--	57	--	2X	Chapman and Casida, 1957
--	54	--	2X	Wiltbank and Cook, 1958
--	32	38	2X	Menge et al., 1962
15	--	23	2X	Morrow et al., 1966
16	45	53	2X	Saiduddin et al., 1968
13	28	--	2X	Marion and Gier, 1968
14	31	--	2X	Marion and Gier, 1968
16	37	--	2X	Marion and Gier, 1968
20	--	--	2X	Pope et al., 1969
--	32-43	--	2X	Gardner, 1969
--	22	--	2X	Wagner and Hansel, 1969
45	56	--	2X	Wagner and Oxenreider, 1971
23	37	34	2X	Callahan et al., 1971
16	--	0	2X	Hackett et al., 1973
18	55	--	2X	Williams et al., 1973
--	36	--	2X	Edgerton and Hafs, 1973
--	49	75	2X	Hartigan et al., 1974
24	38	25	2X	Britt et al., 1974
30	39	55	2X	Whitmore et al., 1974
--	--	41	2X	Hurnik et al., 1975
20-23	36-64	10-44	2X	King et al., 1976
19	--	--	2X	Carruthers et al., 1977
--	69	--	4X	Clapp, 1937
23	--	--	4X	Carruthers et al., 1977
--	72	--	S	Clapp, 1937
--	84	--	S	Wiltbank and Cook, 1958
--	28	--	S	Wagner and Hansel, 1969
52	54	--	S	Wagner and Oxenreider, 1971
39	--	--	S	Carruthers et al., 1977

In general, it appears that first ovulation occurs before day 20 postpartum, and in most cases, is not accompanied by identifiable signs of estrus. Similarly, first ovulation without estrus has been reported for suckled beef cows (Casida and Wisnicky, 1950; Short et al., 1972; Bellows et al., 1974). Incidence of ovulation with estrus was the same in cows returning to estrus early or late during the postpartum period, regardless of their health status (Morrow, 1969). This indicated that frequency of "silent" estrus was affected by factors related to the number of postpartum ovulations rather than the length of the postpartum interval or health status of the animal.

These data also indicate that increasing frequency of milking from two to four times daily or allowing calves to suckle cows further increased the interval from calving to first ovulation. Further discussion of these effects are reviewed in a later section.

Interestrus Interval and Estrous Behavior

The interval between first and second ovulation in the postpartum period is shorter than subsequent interovulatory intervals (Morrow et al., 1966, Marion and Gier, 1968; Pope et al., 1969; Corah et al., 1974). This interval is shorter than the normal estrous cycle length when it occurs first before 20 to 25 days postpartum, indicating the interval between first and second ovulation is influenced by the length of time from calving to first ovulation. Furthermore, cows which return to estrus early postpartum have a higher frequency of shorter cycles, while those cows which ovulate at later times postpartum have average to long first cycles (Menge et al., 1962; Williams et al., 1973). Other factors including metritis, dilation of the uterus, and reduced

corpus luteum weights during luteal phase have been shown to shorten duration of an estrous cycle. This suggests that uterine cellular debris or infection may inhibit both neural and hormonal feedback mechanisms and thereby alter the cycle length (Lauderdale et al., 1968; Callahan et al., 1971).

As the number of postpartum ovarian cycles increases, a gradual increase in mounted and mounting activities and mounting/mounted ratios occurs (Hurnik et al., 1975). This indicates that estrous related activities are sub-normal early in the postpartum period probably due to hormonal imbalances. Incidence of standing estrus increases as the postpartum period advances until all cows exhibit estrus prior to third and fourth ovulations (Britt et al., 1974; King et al., 1976).

Cows producing over 7272 kg of milk per lactation have more silent estrous periods during the 60-day postpartum period than do those cows producing less milk (Morrow, 1969). This may be due to the added physiological stress of lactation and increased demand for body nutrients.

Ovarian Histology

During the course of pregnancy the ovaries remain quiescent with the exception of progesterone secretion by the corpus luteum of pregnancy. Ovarian follicles decrease in size during late gestation and are very small at parturition (Nalbandov and Casida, 1940; Labhsetwar et al., 1964). Both histological examinations and palaptions per rectum revealed that follicles are present within a few days after parturition as the ovaries become more responsive to endogenous gonadotropin secretions (Cole and Hughes, 1946; Allen et al., 1957; Marion and Gier, 1968; Saiduddin et al., 1968).

As early as one week postpartum ovarian follicles equal to 10 mm in diameter are present and these increase in size until day 14, but no further increase in size occurs by day 30 postpartum (Wagner and Hansel, 1969). Similar follicular development has been observed on ovaries of suckled and anemic cows suggesting that adequate FSH is present, while continued presence of nearly mature follicles suggests a possible deficiency in LH. Some thickening and infolding of granulosa cells in these follicles may indicate unsuccessful attempts at luteinization (Wagner and Hansel, 1969).

More first ovulations occur on the ovary contralateral to the ovary which bore the corpus luteum of pregnancy, and the right ovary is apparently more active than the left ovary for ovulations occurring during the first 40 days postpartum (Morrow et al., 1966; Saiduddin et al., 1967; Foote and Peterson, 1968; Hartigan et al., 1974). An increased incidence of follicular cysts prior to and during the first ovarian cycle was observed among cows during winter compared to summer months (Morrow et al., 1966; Marion and Gier, 1968). These conditions indicate a possible insufficiency of LH or refractoriness of developing follicles to hormone stimulation.

The corpus luteum (CL) of pregnancy regresses rapidly and is palpable from 4 to 14 days following parturition. By two weeks postpartum the CL becomes difficult to palpate and exists as a small firm mass (Morrow, 1969). Histological evidence indicates that by day 7 postpartum, no luteal cells appear viable or functional, but a large amount of vascular and connective tissue remnants remain (Wagner and Hansel, 1969). Blood concentrations of progesterone confirm an abrupt cessation of progesterone secretion at parturition (Labhsetwar et al., 1964;

Gomes and Erb, 1965; Erb et al., 1968; Stabenfeldt, 1969; Edqvist et al., 1973; Smith et al., 1973; Corah et al., 1974).

Uterine Involution

Uterine involution is difficult to evaluate except by direct histological examination. Rectal palpation yields information on size and tone, but cannot confirm whether epithelial regrowth has occurred in the uterus. Most values reported are based on rectal palpation and indicate a range of 25 days (Morrow et al., 1966) to 50 days (Buch et al., 1955) from parturition to uterine involution. Slow regression occurs during the first week followed by an accelerated regression during the second week. Studies based upon histological criteria suggest that by 20 to 30 days postpartum most cows have nearly complete uterine involution (Gier and Marion, 1968; Wagner and Hansel, 1969).

Uterine weights at slaughter reveal a reduction in weight of uterine horns from lactating cows (milked or suckled) compared to non-lactating cows (Wagner and Oxenreider, 1971). Following ovulation, weight of the previously gravid horn of lactating cows increases compared to that in cows which did not ovulate. However, uterine horns of cows which are cycling are still smaller than those observed in non-lactating cycling cows.

Postpartum Endocrine Gland Content and Hormonal Changes

Anterior Pituitary Gonadotropin Content

Based on bioassay techniques, pituitary LH content is low at parturition and steadily increases from parturition until ovulation, while FSH

content is comparatively high and decreases over the same interval (Labhsetwar et al., 1964; Saiduddin and Foote, 1964; Saiduddin et al., 1968; Wagner et al., 1969). Immediately after calving the pituitary contains more FSH and less LH than at 20 days postpartum. LH content is low on day 1 when compared to days 10, 20, and 30 postpartum, while FSH decreases from day 1 to 20 and is lower on day 20 compared to either days 10 or 30 postpartum. Suckling or milking does not affect anterior pituitary weight.

During pregnancy a steady decrease in pituitary gonadotropin activity occurs concomitantly with a rise in placental estrogen production and decline in follicular growth (Nalbandov and Casida, 1940). At parturition pituitary LH content is less than that found at advanced stages of preganacy or at 20 days postpartum. This suggests that reduced synthesis of LH occurs before or within a few days after calving (Erb et al., 1971a). In contrast, FSH content at parturition is even higher than that found during the first and second postpartum ovarian cycles. Higher pituitary FSH content at parturition apparently represents releasable stores since follicles decrease in size during late pregnancy and are small at parturition, but become palpable within 5 to 7 days after parturition (Morrow et al., 1966; Marion and Gier, 1968).

Presumably the only influence or feedback on pituitary gonadotropin content appears to be limited to ovarian hormones (Foote, 1971), since ovariectomy at parturition decreases pituitary LH content and increases FSH content, while hysterectomy has no effect (Quevedo et al., 1967).

Blood LH and FSH Concentrations

During the last stages of pregnancy and the periparturient period, LH concentrations are low in blood collected daily or weekly (Erb et al., 1971a; Arije et al., 1971; Edgerton and Hafs, 1973). LH increases in blood within one to two weeks postpartum (Echternkamp and Hansel, 1973; Randel et al., 1976) and parallels the increase in pituitary LH content. FSH concentrations in blood have not been measured in the postpartum period due to lack of a workable radioimmunoassay for bovine FSH. However, blood FSH must be similar to that found in normal cycles due to the increase in follicular growth previously reviewed.

In blood samples collected at 15, 30, or 60 minute intervals, distinct LH peaks or episodic release of LH have been reported to occur prior to first ovulation (Schams et al., 1972). Number of LH peaks per week and magnitude of the largest peak per week increases in suckled beef cows as first estrus approaches (Humphrey et al., 1976). Similarly in dairy cows, suckling compared to milking depressed average blood LH and retards the onset of episodic LH release (Carruthers et al., 1977).

Pituitary TSH Content and the Thyroid Gland

Bioassay results indicate that pituitary TSH content is variable among postpartum cows (Wagner et al., 1969; Wagner and Oxenreider, 1971). Animals fed thyroprotein showed a marked increase in thyroid cell height and decreased pituitary TSH content. Similarly, when thyroxin was given, pituitary TSH content was decreased (McQuillan et al., 1948; D'Angelo, 1958; Bakke et al., 1964). In general, the thyroid-pituitary relationship appears to have some role in the inhibition of postpartum

ovulation, since thyroprotein fed animals had increased intervals to first estrus when compared to control animals (Wagner and Hansel, 1969). However, conception rates were similar in control and treated cows once estrous cycles were re-established indicating that the thyroid-pituitary role is probably mediated by the influence of thyroid hormones on basal metabolic rates.

Posterior Pituitary and Oxytocin

Studies on the role of the posterior pituitary and oxytocin during the postpartum period have mostly been limited to the effects of oxytocin on milk ejection. However, in one study infusions of oxytocin during this interval caused an increase in plasma cortisol concentrations in non-lactating cows (Wagner and Oxenreider, 1971) but in another study no effect on plasma cortisol was observed (Cameron and Fosgate, 1964). While oxytocin was shown to affect plasma cortisol in non-lactating postpartum cows, similar studies in lactating cows did not yield consistent responses. These data suggest that oxytocin may not be acting directly on the hypothalamus or pituitary to affect the postpartum interval.

Adrenal Gland and Blood Glucocorticoid Concentrations

In postpartum cows milked twice daily, blood glucocorticoid concentrations vary greatly (Edgerton and Hafs, 1973). However suckled cows have lower adrenal contents of both progesterone and cortisol than do milked animals indicating that in the suckled cow there may be an increase secretion or decreased synthesis of adrenal steriods (Wagner and Hansel, 1969).

Milking or suckling resulted in an increase in blood glucocorticoid concentrations (Wagner and Oxenreider, 1971), but no differences were

found between suckled and milked animals. Failure to find any consistent changes in glucocorticoids during the early postpartum period may indicate that the adrenal has a passive role in terms of its effect on the postpartum interval. However, since adrenal gland stimulation occurs more often in suckled cows adrenal gland function may have an important role in controlling postpartum ovarian activity since both corticoids and progesterone are secreted when the gland is stimulated (Balfour et al., 1957; Short, 1960; Gwazdauskas et al., 1972).

Estrogen Concentrations in Blood and Urine

During pregnancy estrone, estradiol-17α, and estradiol-17β have been identified in placental extracts (Gorski et al., 1959) and increased estrogen concentrations in both feces and urine occur coincident with increased estrogen content of fetal cotyledons (Mellin et al., 1966). During the last 30 days of gestation, estrogen excretion in urine remains relatively low and increases gradually as parturition approaches. During an 8 hour period including parturition urinary excretion of estrogens is higher than during the 40 hour period before or after calving. Following parturition estrogen excretion is two to six times higher during the first 42 days postpartum than at 0, 7, and 14 days after conception (Erb, et al., 1971b; Randel and Erb, 1971). However, rate of excretion of total estrogens decreases rapidly from 0.5 to 8 days postpartum (Hunter et al., 1970). In spite of high urinary estrogen excretion, few cows show estrus at this time.

Serum estradiol-17β, estrone and total estrogen concentrations increase linearly during the last 25 to 30 days of gestation (Smith et al., 1973; Hendricks et al., 1972; Stellflug, 1972; Edqvist et al.,

1973). Serum estrogen concentrations peak one to two days prior to parturition and then decline by four to eight days postpartum (Holm and Galligan, 1966; Robinson et al., 1970; Arije et al., 1971; Echternkamp and Hansel, 1973; Smith et al., 1973; Corah et al., 1974). Estradiol concentrations are higher at calving than at days 1, 4, 5, 6, 8, 9, and 12 postpartum (Echternkamp and Hansel, 1973).

Estradiol concentrations fluctuate considerably in blood collected from some cows between parturition and first estrus, while in others concentrations remain low until two to three days before estrus and then increase. Apparently, pituitary FSH content is also decreasing during this interval and can account for the erratic variation in circulating estrogen concentrations as follicles mature and then undergo atresia (Echternkamp and Hansel, 1973). The variable follicular growth known to occur during this period (Wagner and Oxenreider, 1971; Morrow et al., 1966) may be reflected in variable estrogen secretion.

Systemic Progesterone Concentrations

During the last two weeks of gestation, changes in serum progesterone concentrations were small relative to the precipitous decline that occurs one to two days prior to parturition (Stabenfeldt et al., 1970; Hendricks et al., 1972; Schams et al., 1972; Smith et al., 1973). Following parturition, serum progesterone concentrations are low or undetectable until development of the first corpus luteum after first ovulation (Arije et al., 1971; Echternkamp and Hansel, 1973). In contrast, several investigators have reported a pre-estrus rise in progesterone which exceeds 1 ng/ml for three to five days and then declines at estrus (Pope et al., 1969, Donaldson et al, 1970; Corah et al., 1974; LaVoie et al., 1976; Humphrey et al., 1976).

Progesterone concentrations in plasma are lower during the first compared to the second postpartum estrous cycle (Edgerton and Hafs, 1973). This apparently indicates a lack of sufficient luteotropin, luteinization or general imbalance of hormone synchrony during the first estrous cycle compared to most normal cycles.

Hormone Therapy

Numerous studies have been conducted in an effort to alter postpartum physiology by administering exogenous hormones. Until synthetic gonadotropin-releasing hormone (GnRH) was available for research purposes, most therapy yielded negative results. Progesterone (2.2 mg/kg body weight) given 14 days postpartum was shown to delay postpartum ovulation and estrus in beef cows (Foote et al., 1960). However, smaller doses of progesterone had the opposite effect (Foote and Hunter, 1964).

Estradiol or estradiol following progesterone decreased the interval from parturition to estrus and ovulation in suckled beef cows (Ulberg and Lindley, 1960; Saiduddin et al., 1968; Brown et al., 1972). Treatment of suckled beef cows and dairy cows milked twice daily with single injections of estradiol between 9 and 15 days postpartum demonstrated that estrogen treated cows returned to estrus and ovulated earlier than controls (Foote, 1971). Milked cows were more responsive to estrogen treatment than suckled cows since ovulation occurred one to two days post treatment whereas suckled cows ovulated an average 20 to 40 days after estradiol treatment.

Several studies in which GnRH was given at intervals ranging from 1 to 40 days postpartum demonstrate a decreased interval to first ovulation in dairy cattle (Britt et al., 1974; Manns and Richardson, 1976; Fernandes et al., 1976; Kesler et al., 1976). It appears that pituitary responsiveness

to GnRH is not restored until 7 to 8 days postpartum, suggesting that until then, releasable stores of LH are not available in sufficient quantity to induce ovulation.

When similar GnRH treatments were given to suckled beef cows at one month postpartum, only two of six cows which ovulated had regular estrous cycles subsequent to treatment (Britt et al., 1975). However, when similar treatments of either GnRH or PMSG (pregnant mare serum gonadotropin) were administered to beef cows 42 days postpartum or 7 days post-weaning, pituitary responsiveness to exogenous stimulation was demonstrated (Echternkamp, 1974). Thus, by some unknown mechanism suckling blocked establishment or normal estrous cycles after GnRH in early postpartum suckled cows but not in cows whose calves were weaned several days before GnRH or gonadotropin treatment.

Non-endocrine Factors Affecting Postpartum Ovulation

Level of Energy and Nutrition

Limiting energy intake while feeding recommended levels of protein and minerals significantly lengthens the postpartum interval to the appearance of follicles greater than 10 mm in diameter (Wiltbank et al. 1964; Wagner and Oxenreider, 1971). Energy intake has been shown to be important in the early return of postpartum ovarian activity.

Reduced energy intake has been reported to cause mild hypoglycemia in cattle (McClure, 1968) which may limit glucose availability to glucose-dependent brain tissue such as the hypothalamus and pituitary. Glucose is also rate limiting to milk secretion and lactation (Hardwick et al., 1961; Linzell, 1967; Kronfeld et al., 1968). Thus, glucose is in great demand during early lactation and could account for lack of gonadotropin

synthesis and release from pituitary tissue. It would appear that competition for blood glucose by mammary and endocrine glands may further increase the possibility of reduced function of all endocrine glands, thus causing a delay in the interval to first postpartum ovulation. However, this apparently is not the case since most studies indicate that low energy intake has no delaying effect on initiation of postpartum ovarian activity in milked cows (Gardner, 1969b; Whitmore et al., 1974; Corah et al., 1974; Ray et al., 1974), nor in cows not subject to lactational stress (Oxenreider and Wagner, 1971).

Body condition at parturition may be an important factor affecting interval to first postpartum ovulation (Wiltbank et al., 1962). Since body stores of metabolizable energy may be used to supplement deficient postpartum energy intake, it is impossible to conclude that energy intake per se is a factor affecting postpartum interval without knowing that energy balance of the animal.

Changes in Body Weight and Condition

Suckled and milked cows have larger postpartum body weight losses than non-lactating cows, however body condition at calving is an important factor in determining the amount of body weight lost following parturition (Oxenreider and Wagner, 1971). It appears that body condition at parturition is more important than postpartum energy intake, since cows that are subject to high prepartum energy levels (high) followed by reduced postpartum energy levels (low) return to estrus sooner than cows that receive low to high, low to low, or high to high feed group treatments (Wiltbank et al., 1964). If energy were the complete answer then cows changed from low to high feed groups would be expected to ovulate sooner before energy was used to put on body weight and condition.

Most milked cows apparently regain their calving weight from 8 to 36 weeks postpartum depending upon their level of postpartum energy intake and milk yield (Gardner, 1969a). Body weight change apparently follows energy balance, since body weight is highly correlated to energy balance (Carstairs, 1975). Cows which lose less weight during the postpartum period may have a shorter interval to the appearance of the first postpartum corpus luteum (Menge et al., 1962).

Level of Milk Yield and Suckling

In general, suckling and increased milking frequency (4X vs 2X daily) increases the postpartum interval to first ovulation and estrus (Clapp, 1937; Wiltbank and Cook, 1958; Carruthers et al., 1977). Cows suckled by three or four calves had their first postpartum ovulation 23 days later than cows milked twice daily even though suckled cows were nursed for only one hour twice daily (Moller, 1970). Thus, the effect of suckling on postpartum ovarian activity may differ from that of milking per se. Removal of the calf at birth or a few days later (early weaning) causes rapid initiation of cyclic ovarian activity in cows (Oxenreider, 1968; Saiduddin et al., 1968; England et al., 1973; Laster et al., 1973; Bellows et al., 1974).

The interval from parturition to first estrus is shorter in cows mastectomized prior to calving compared to cows with intact mammary glands suggesting that presence of the gland itself may have an effect on duration of postpartum anestrus (Short et al., 1972). Reports concerning the effect of either the whole or 120 day lactations on the interval to first estrus indicate that level of milk yield is either slightly or not at all correlated with the interval to first estrus (Herman and Edmondson, 1950;

Olds and Seath, 1953; Menge et al., 1962). However, when cows were divided into different production groups based on daily yields of low (<22 kg), medium (22 to 30 kg), and high (>30 kg) producers, a significant increase in days to first ovulation and first estrus was associated with each increased increment in daily milk yield (Marion and Gier, 1968). In another large study where different nutritional levels and levels of genetic ability for milk production were studied, postpartum ovarian activity was concluded to be more closely associated with milk production than with average daily TDN intake (Whitmore et al., 1974). These results indicate that milk yield in general is negatively related to the interval to initiation of postpartum ovarian activity.

Genetics

Genetic variation in interval to first ovulation and incidence of quiet ovulations during the postpartum period has been measured. Length of postpartum anestrus for all cows showed intra-cow correlations (repeatability) of nearly .20, while data from cows that exhibited estrus at first ovulation showed a repeatability of .48 (Menge et al., 1962; Labhsetwar et al., 1963; Saiduddin et al., 1968). These differences indicate that genetic variation in these reproductive traits exists among different lines of cattle since females produced by inbreeding had postpartum intervals to first estrus ranging from 21 to 49 days, while outbred females only varied from 30 to 36 days.

Variation in interval from parturition to first estrus for groups of cows with genetically high or low milk producing ability has also been compared. The interval to first estrus was longer for cows with a higher compared to low genetic potential for milk production (Whitmore et al., 1974).

Season

In some studies, season of calving has been observed to affect the interval from parturition to first estrus (Chapman and Casida, 1937; Buch et al., 1955; Carman, 1955), while in others no seasonal effect was observed (Herman and Edmondson, 1950; Wiltbank and Cook, 1958; Morrow, 1969). In those studies where an effect was observed, cows calving during the winter had the longest interval to first estrus; followed by spring, autumn and summer, respectively. Seasonal differences in incidence of cystic follicles, cystic corpora lutea, and quiet ovulations have also been reported (Wiltbank et al., 1953; Roberts, 1955; Henricson, 1957; Morrow, 1969). Cystic corpora lutea occur more frequently during August to January than during the rest of the year. This fluctuation is similar to the increased incidence of cystic follicles occurring from November to January. "Silent" ovulations appear to occur most often during the early spring and summer months, probably due to increased number of cows calving during that period of time. Thus, season appears to play only a minor role in postpartum anestrus.

Age and Parity

Parity has been reported to influence interval to first estrus in some studies (Hammond and Sanders, 1923; Casida and Winsnicky, 1950), but not in others (Buch et al., 1955; Warnick, 1955; Foote et al., 1960; Morrow, 1969). If parity does affect the postpartum interval, cows with four or more calving intervals tend to have slightly longer intervals to first estrus and ovulation than do younger multiparous cows, and first calf heifers, 1.5 to 2.5 year olds have longest intervals. The prolonged interval for younger cows may be due to insufficient energy provided to

meet the body requirements of growth and concurrent lactation (Menge et al., 1962; Wiltbank et al., 1964).

Postpartum Abnormalities

The effect of disease during the periparturient period on the interval to first ovulation has been reviewed (Morrow, 1971). Cows were classified as "abnormal" if abortion, dystocia, retained fetal membranes, metritis, milk fever, acute mastitis, ketosis, or other debilitating disease occurred during the postpartum period. In general, "abnormal" cows had longer intervals to first estrus than "normal" cows, however, cows with only clinically detected uterine disease have been reported to have a shorter interval to first estrus (Hartigan, 1974). "Abnormal" cows produced more milk and had completed more lactations than cows with "normal" parturitions indicating that influence of increased milk yield, parity, age and disease may have increased the postpartum interval.

In another study, metritis, cystic ovaries, and combination of both pathologies were shown to generally increase the interval to first ovulation, first estrus, first 1.5 cm corpus luteum, first 1.0 cm follicle and duration of the first estrous cycle (Callahan et al., 1971). Regularity of estrous cycle length in cows following retention of fetal membranes was not different from that in clinically normal cows (Erb et al., 1958a), but subsequent genital infections in cows with retained placentas undoubtedly increased cycle lengths from 26 to 35 days in most cows (Erb et al., 1958 b).

Summary

The postpartum interval to first ovulation is approximately three weeks in dairy cows milked twice daily, but increasing frequency of milking or suckling prolongs the period. Interval between first and second ovulations is positively correlated with duration of postpartum anestrus. Cows which return to estrus early following calving have a higher frequency of shorter first cycles than those cows which ovulate at later postpartum periods. "Silent" estrus occurs frequently prior to first ovulation and decreases at subsequent periods of estrus. Inherent cow differences in occurrence and intensity of estrous behavior are evident, while the intensity of behavior associated with estrus increases as the postpartum period progresses.

Follicular growth is evident within a few days after calving, due to FSH released from the pituitary gland. Pituitary FSH content declines and LH content increases during the first 3 to 4 weeks postpartum. Pituitary responsiveness to GnRH is restored in milked cows by 10 to 12 days postpartum, while suckling depresses both pituitary responsiveness and subsequent normal ovarian responses to exogenous gonadotropins. Serum LH concentrations increase in an episodic pattern as ovulation approaches and follicles mature and ovulate due to this LH stimulation. The adrenal and thyroid glands play a passive role in regulating duration of postpartum anestrus and these effects are mediated by the influence of thyroid and glucocorticoid hormones on basal metabolism. Ovarian estrogens in serum decline in most cows following calving but remain increased in urine above that normally found during early gestation. Erratic estrogen secretion is observed during the anestrous period until a pre-estrus rise in serum estrogens occurs prior to first ouvlation.

The corpus luteum of pregnancy regresses rapidly and progesterone concentrations remain low or undetectable following calving until corpus luteum development after first ovulation. A pre-estrus rise in progesterone has been noted in some studies.

Limiting postpartum energy may prolong the interval to first estrus, however prepartum levels of energy and body condition at calving appear to be more important in affecting the occurrences of postpartum ovarian activity while body weight loss postpartum follows postpartum energy balance. Other parameters such as genetic variation, season, parity and disease appear to have only minor effects on the interval to first ovulation after calving.

MATERIALS AND METHODS

This study was originated to describe several factors which may be responsible for initiation of postpartum ovarian activity. Specifically, the objectives of this study were 1) to describe changes in serum concentrations of estradiol, progesterone, LH and glucocorticoids from parturition until first ovulation in lactating cows; 2) to investigate relationships between hormone concentrations and energy balance; and 3) to determine effects of progesterone, estradiol, LH and glucocorticoids on interval to first ovulation.

Animal Handling

Primiparous (n = 12) and multiparous (n = 16) Holstein cows housed in a stanchion barn were used for this study. Cows were assigned to this project as they calved between February 29 and July 1, 1976 and remained on this project until the third postpartum corpus luteum (CL) was detected. All animals were individually fed a ration consisting of corn silage (30% dry matter), concentrate mix (11% to 15% crude protein), alfalfa hay (88% dry matter) and haylage (60% dry matter) designed to provide at least 100% of National Research Council (1971) requirements. All cows were milked twice daily at 0400 hours and 1600 hours. Body weights were measured weekly for all cows beginning 3 to 5 days after parturition.

Ovarian Activity and Blood Collection

Ovaries of each cow were palpated per rectum twice weekly to monitor follicular and corpus luteum development until the appearance of the third postpartum luteum. During this same period, blood samples were collected twice weekly by coccygeal arterial or veni-puncture to monitor ovarian activity by radioimmunoassay of serum progesterone (Convey et al., 1977; Appendix A). Serum estradiol was measured by radioimmunoassay (Stellflug et al., 1977; Oxender et al., 1977; Appendix B) in blood samples collected between parturition and the first postpartum ovulation.

Cows were monitored for estrous behavior between 0600 and 0800 hours following AM milking and between 1700 and 1800 hours following the PM milking. Each animal was fitted with a MateMaster rump-mounted heat detector (Stevenson and Britt, 1977) and exposed to a testosterone-treated marking heifer (Kiser et al., 1977; Stevenson and Britt, 1977) to aid in detection of estrus. Animals were recorded in estrus if they stood for a mounting herdmate, for the marking heifer, or if the detector was activated.

Cows were assigned to one of three calving days (Tuesday, Thursday, or Saturday) as they calved to increase efficiency of collecting blood samples one week (day 7 postpartum) and two weeks (day 14 postpartum) later. Due to calving day assignments cows may have been bled on days 6, 7, or 8, and on days 13, 14, or 15 postpartum. On days when blood samples were collected frequently, samples were taken via indwelling jugular cannulae at 15 minute intervals for 4 hours. Cows which ovulated prior to day 14 were not bled frequently on that day. Luteinizing hormone (LH) was monitored by radioimmunoassay (Convey et al., 1976; Appendix C) in all 15-minute blood samples (n = 16) collected from each

cow on days 7 and 14 postpartum. Glucocorticoids were measured by competitive protein binding (Smith et al., 1972, 1973; Appendix D) in alternate 15 minute samples (n = 8) for each animal.

Energy Balance

Feed samples were taken and analyzed for dry matter and protein percentage. From these measurements and National Research Council (N.R.C.) feed composition tables, a Net Energy (NE) value was calculated for each feedstuff in the ration (Appendix E). Feedstuff composition, feed intake, milk yield and composition, and changes in body weight were used to calculate energy balance for each cow once weekly during the first 6 weeks of lactation.

Energy requirements (Mcals NE lactation/day) were calculated from N.R.C. (1971) tables using weekly body weight, milk production and milk fat percent. Then energy intake was calculated (Mcals NE lactation/day) using feed intake data and the energy values of the feeds. Thus, energy balance indicates the difference between requirements and actual intake, or represents the difference between input and output of energy. A requirement was estimated according to N.R.C. standards to account for the energy needed for maintenance and milk production. If the energy consumed was not enough to meet these requirements then the cow had to supply this energy difference from endogenous sources or decrease her milk production. Under such circumstances, the cow would be in negative energy balance. If the amount consumed was equivalent to or greater than the amount required then the animal was at zero balance (100% N.R.C.) or positive balance (>100% N.R.C.) respectively.

Statistical Analysis

Cows were divided into ovulation interval groups according to the time of first postpartum ovulation. Cows which ovulated before day 15 (9 to 14), between days 15 and 20 (15 to 20), and after day 20 (21 to 34) were assigned to intervals I, II and III, respectively. Periods were used to designate either day 7 postpartum (Period I) or day 14 (Period II) when blood samples were collected frequently.

Analysis of variance for repeat measurements (Gill and Hafs, 1971) was used to analyze glucocorticoid and LH data. Number of LH peaks, number of samples per LH peak, and magnitude of largest LH peak were determined using a subjective method of choice by ten judges. The judges included were physiologists who had some experience and understanding of hormone secretion and its variation. LH profiles of each cow were plotted separately by period (day 7 and 14) with LH on the ordinate without concentration marks and time indicated on the abcissa representing 15 minute intervals for 4 hours. The graphs were randomized and given to the judges to examine. Each judge was told to circle all "peaks" and indicate all samples that fell within any peak. Data were analyzed by analysis of variance for repeat measurement where judge was the repeat measurement variable. A split-plot analysis of variance was employed to analyze estradiol, body weight, and energy balance by interval and period.

Significant differences between all interval and period means were determined using Bonferroni's t-test (Miller, 1966) for non-orthogonal comparisons. A Chi Square test was used for data analysis where appropriate. When multiple comparisons of Chi Square data were made, a Bonferroni Chi Square procedure was used (Kramer, 1972). Comparisons of other data

with only two means were by t-test if two means had homogeneous variances or appropriate t-test if variances were heterogeneous (Sokal and Rolhf, 1969).

Multiple regression analysis was employed to determine relationships between LH, glucocorticoids, estradiol and energy balance by period (day 7 and 14) and their effect on days to first ovulation (dependent variable). Several models were attempted to obtain the best model to fit the data. Variables that entered at earlier stages of the procedure could be rejected at later stages. The process continued until no more variables were admitted or rejected (Draper and Smith, 1966).

RESULTS AND DISCUSSION

First Ovulation, First Estrus, and Postpartum Estrous Cycles

Interval to first ovulation averaged 17.6 days (range: 9 to 34 days) for all cows and tended to be longer ($P<.10$) for pluriparous compared to primiparous cows (18.7 vs. 16.3 days, Table 2). Interval to first estrus did not differ between young and old animals (Table 2). These results are consistent with previous observations in beef and dairy cows (Buch et al., 1955; Warnick, 1955; Foote et al., 1960).

In the present study, nearly 30% of the first, 86% of the second and 87% of the third postpartum ovulations were preceded by estrus. Similar postpartum increases in estrous behavior, mounting and mounting/mounted ratios have been reported (Britt et al., 1974; Hurnik et al., 1975; King et al., 1976). Morrow (1969) observed that incidence of ovulation without estrus was not different in cows returning to ovarian activity early or late postpartum, regardless of their health status.

The first postpartum estrous cycle was shorter ($P<.05$) in duration than the second cycle (17.7 vs. 20.1 days, Table 3). Nine of 28 cows (7 primiparous and 2 pluriparous) had first estrous cycles less than 16 days in length. Similar observations of short first cycles have been made (Morrow et al., 1966; Marion and Gier, 1968; Pope et al., 1969; Corah et al., 1974) and tend to indicate that the first interovulation interval is shorter in duration than subsequent intervals for some cows. Cows which return to estrus early following parturition have a higher

frequency of short first cycles than those which ovulate at later postpartum intervals (Menge et al., 1962; Williams et al., 1973). In the present study four of nine animals which had first cycles less than 16 days in duration ovulated before day 15 postpartum.

Table 2. -- Effect of parity on interval to first ovulation and first estrus in postpartum dairy cows.

Parity	Interval in Days[a]	
	First Ovulation	First Estrus
Primiparous (n = 12)	16.3 ± 1.1[b]	27.7 ± 2.8
Pluriparous (n = 16)	18.7 ± 1.5[c]	26.1 ± 3.1
All cows	17.6 ± 1.0	26.4 ± 2.1

[a]Mean ± S.E.

[b,c]Means with dissimilar letters are different (P<.10)

It has been suggested that short first cycles could be due to decreased corpus luteum weight and function (Morrow, 1969). In the present study, primiparous cows had smaller (P<.05) diameter corpora lutea during the first estrous cycle than older cows. Furthermore, peak serum progesterone concentrations were decreased in primiparous cows compared to pluriparous cows during the same interval (P<.05, Table 3). Corpus luteum size or weight is directly related to its ability to synthesize and secrete progesterone. Progesterone content of corpora lutea on day 17 to 19 after first postpartum ovulation was 25% of that found during later cycles in control animals (Wagner, 1968). This indicates luteal function may not be well established during the first postpartum luteal phase.

Table 3. -- Effect of parity on corpus luteum diameter, peak progesterone concentrations and interval between first, second and third postpartum ovulations.

Parity	First Cycle[d]			Second Cycle		
	CL Diameter[a]	Progesterone Peak[b]	Cycle Length[c]	CL Diameter	Progesterone Peak	Cycle Length
Primiparous (n=12)	1.9 ± .1[e]	3.0 ± .5[e]**	16.4 ± 1.7*	2.1 ± .1	4.7 ± .6**	19.8 ± .7*
Pluriparous (n=16)	2.3 ± .1[f]	4.1 ± .3[f]	18.6 ± .8*	2.4 ± .1	4.6 ± .4	20.3 ± .6*
All Cows	2.1 ± .1	3.7 ± .3**	17.7 ± .5**	2.2 ± .1	4.7 ± .4**	20.1 ± .5**

[a]Maximum diameter (cm) [b]ng/ml [c]days [d]Mean ± S.E.

[e,f] (P<.05) within category

*(P<.10) between cycles

**(P<.05) between cycles

Overall, corpora lutea diameters were not found to be different between first and second postpartum cycles ($P>.10$, Table 3). However, serum progesterone concentrations were higher ($P<.05$) during the second cycle (Table 3). A similar increase in serum progesterone concentrations during the second cycle has been reported (Edgerton and Hafs, 1973). Data from the present study indicate that while normal development in corpus luteum size did not change from first to second cycle, luteal secretion of progesterone did not become well established during the first cycle. Early regression or failure of the corpus luteum to develop in a "normal" manner shortens the estrous cycle (Lauderdale et al., 1968).

Early regression of the first postpartum corpus luteum may be characteristic of normal ovarian activity associated with the period of rapid uterine involution. Postpartum uterine infections may account for shortened cycles and early regression during uterine involution (Callahan et al., 1971). Early regression may be due to earlier production of a uterine luteolysin or to reduced luteotropin or LH concentrations (Morrow, 1969). However, it has been reported that LH concentrations were higher during the luteal phase of the first postpartum cycle compared to the second cycle (Edgerton and Hafs, 1973). This may not account for decreased progesterone concentrations observed in the first estrous cycle, and for the reduced lifespan of the first postpartum corpus luteum. A significant negative correlation between LH and progesterone concentrations from day 1 to day 15 of the first cycle may be evidence for a negative feedback of progesterone on LH release (Echternkamp and Hansel, 1973). Decreased negative feedback would appear to account for higher LH and lower progesterone concentrations during

the first short cycle while increased negative feedback may have developed during the second estrous cycle and when lower LH and higher progesterone concentrations have been reported.

Progesterone Concentrations

Serum progesterone concentrations remained below 0.2 ng/ml from parturition until formation of the first postpartum corpus luteum (Figure 1). Within 2 to 3 days after first ovulation in all cows, serum progesterone increased ($P<.01$) above pre-ovulation concentrations. The progesterone profile for the first two postpartum estrous cycles is in Figure 2. The bimodal profile for progesterone during the first cycle indicated that several cows had short first cycles, then reovulated and were in the mid-luteal phase of their second cycle when the remaining animals were in estrus around 20 days after first ovulation. Since time of second ovulation was more varied than time of first ovulation, the overall progesterone profile for the second estrous cycle is somewhat extended (Figure 2) in comparison to that for any individual animal.

Prior to first observed estrus, nine cows had increased serum progesterone concentrations which exceeded 0.8 ng/ml for several days. Increase was apparently due to ovulation since corpora lutea were palpable on ovaries of 8 of 9 cows during the time progesterone concentrations were increased. Other reports indicated that rise in progesterone prior to first estrus and ovulation was absent in the cow (Arije et al., 1971; Echternkamp and Hansel, 1973).

In contrast to these data, a rise in blood progesterone concentrations prior to first observed estrus has been reported (Pope et al., 1969; Donaldson et al., 1970; Corah et al., 1974; LaVoie et al., 1976;

Figure 1. -- Serum progesterone concentrations collected in blood samples between parturition and end of first postpartum estrous cycle.

⊖ Mean progesterone concentration of blood samples from 28 lactating cows.

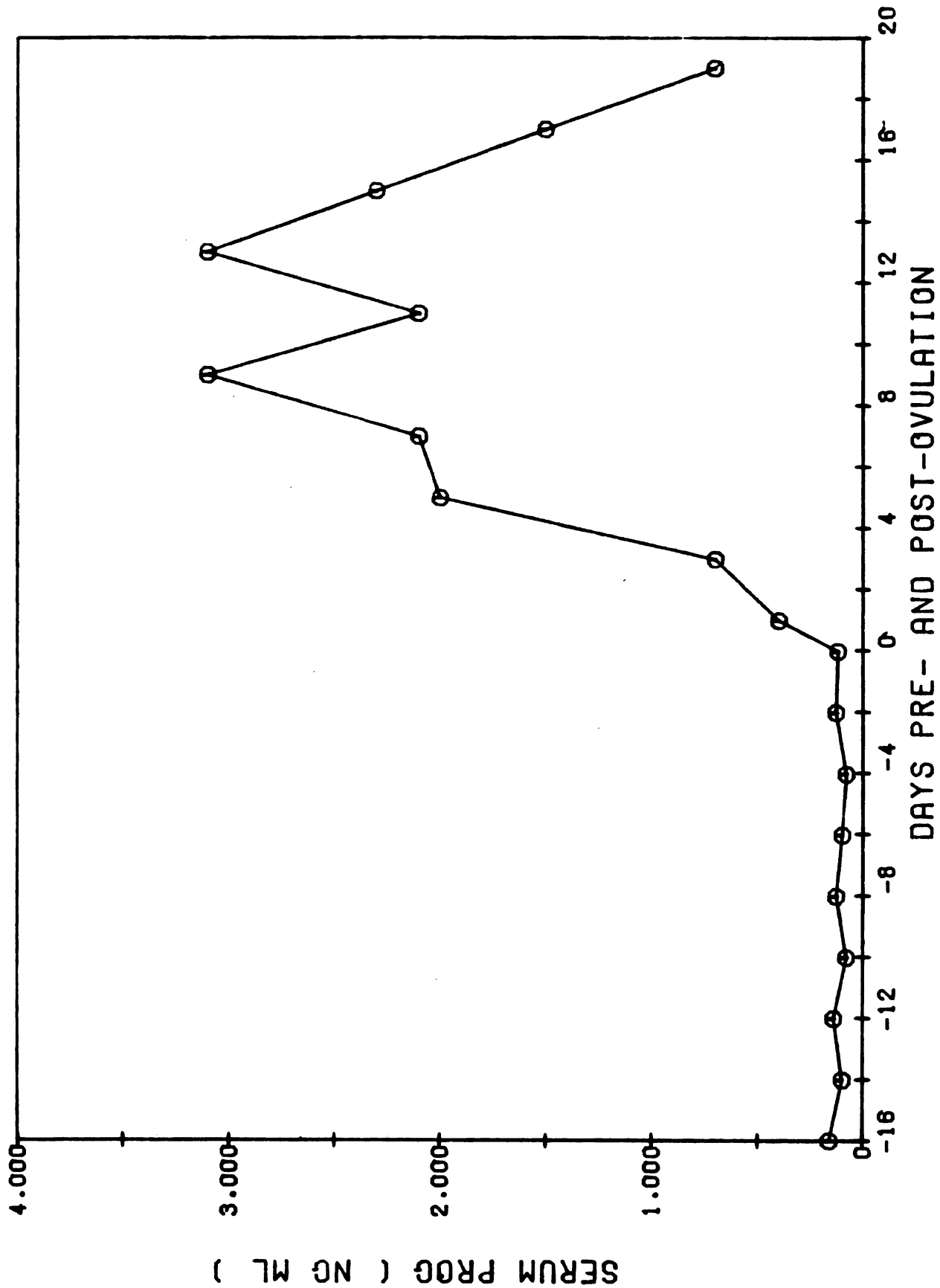
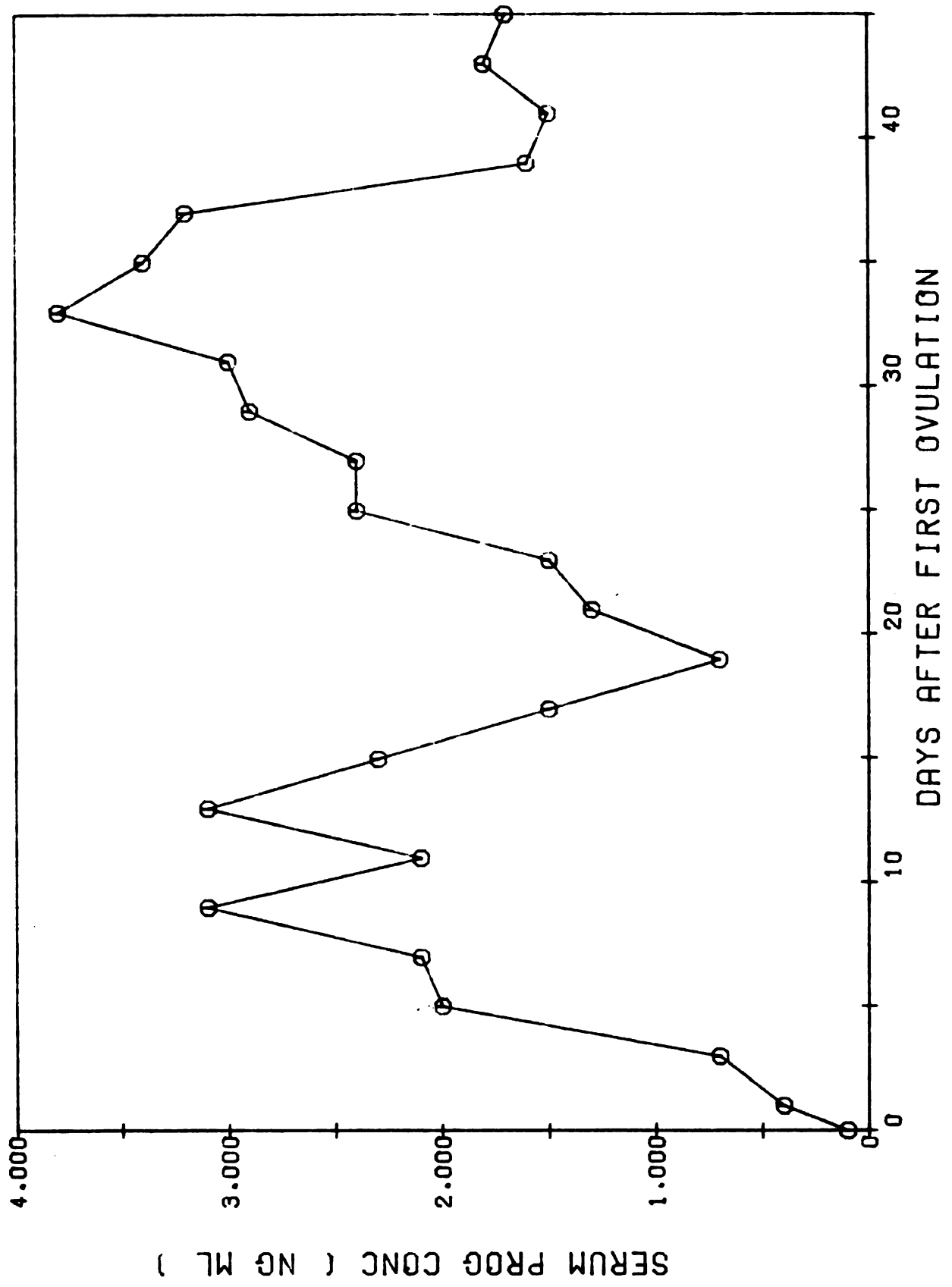

SERUM PROG (NG ML)
4.000
3.000
2.000
1.000
0
-16
-12
-8
-4
0
4
8
12
16
20
DAYS PRE- AND POST-OVULATION

Figure 2. -- Serum progesterone concentrations during first two postpartum estrous cycles.

⊖ Mean progesterone concentration of blood samples collected from 28 cows.


SERUM PROG CONC (NG ML)
4.000
3.000
2.000
1.000
0.0
0
10
20
30
40
DAYS AFTER FIRST OVULATION

Humphrey et al., 1976). However, lack of regular examination of ovaries for progesterone secreting structures may have led to the suggestion that the rise in progesterone may be due to adrenal secretion of progesterone, since the adrenal gland has been observed to contribute significantly to plasma progesterone concentrations (Gwazdauskas et al., 1972). Recently Castenson et al., (1976) indicated that the pre-estrus rise in progesterone was due to secretion from a corpus luteum in non-suckled postpartum beef heifers. Heifers were laparotomized following two consecutive days of increased plasma progesterone. Peripheral rise in progesterone to 2.5 ng/ml was due to recent CL formation resulting from ovulation in 7 of 8 and a luteinized follicle in 1 of 8 heifers. Following ovariectomy, progesterone concentrations were not detectable in plasma. This indicated that the rise in progesterone which proceded the first observed estrus in some cows was due to prior "silent" ovulation and recent corpus luteum formation.

Estradiol and Follicular Growth

Consistent patterns of estradiol secretion among cows within the same interval to first ovulation provided some conclusive evidence for the postpartum role of estradiol in spite of limited sensitivity of the assay in known physiological ranges. Pre-ovulation estradiol data from all cows was centered on the day of first ovulation. Sixteen days before ovulation (2 to 3 days postpartum), serum estradiol was higher ($P<.05$) compared to estradiol concentrations at subsequent sampling intervals. Just prior to estrus, estradiol increased ($P<.05$) to concentrations similar to those observed 2 to 3 days postpartum. Since changes in estradiol appeared to depend on the time of ovulation (i.e. Intervals

I, II and III), interval to first ovulation was confounded with the pattern of estradiol secretion. Therefore, profiles from individual cows within interval groups were examined.

Serum estradiol is plotted for all cows that ovulated 9 to 14, 15 to 17, 18 to 20 and 21 to 34 days after parturition (Figures 3, 4, 5, 6). Estradiol fluctuated considerably in blood samples collected from some cows between parturition and first ovulation, while in others the concentrations remained low until 2 to 3 days before estrus. Pituitary FSH content is known to decrease from parturition until 20 days postpartum and may account for follicular growth and estrogen secretion (Wagner et al., 1969; Echternkamp and Hansel, 1973).

Analysis of estradiol levels by interval to first ovulation and by period (day 7 and day 14) revealed a significant ($P<.03$) interval by period interaction (Table 4). Cows which ovulated after day 20 postpartum had higher estradiol concentrations on day 7 and these decreased ($P<.01$) to levels similar to those observed in other cows on day 14. Higher estradiol concentrations observed in these cows may have suppressed LH and FSH on day 7 and inhibited early ovulation.

Follicular growth was estimated by palpation during the first week postpartum (Table 5). There were no differences in follicular growth among cows in different intervals to first ovulation. Only two cows had palpable follicles greater than 10 mm in diameter by day 7 postpartum. One cow which ovulated on day 9 and two cows which ovulated on day 11 postpartum had no palpable follicles greater than 10 mm in diameter prior to ovulation. Some ovaries were difficult to reach and palpate during the first week postpartum and made determination of ovarian structures difficult or not possible in many cows and may have accounted

Figure 3. -- Serum estradiol concentrations of eight cows which ovulated between day 9 and day 14 postpartum.

Each data point is the mean estradiol concentration of three serum replicates of one cow measured during the postpartum period prior to ovulation. All data points were centered on day 14 postpartum to represent the day of first ovulation.

Legend	Cow No.	Days to First Ovulation
Q	1368	9
◱	1367	11
✳	1289	14
◈	1452	14
✿	1444	11
×	1340	14
◭	1466	11
+	1438	14

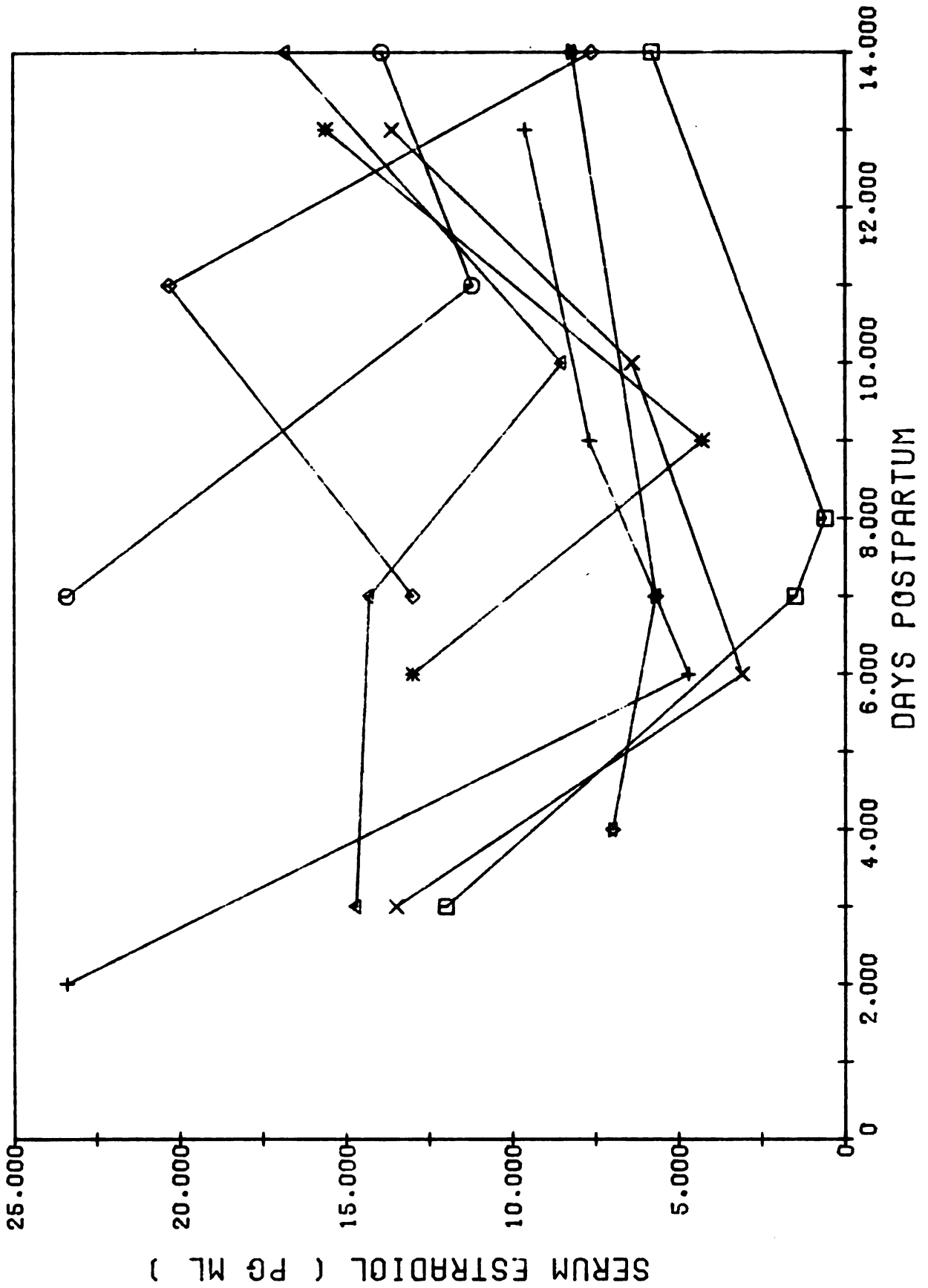

SERUM ESTRADIOL (PG ML)
25.000
20.000
15.000
10.000
5.000
0
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
DAYS POSTPARTUM

Figure 4. -- Serum estradiol concentrations of eight cows which ovulated between day 15 and day 17 postpartum.

Each data point is the mean estradiol concentration of three serum replicates of one cow measured during the postpartum period prior to ovulation. All data points were centered on day 17 postpartum to represent the day of first ovulation.

Legend	Cow No.	Days to First Ovulation
□	1351	17
○	1349	17
△	1229	15
+	1448	16
×	1274	16
◇	1435	15
☆	1416	17
✳	1411	17

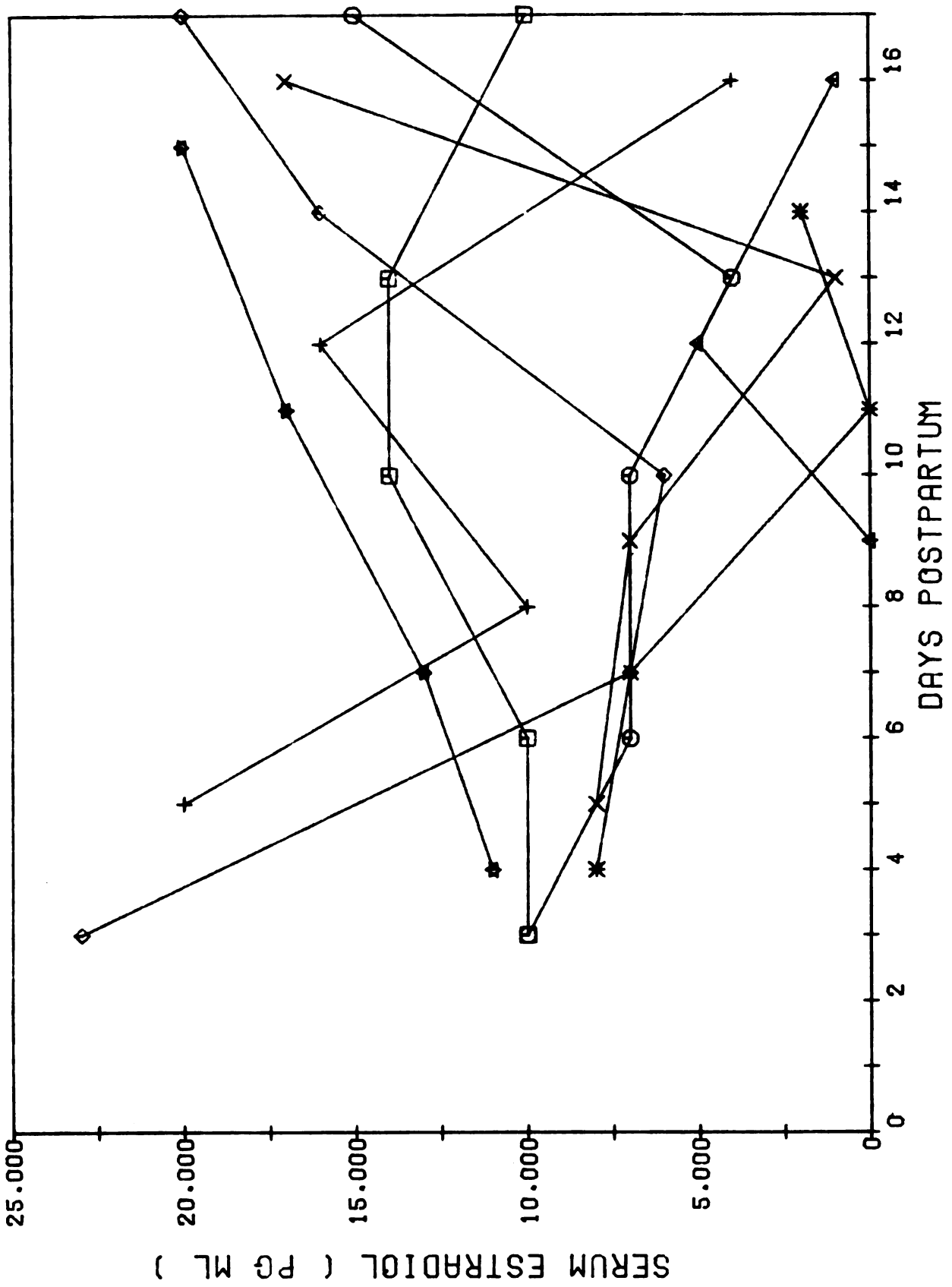

SERUM ESTRADIOL (PG ML)
25.000
20.000
15.000
10.000
5.000
0
0
2
4
6
8
10
12
14
16
DAYS POSTPARTUM

Figure 5. -- Serum estradiol concentrations of seven cows which ovulated between day 18 and day 20 postpartum.

Each data point is the mean estradiol concentration of three serum replicates of one cow measured during the postpartum period prior to ovulation. All data points are centered on day 20 postpartum to represent the day of first ovulation.

Legend	Cow No.	Days to First Ovulation
⊟	1401	19
⊖	1259	20
+	1280	18
◭	1364	18
✳	1424	18
×	1450	18
◈	1224	19

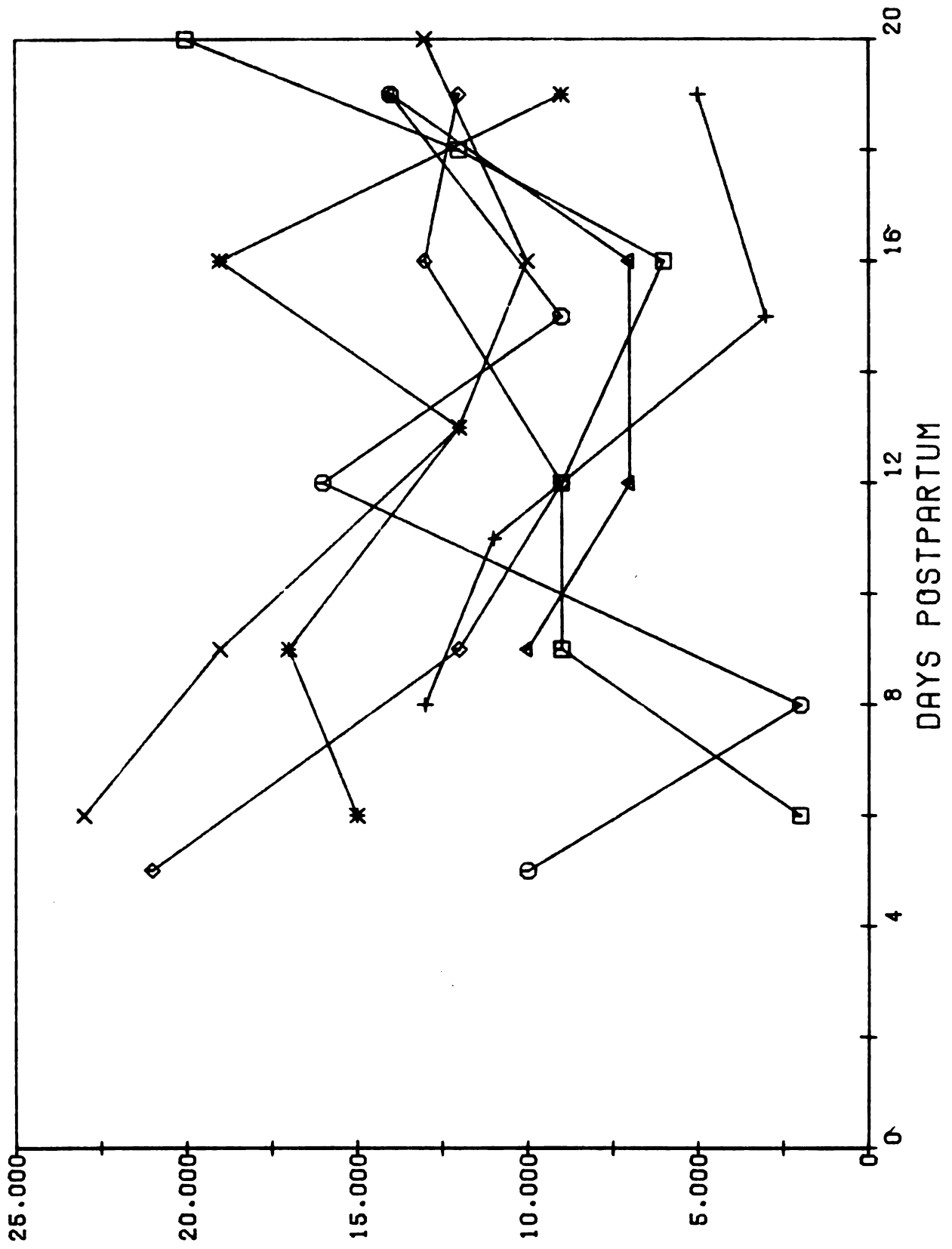
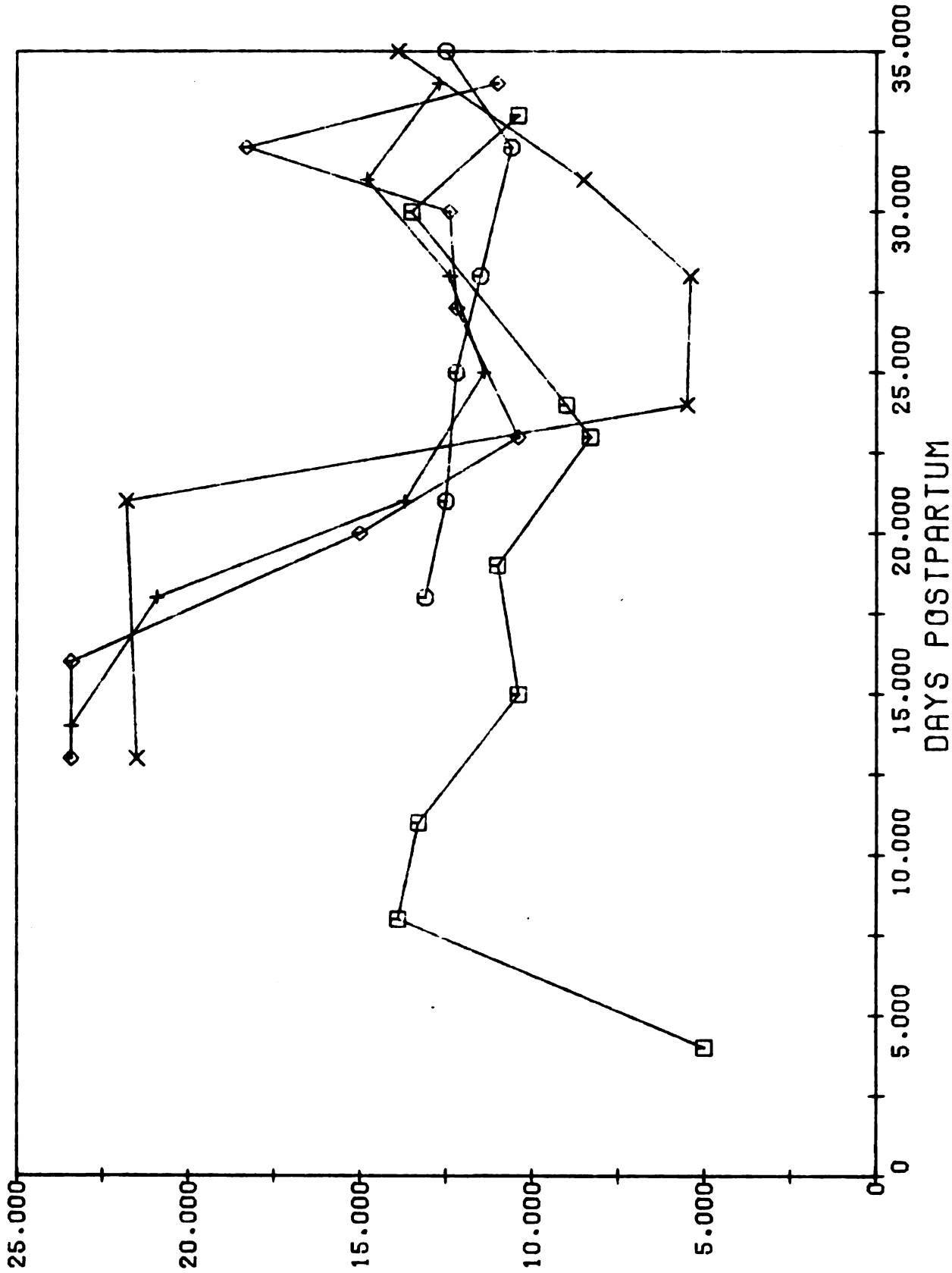

SERUM ESTRADIOL (PG ML)
25.000
20.000
15.000
10.000
5.000
0
4
8
12
16
20
DAYS POSTPARTUM

Figure 6. -- Serum estradiol concentrations of five cows which ovulated between day 21 and day 34 postpartum.

Each data point is the mean estradiol concentration of three serum replicates of one cow measured during the postpartum period prior to first ovulation. All data points were centered on day 35 postpartum to represent the day of first ovulation.

Legend	Cow No.	Days to First Ovulation
⌀	1374	22
⍁	1345	34
⌖	1359	28
+	1442	25
×	1331	24


SERUM ESTRADIOL (PG ML)
25.000
20.000
15.000
10.000
5.000
0
5.000
10.000
15.000
20.000
25.000
30.000
35.000
DAYS POSTPARTUM

for missing some existing follicles. At the end of the second week, a significantly greater (P<.05) number of cows which ovulated before day 21 postpartum had more 10 mm follicles compared to those which ovulated at later intervals. Follicular growth which occurred during this period may be reflected in variable estradiol concentrations observed in blood collected from most cows between parturition and first ovulation.

Table 4. -- Serum estradiol concentrations on day 7 and day 14 postpartum.

	Days to First Ovulation		
Period	9 - 14	15 - 20	21 - 34
Day 7	7.2 ± 1[b]	7.2 ± 1[b]	15.4 ± 2[c]
Day 14	10.5 ± 2[b]	8.6 ± 1[b]	8.8 ± 2[b]

[a] pg/ml

[b,c] Means with dissimilar letters are different (P<.01).

Table 5. -- Follicular growth and interval to first postpartum ovulation.

Days to Ovulation	Week 1	Week 2	Week 3
I. 9 - 14	1/8[a]	6/8[b]	--
II. 15 - 20	1/15	12/15[b]	13/15
III. 21 - 34	1/5	1/5	3/5

[a] Number of cows with follicle ≥ 10 mm in diameter.

[b] Cows ovulating by day 20 had more (P<.05) 10 mm follicles than cows ovulating after day 20 $\chi^2 = 6.39$, 1 d.f.

Other sources of serum estrogens are possible. Fetal cotyledons are rich in estrogen (Nalvandov and Casida, 1940) and urinary estrogens during the first seven weeks postpartum are higher than those found during early gestation (Erb et al., 1971b; Randel and Erb, 1971). In spite of high urinary estrogen excretion, few cows showed estrus before two weeks postpartum (Randel and Erb, 1971). It appears that the source of estradiol secretion prior to estrus is ovarian follicles since follicles were palpable early postpartum in this and other studies (Wagner and Hansel, 1969; Morrow, 1969).

Evidence for feedback of estradiol on the hypothalamus and pituitary during the period from parturition to first ovulation has been suggested. Single injections of 10 mg estradiol 17-β between 9 and 15 days postpartum in dairy cows induced ovulation one to two days after treatment (Foote, 1971). Estradiol may have caused increased LH synthesis and release to initiate follicular maturation and ovulation. However, when 10 mg estradiol-17β was administered with GnRH approximately 11 days postpartum, GnRH-induced LH release appeared to be inhibited (Manns and Richardson, 1976). Similarly, high serum estrogen concentrations are known to inhibit LH production in the anterior pituitary (Barraclough, 1973), whereas low concentrations of estrogen resulted in increased LH production (Hobson and Hansel, 1972). Variable low concentrations of estradiol observed in this study could have accounted for increased LH synthesis and release to initiate ovulation.

Changes in Luteinizing Hormone

Increased serum LH concentrations and increased numbers of episodic LH peaks during the first three weeks postpartum were associated with

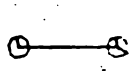
early postpartum ovulation. The relation between LH concentrations and interval to first ovulation is graphically depicted in Figures 7 and 8. Overall, animals which ovulated between 9 and 14 days postpartum had higher ($P<.01$) LH concentrations in blood samples collected on days 7 and 14 postpartum compared to values for animals which ovulated after day 14 (Table 6). More LH peaks ($P<.05$) and increased ($P<.05$) magnitude of the largest LH peak were observed in blood collected from cows which ovulated before day 15 compared to those ovulating after day 15. Similarly, more LH peaks ($P<.05$) were observed in blood collected from cows in interval II compared to animals which ovulated after day 20 postpartum. There was no apparent relationship between duration of the LH peak and interval to first ovulation in any interval while magnitude of the largest LH peak increased in those cows which ovulated before day 15 postpartum. Individual plots by period for three cows, one cow from each interval group, are depicted in Figures 16 to 21 (Appendix G). Increase in number of episodic LH peaks and magnitude of the largest LH peak have been reported to occur in postpartum beef cattle prior to first estrus (Humphrey et al., 1976).

Table 6. -- Effect of interval to first postpartum ovulation on serum LH.

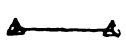
Days to Ovulation	LH[a]	No. Episodic LH Peaks	Duration of LH Peak[b]	Magnitude of Largest LH Peak[a]
I. 9 - 14	2.04 ± 0.31[c]	2.1 ± 0.2[e]	33 ± 2.0	5.5 ± 1.1[e]
II. 15 - 20	0.95 ± 0.19[d]	1.5 ± 0.1[f]	36 ± 1.0	2.8 ± 0.7[f]
III. 21 - 34	0.73 ± 0.33[d]	1.1 ± 0.2[g]	35 ± 2.0	1.3 ± 1.1[f]

[a]ng/ml (average of samples collected on days 7 and day 14) [b]minutes
[c,d]Means with dissimilar letters within category are different ($P<.01$).
[e,f,g]Means with dissimilar letters within category are different ($P<.05$).

Figure 7. -- Average LH concentrations on day 7 postpartum depicted by interval to first ovulation.



Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of eight cows which ovulated between day 9 and day 14 postpartum (Interval I).



Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of 15 cows which ovulated between day 15 and day 20 postpartum (Interval II).

Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of five cows which ovulated between day 21 and day 34 postpartum (Interval III).

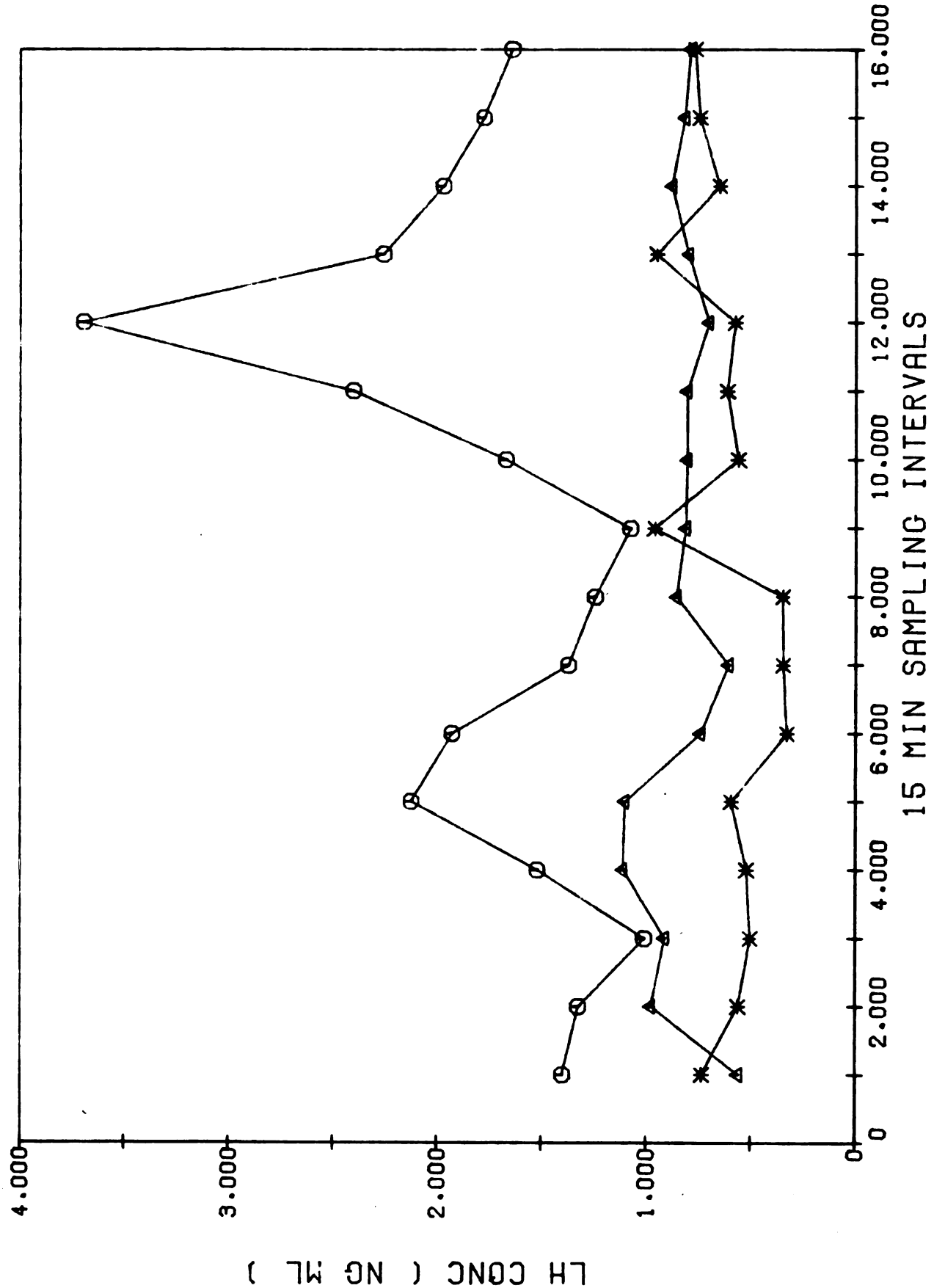
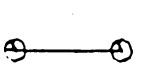
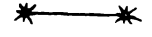

LH CONC (NG ML)
4.000
3.000
2.000
1.000
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

Figure 8. -- Average LH concentrations on day 14 postpartum depicted by interval to first ovulation.

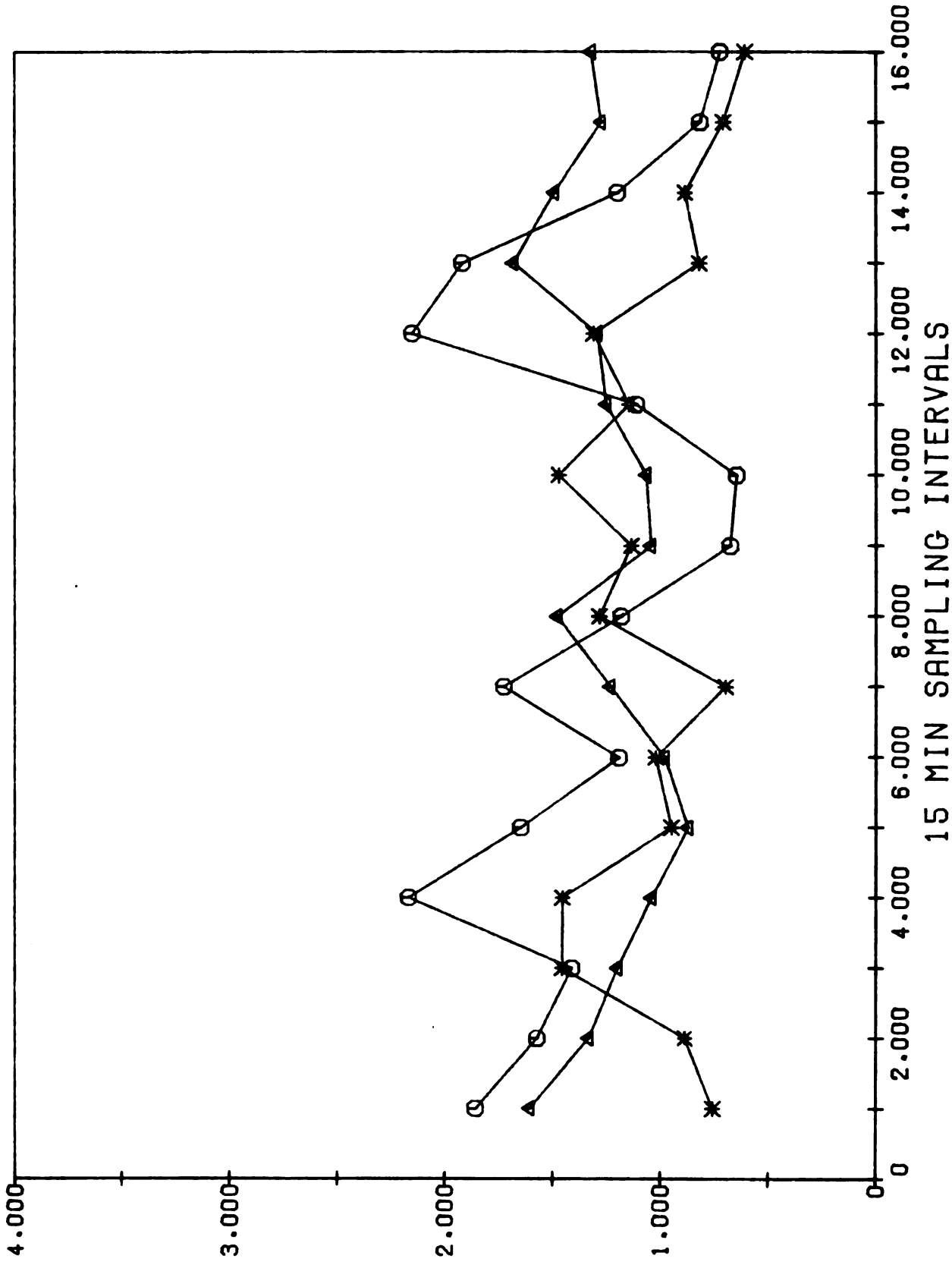


Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of three cows which ovulated between day 9 and day 14 postpartum (Interval I).

Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of 15 cows which ovulated between day 15 and day 20 postpartum (Interval II).



Mean LH concentrations in blood collected at 15 minute intervals for 4 hours of five cows which ovulated between day 21 and day 34 postpartum (Interval III).


LH CONC (NG ML)
4.000
3.000
2.000
1.000
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

It appears from data in this study that when ovulation occurred during the first two weeks postpartum, LH concentrations may not have been limiting for stimulation of follicular maturation and ovulation while LH availability may have been limiting in cows which ovulated at later postpartum intervals. Occurrence and timing of episodic LH peaks may have stimulated key processes leading to ovulation. Perhaps follicles required periodic stimulation by LH to promote further development. In previous studies, early postpartum follicular growth suggested that FSH was not rate-limiting, while continued presence of mature-like follicles indicated that LH was probably deficient (Wagner and Oxenreider, 1971). Histological examination of granulosa cells in similar follicles found some thickening and infolding of granulosa cells which may have indicated unsuccessful attempts at luteinization (Wagner and Hansel, 1969).

Quevedo et al., (1967) reported that ovariectomy at parturition decreased pituitary LH content and increased FSH content, while hysterectomy had not effect. Reduced pituitary LH content in ovariectomized animals may have been due to lack of ovarian estrogens to stimulate LH synthesis, while increased FSH content resulted from lack of steriod negative feedback. Early postpartum follicular growth observed in the present study and appearance of histologically normal nature follicles in postpartum cows in other studies (Wagner and Hansel, 1969; Wagner and Oxenreider, 1971) indicated that increased estradiol secretion is possible soon after calving. In another study, variation in LH and estradiol within animals suggested possible early attempts at cyclic ovarian activity from 5 to 13 days postpartum (Gaverick et al., 1973). Increased estradiol concentrations may have differentially inhibited FSH synthesis and release while concomitantly stimulating LH synthesis and release

in the absence of progesterone. The presence of more follciles ≥ 10 mm (Table 5) in cows which ovulated prior to day 21 postpartum in this study could be evidence for such a mechanism. However, serum estradiol on day 7 postpartum was higher in those cows which ovulated after day 20 postpartum. This estradiol may have been a carry-over from the high levels at parturition and may have limited LH and FSH secretion.

Concentration of LH in blood samples collected from all cows on day 14 postpartum was higher ($P<.001$) than that in samples collected on day 7 (Appendix F, Table 7). This is depicted in Figure 9. Number of episodic LH peaks increased ($P<.08$), while magnitude of the largest LH peak tended to increase ($P<.10$) with days postpartum. Increase in serum LH concentrations during the first two weeks postpartum paralleled increases in pituitary LH content observed during the first 20 days after calving in other studies (Labhsetwar et al., 1964; Saiduddin and Foote, 1964; Saiduddin et al., 1968). This suggests that increased pituitary LH content represented both synthesis and release of hormone. While pituitary responsiveness to GnRH was not fully restored until 7 to 8 days postpartum (Kesler et al., 1976; Fernandes et al., 1976), prior to then sufficient releasable quantities of LH were available to cause ovulation in some cows. Eight cows in the present study ovulated before day 15, three of which ovulated prior to day 12 postpartum.

Regression analysis of LH concentrations on day 7 and day 14 from all cows on days to first ovulation is shown in Figures 10 and 11, respectively. On day 7 average serum LH concentrations were negatively correlated with days to first ovulation ($r = -.40$, $P<.05$), but no significant relationship was observed one week later. This negative relationship was

Figure 9. -- LH concentrations on day 7 and day 14 postpartum

⊖ Average LH concentration of 16 serum samples collected at 15 minute intervals for 4 hours on day 7 (n = 28 cows) and day 14 (n = 23 cows).

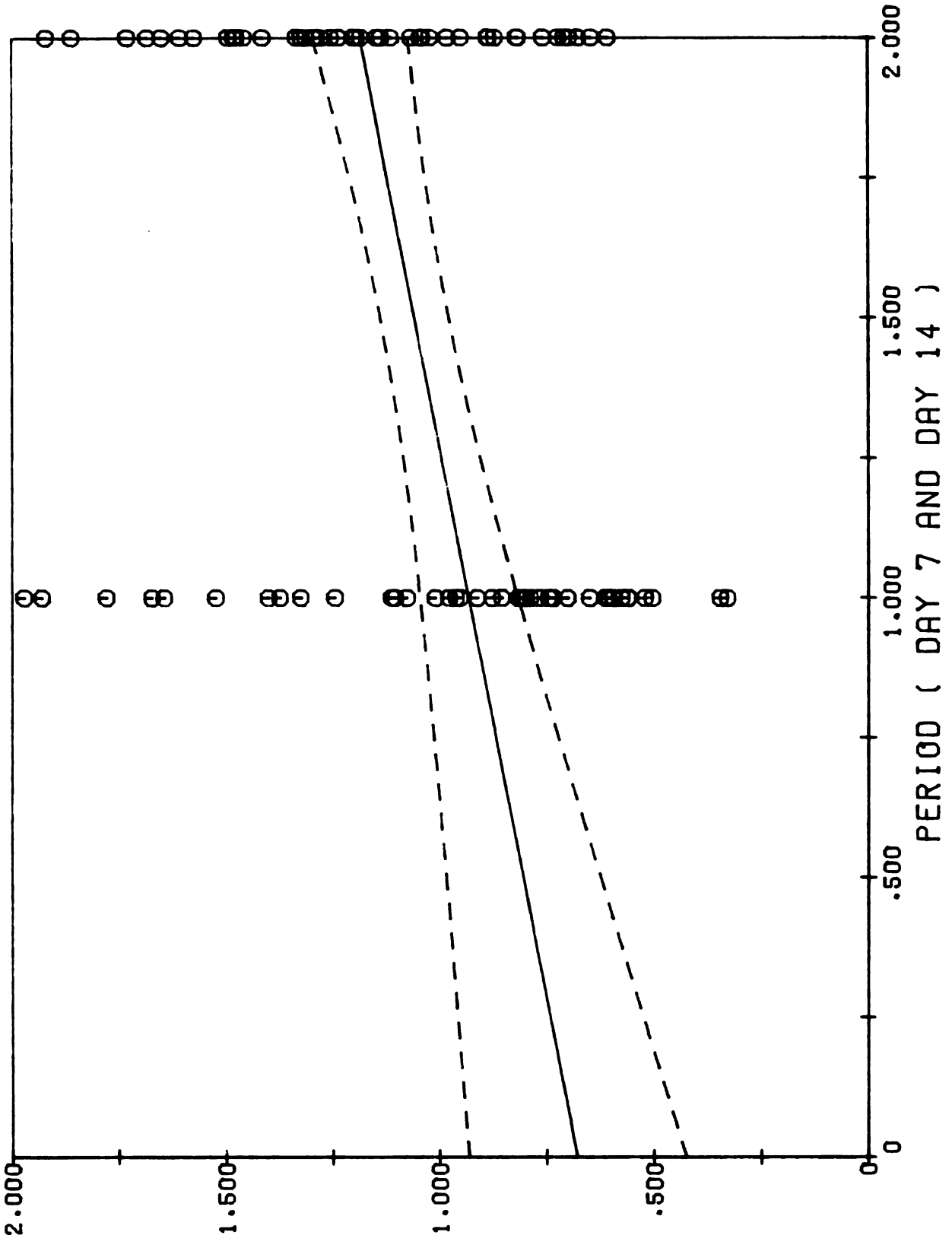

LH CONC (NG ML)
2.000
1.500
1.000
.500
0
0
.500
1.000
1.500
2.000
PERIOD (DAY 7 AND DAY 14)

Figure 10. -- Regression of LH concentrations on day 7 with days to first postpartum ovulation.

Regression line with 95% confidence interval:

$y = 2.329 - 0.072X$

y = average LH concentration on day 7

X = days to first ovulation

$r = -.40$ $(P<.05)$

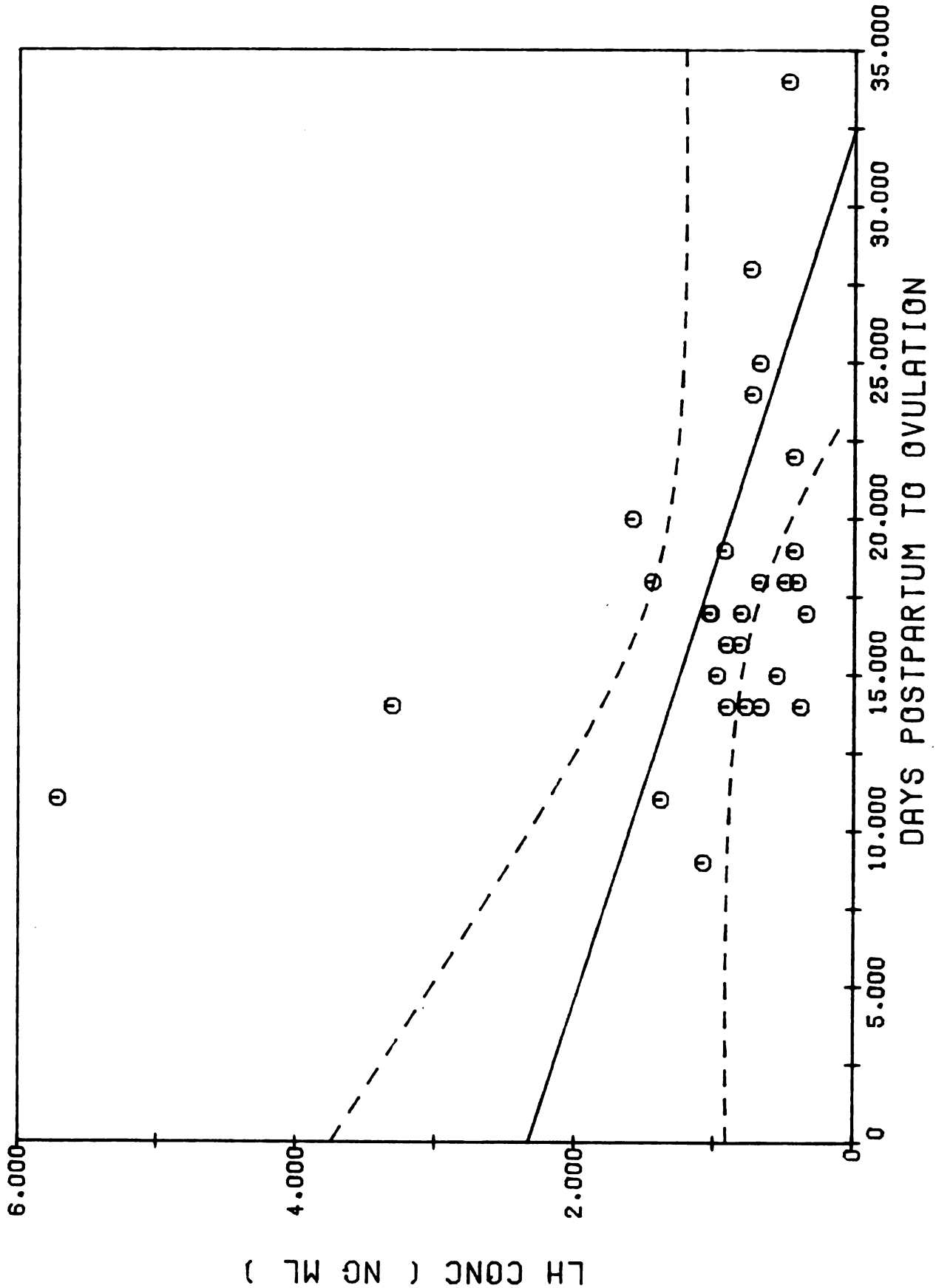

LH CONC (NG ML)
6.000
4.000
2.000
0
0
5.000
10.000
15.000
20.000
25.000
30.000
35.000
DAYS POSTPARTUM TO OVULATION

Figure 11. -- Regression of LH concentrations on day 14 with days to first postpartum ovulation.

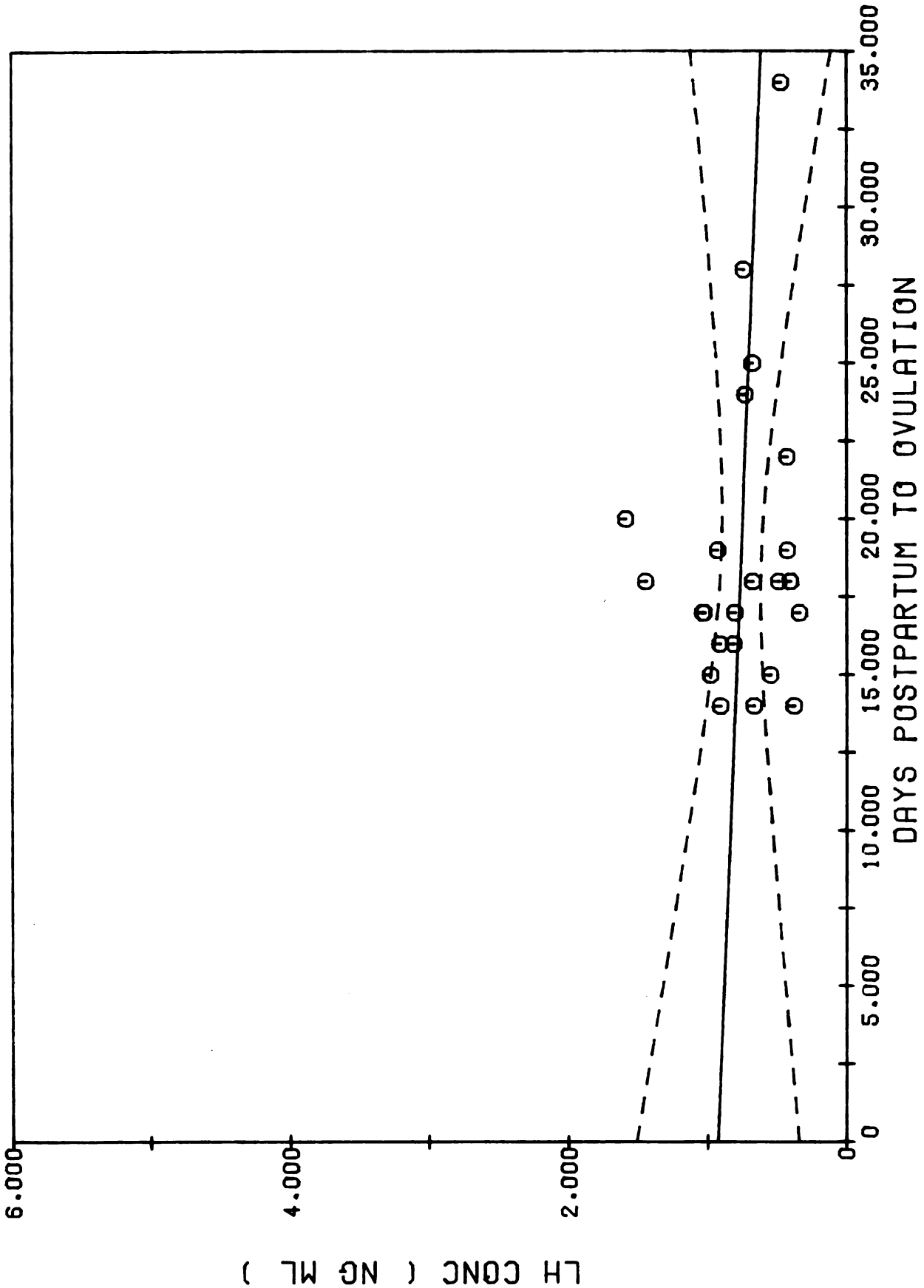
Regression line with 95% confidence interval.

$y = 1.518 - 0.015X$

y = average LH concentration on day 14

X = days to first ovulation

$r = -0.19$ $(P>.10)$


LH CONC (NG ML)
6.000
4.000
2.000
0
0
5.000
10.000
15.000
20.000
25.000
30.000
35.000
DAYS POSTPARTUM TO OVULATION

apparently due to several cows which had higher LH concentrations and ovulated early postpartum, while those which ovulated at later intervals had lower LH concentrations during the first week after calving. By day 14, this relationship was no longer observed due to overall increases in serum LH in all remaining cows which had not ovulated by the end of the second week.

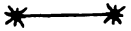
Changes in Glucocorticoids

Serum glucocorticoid concentrations alone were not related to the interval to first postpartum ovulation (Appendix F, Table 8) when glucocorticoids were measured at 30 minute intervals for 4 hours in blood samples collected on postpartum days 7 and 14, respectively (Figures 12 and 13). There was no increase from first to second period in basal secretion as shown in Figure 14 and in Table 9 (Appendix F). However, significant ($P<.0005$) sample to sample variation was observed. Sample to sample variation in basal glucocorticoid concentrations is depicted by period for two cows in Figures 22 to 25 (Appendix G). Active secretion of glucocorticoids in response to exteroceptive stimuli and the milking stimulus had been observed where an unmilked cow responded by releasing serum glucocorticoids in the presence of a herdmate being milked (Smith et al., 1972). While cows in the present study were not sampled during milking, basal glucocorticoid concentrations were variable. Decreases in glucocorticoids observed during the first hour on day 7 and day 14 were probably due to initial handling of cows, while further apparent increases in most cows observed on day 14 postpartum during the second and third hours cannot be similarly explained. Koprowski (1973) concluded that basal concentrations of glucocorticoids were not related to milk

Figure 12. -- Average glucocorticoid concentrations on day 7 postpartum depicted by interval to first ovulation.

Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of eight cows which ovulated between day 9 and day 14 postpartum (Interval I).

Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of 15 cows which ovulated between day 15 and day 20 postpartum (Interval II).



Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of five cows which ovulated between day 21 and day 34 postpartum (Interval III).

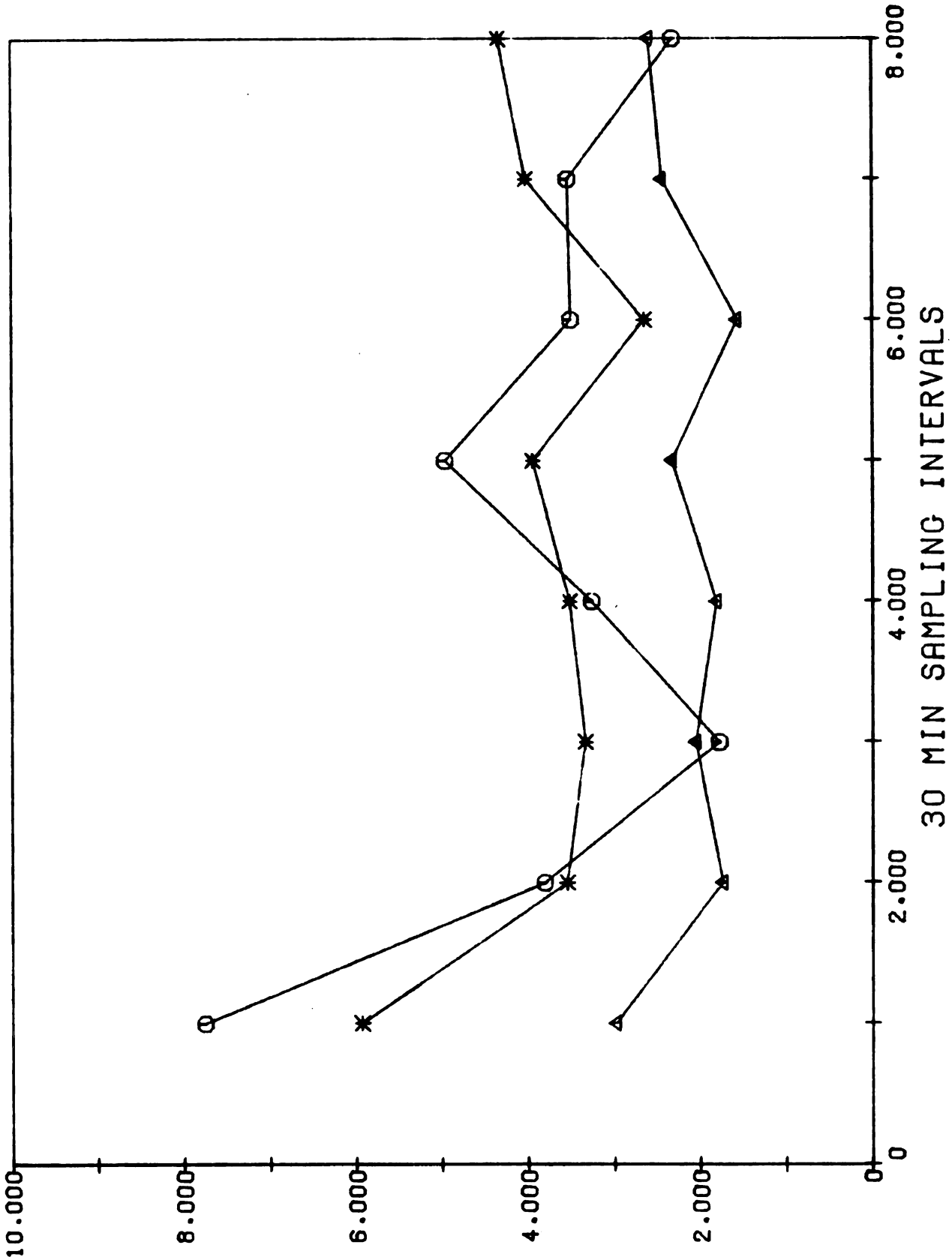
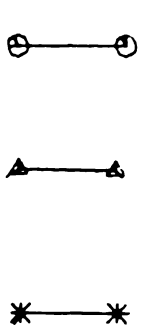

GLUCOCORTICOID CONC (NG ML)
10.000
8.000
6.000
4.000
2.000
0
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

Figure 13. -- Average glucocorticoid concentration on day 14 postpartum depicted by interval to first ovulation.



Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of three cows which ovulated between day 9 and day 14 postpartum (Interval I).

Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of 15 cows which ovulated between day 15 and day 20 postpartum (Interval II).

Mean glucocorticoid concentrations in blood collected at 30 minute intervals for 4 hours of five cows which ovulated between day 21 and day 34 postpartum (Interval III).

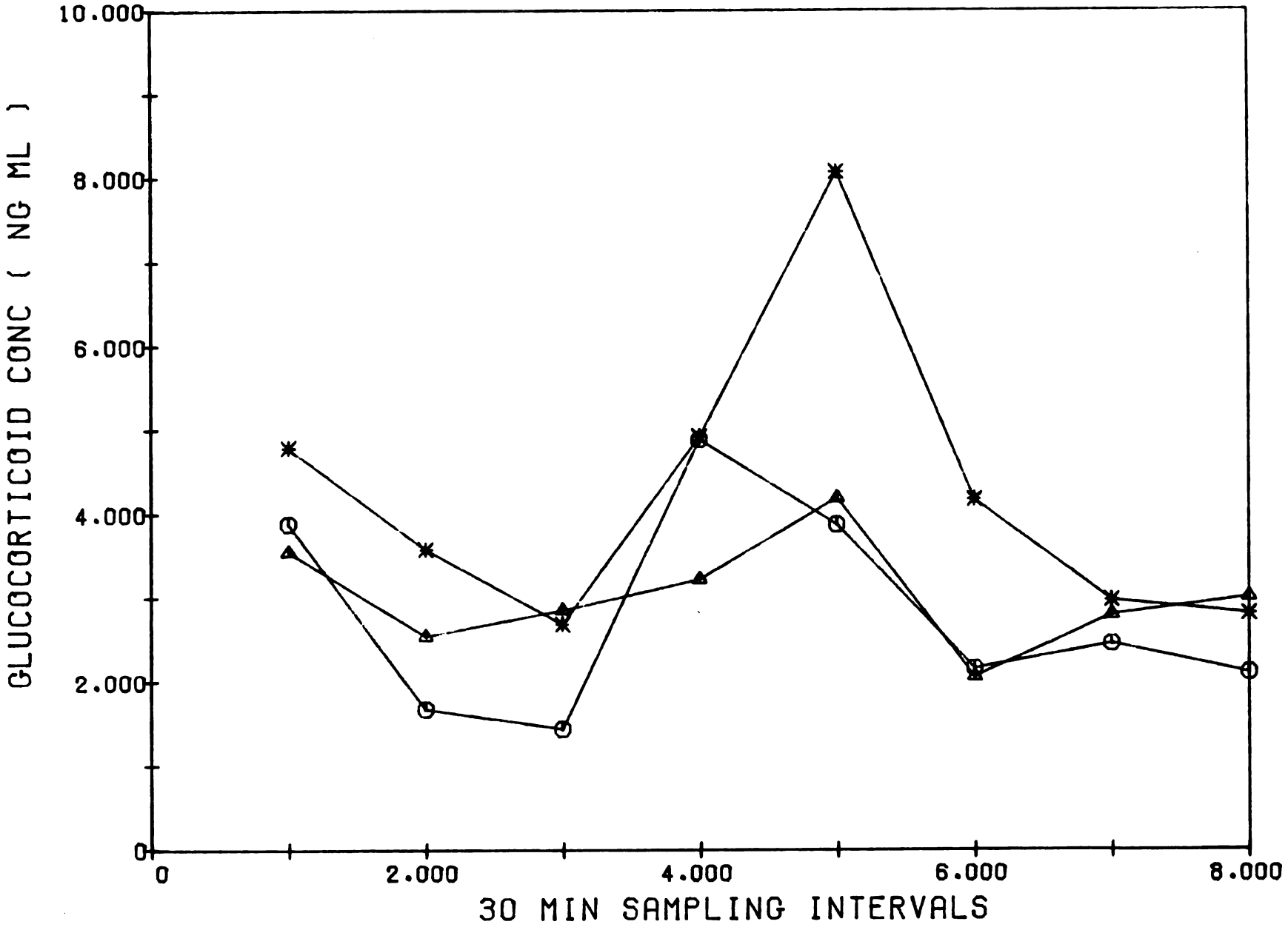
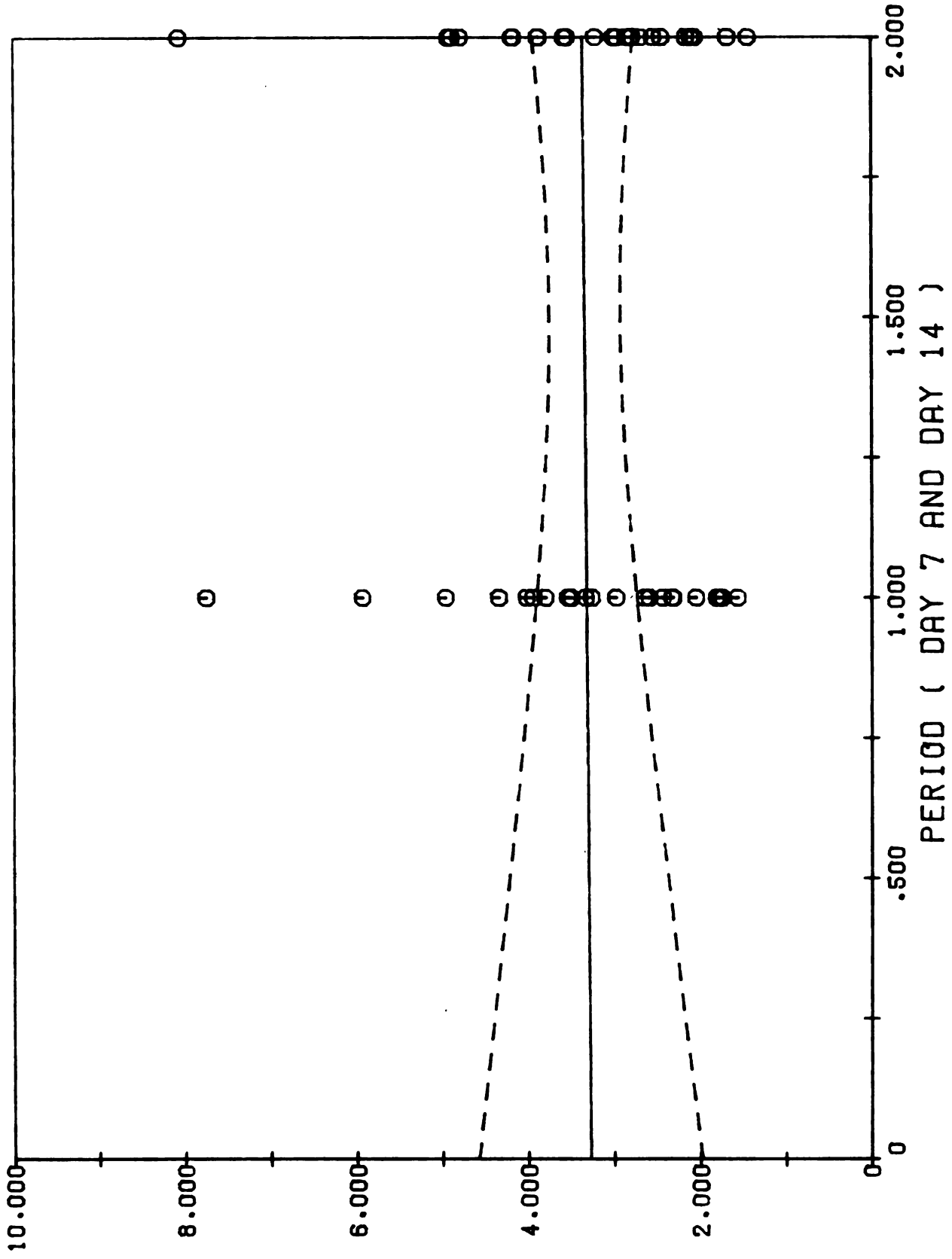

10.000
8.000
6.000
4.000
2.000
0
GLUCOCORTICOID CONC (NG ML)
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

Figure 14. -- Glucocorticoid concentrations on day 7 and day 14 postpartum.

⊗ Average glucocorticoid concentration of eight serum samples collected at 30 minute intervals for 4 hours on day 7 (N = 28 cows) and day 14 (n = 23 cows).


GLUCOCORTICOID CONC (NG ML)
10.000
8.000
6.000
4.000
2.000
0
0
.500
1.000
1.500
2.000
PERIOD (DAY 7 AND DAY 14)

production similar to results in this study where milk yield was neither correlated with glucocorticoid response on day 7 ($r = -.25$, $P>.10$) nor on day 14 ($r = -.03$, $P>.50$).

Effect of Milk Yield

Interval to first ovulation increased in direct proportion to average daily milk yield to day 40 postpartum. Cows which ovulated early (9 to 14 days postpartum) produced less ($P<.05$) 4% fat-corrected-mature-equivalent milk than those which ovulated between days 15 to 20, while both of these groups of cows produced less ($P<.05$) milk than cows which ovulated after day 20 postpartum (Table 10). Proportion of cows in ovulation intervals with either above average (best) or below average (worst) levels of genetic ability for milk production were not different ($P>.10$, Appendix F, Table 11). However, all cows ($n = 5$) which returned to estrus after day 20 postpartum were above average in genetic ability to produce milk. These data indicated a negative relationship between the level of milk yield and postpartum ovarian activity. Other reports have indicated similar results (Marion and Gier, 1968; Whitmore et al., 1974).

Table 10. -- Daily milk yield to day 40 of lactation and the interval to first postpartum ovulation.[a]

Days to Ovulation	Kg Milk/Day
I. 9 - 14 (n = 8)	22.9 ± .2[b]
II. 15 - 20 (n = 15)	27.2 ± .3[c]
III. 21 - 34 (n = 5	29.8 ± .5[d]

[a] 4% F.C.M. - Mature Equivalent

[b,c,d] Means with dissimilar letters are different ($P<.05$).

Regression of average daily milk yield to day 40 postpartum on number of days to first ovulation yielded a significant linear regression ($r = +.33$, $P<.05$). Previous studies have reported smaller correlations with respect to days to first estrus (Herman and Edmondson, 1950; Olds and Seath, 1953; Menge et al., 1962). Since milk yield only accounted for 10% of the variation of number of days to first ovulation in the present study, this may indicate that although milk yield negatively influenced interval to postpartum ovulation, its role tended to be only minor in significance.

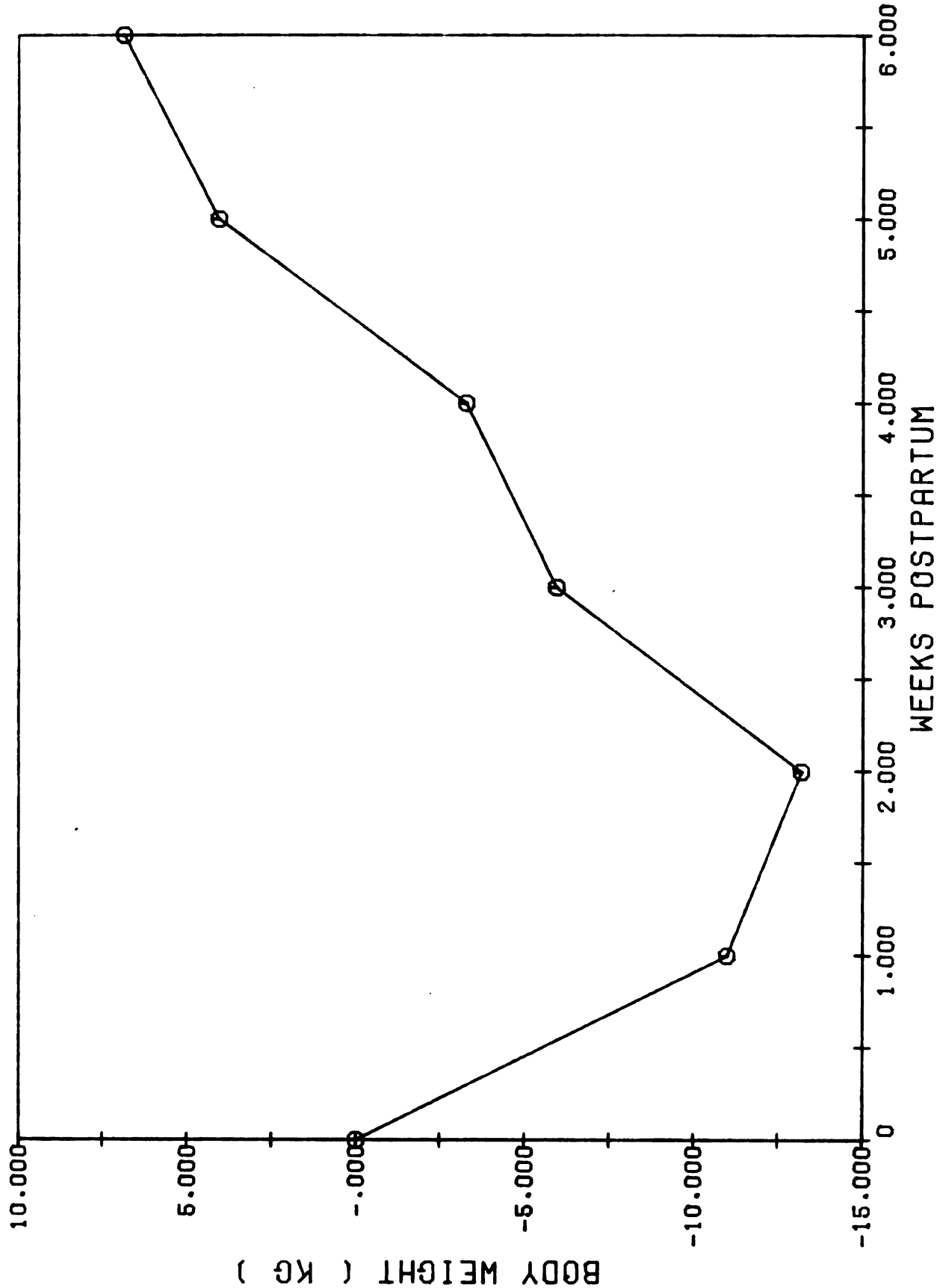
Changes in Body Weight and Energy Balance

Loss of body weight was greatest between parturition and the second week of lactation and reached a maximum cumulative loss of 13.2 kg by the end of the second week after calving (Figure 15). Analysis of overall loss in body weight and percent loss in body weight in reference to body weight during the first week postpartum revealed no significant effect of change in body weight due to parity or interval to first ovulation (Appendix F, Table 12). Week to week analysis of results indicated that cows began to regain body weight during the third week of lactation ($P<.05$). Sometime during the fifth week postpartum, all cows, on the average, had regained body weight lost since the first postpartum body weight measurment.

Overall, energy balance never decreased to less then 100% of N.R.C. requirements (Appendix F, Table 13). All cows were in lower energy balance during the second week of lactation corresponding to the period of greatest cumulative loss in body weight. Analysis of weekly energy balance by interval to first ovulation resulted in no significant differences among groups of cows which ovulated during different intervals

Figure 15. -- Cumulative change in body weight in reference to body weight measured during first week postpartum.

Average body weight of 27 cows. Week 0 body weight was measured 3 to 5 days postpartum.


BODY WEIGHT (KG)
10.000
5.000
-.000
-5.000
-10.000
-15.000
0
1.000
2.000
3.000
4.000
5.000
6.000
WEEKS POSTPARTUM

(Appendix F, Table 7). However, cows which ovulated after day 20 postpartum had numerically the lowest energy balance during the first six weeks of lactation. The same animals produced more milk during this period (Table 10).

Analysis of energy balance by milk production group revealed that cows which produced more than 26 kg of milk per day had the lowest energy balance (Table 14).

Table 14. -- Average daily milk yield and energy balance during first six weeks postpartum[a].

Daily yield[b]	% N.R.C.[c]
> 26	114 ± 3[d]
24 - 26	126 ± 3[e]
< 24	131 ± 3[e]

[a] 4% F.C.M. - Mature Equivalent

[b] kg (9 cows per group)

[c] Intake ÷ Requirement) X 100%

[d,e] Means with dissimilar letters are different (P<.01).

There was a significant interaction between parity and week of lactation on energy balance. Primiparous cows had a higher (P<.05) energy balance during weeks 1 to 5 postpartum than pluriparous cows (Appendix F, Table 15). In younger cows, a decrease (P<.10) in energy balance between weeks 1 and 2 may have been due to an increased demand for nutrients for lactation and growth, or it may have reflected a change in environment and habitat associated with first lactation. Energy balance remained rather constant after the second week in all cows. This indicated that all cows, including first lactation heifers were

probably consuming enough feed to meet energy demands of maintenance and lactation.

Body weight losses tended to decrease as energy balance increased indicating that body weight followed energy balance as previously reported (Carstairs, 1975). In constrast to these data, other work has indicated that during early lactation, high producing milk cows are in negative energy balance (Reid et al., 1966; Moe et al., 1971), and prolonged postpartum anestrus occurred when available energy in rations was at least 25% below calculated requirements (Wiltbank et al., 1962; Folman et al., 1973; Wiltbank, 1974). However, postpartum energy levels appeared to have no effect on return to estrus for cows in good body condition at calving (Wiltbank et al., 1964). Although prepartum body condition was not measured in the present study all cows including first lactation heifers appeared to consume enough feed to maintain a positive energy balance. Therefore, changes in postpartum energy balance and loss in body weight alone appeared to have little overall effect on resumption of ovarian activity. However, an important interaction effect between energy balance and glucocorticoid concentrations on day 7 and day 14 was observed. This effect is discussed in the following section.

Relationships Between Hormone Concentrations and Energy Balance

Average hormone concentrations measured in blood collected on day 7 and average energy balance during the first week postpartum were the independent variables used in a multiple regression analysis of variance to predict days to first ovulation. Results indicated that concentrations of estradiol and the cross product interaction between glucocorticoid concentrations with energy balance, and LH with estradiol were significant ($P<.003$) variables in accounting

for variation in days to first ovulation (R^2 = .39, Table 16). The respective correlation matrix of all variables is found in Table 17. While estradiol was positively related to days to first ovulation (r = +.38, P<.05), LH was negatively related (r = -.40, P<.05). These data indicated that perhaps a functional pituitary-ovarian relationship may have been involved in controlling onset of postpartum ovarian activity. While neither glucocorticoid concentrations nor energy balance alone affected interval to first ovulation, the interaction between these two variables may indicate that energy processes involved in maintaining energy balance may have been affected by serum glucocorticoid concentrations.

Table 16. -- Multiple regression analysis of variance of hormone concentrations and energy balance on day 7 postpartum.

Model: $\log y = a + b_1X_1 + b_2X_1X_2 + b_3X_3X_4$

y = days to first ovulation	a = 1.19658
X_1 = Estradiol	b_1 = 0.01211
X_1X_2 = Estradiol * LH	b_2 = -0.00305
X_3X_4 = Glucocorticoids * Energy Balance (cross-product interaction)	b_3 = -0.00015

Source of Variance	df	F	Significance
Regression (about mean)	3	6.64	.003
Error	23		

R^2 = .456 Adjusted R^2 = .386

Multiple regression analysis of variance of average hormone concentrations measured in blood collected on day 14 and average energy balance during the second week postpartum demonstrated that glucocorticoid

Table 17. -- Correlation matrices of multiple regression variables by period.

Period I - Day 7

	Days	LH	GL	E_2	EB	GL^2	EB^2	GL*EB	LH*E_2
Days[a]	1.00	-.40*	-.17	.38*	-.15	-.07	-.10	-.40*	-.27
LH[b]		1.00	.12	.12	.04	.04	-.41	.19	.90*
GL[c]			1.00	.19	-.23	.97*	-.21	.84*	.15
E_2[d]				1.00	-.35*	.22	.38*	-.09	.44
EB[e]					1.00	-.28	-.15	.26	-.09
GL^2						1.00	-.25	.76*	.09
EB^2							1.00	.26	-.11
GL*EB								1.00	.12
LH*E_2									1.00

Period II - Day 14

	Days	LH	GL	E_2	EB	GL^2	EB^2	GL*EB	LH*E_2
Days	1.00	-.19	.25	-.04	-.30	.26	-.25	-.11	-.10
LH		1.00	-.21	-.20	.09	-.23	.08	-.15	.47*
GL			1.00	.15	-.13	.97*	-.04	.73*	-.02
E_2				1.00	-.29	.15	-.30	.02	.73
EB					1.00	-.24	.98*	.50*	.57*
GL^2						1.00	-.13	.61*	-.04
EB^2							1.00	.53*	-.21
GL*EB								1.00	-.09
LH*E_2									1.00

[a]Days to first ovulation

[b]Luteinizing hormone

[c]Glucocorticoids

[d]Estradiol

[e]Energy Balance

*Correlation is significant ($P<.05$)

concentrations, energy balance, their first order quadratics, and first order cross product interactions were significant ($P<.03$) variables in accounting for 35% of the variation in days to first ovulation. Correlations between variables are found in Table 16. Similar to multiple regression results for variables measured on day 7 postpartum, glucocorticoid and energy balance interaction was again significant. Overall, cows in interval II had decreased glucocorticoid concentrations and more positive energy balance compared to cows in interval III. In contrast, cows in interval I had increased glucocorticoid concentrations and more positive energy balance compared to all cows which resumed ovarian activity after day 14 (Intervals II and III).

These data suggest that possible relationships governing the control of metabolic processes may have been mediated by glucocorticoids responding to different metabolic demands. Cows which ovulated after day 14 postpartum apparently had greater metabolic demands associated with increased milk production compared to those which ovulated earlier postpartum. Perhaps energy dependent processes may have delayed the events associated with the initiation of postpartum ovarian activity. This may have been possible since older cows appeared to be at zero or in slightly negative energy balance during the first two weeks postpartum (Appendix F, Table 15) when pituitary-ovarian relationships are normally re-established in most cows.

Table 18. -- Multiple regression analysis of variance of hormone concentrations and energy balance on day 14 postpartum.

Model: $\log y = a + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2$

y = days to first ovulation	a = 1.55932
X_1 = Glucocorticoids	b_1 = 0.29824
X_2 = Energy Balance	b_2 = -0.01298
X_1X_2 = Glucocorticoids * Energy Balance (cross-product interaction)	b_3 = -0.00082
X_1 = Glucocorticoids ** 2 (quadratic)	b_4 = -0.02961
X_2 = Energy Balance ** 2 (quadratic)	b_5 = 0.00060

Source of Variance	df	F	Significance
Regression (about mean)	4	3.27	.032
Error	16		

R^2 = .506 Adjusted R^2 = .351

SUMMARY AND CONCLUSIONS

Postpartum ovarian activity was monitored in 28 lactating Holstein cows from parturition until development of the third postpartum corpus luteum. Ovaries were palpated twice weekly to determine follicular growth and corpus luteum development, while changes in estradiol and progesterone were measured in peripheral blood collected twice weekly. Interval to first ovulation averaged 17.6 days and ranged from 9 to 34 days in length. Primiparous cows tended to have shorter ($P<.10$) period of postpartum anestrus compared to older animals, while the interval to first estrus was not different among parity groups. A gradual increase in postpartum estrus behavior and activity was observed at each successive ovulation. These data suggest that duration of postpartum anestrus is probably not a problem which would prevent achievement of calving intervals of 12 months or less in most Holstein cows.

Animals were divided into three postpartum interval groups to study the effect of changes in patterns of hormonal secretion, milk production, and energy balance on interval to first ovulation. Cows which ovulated before day 15, between days 15 and 20, and after day 20 postpartum were assigned to interval groups I, II and III, respectively. Changes in estradiol concentrations were observed between parturition and first ovulation. Generally, estradiol concentrations fluctuated in some cows while in others concentrations remained low until 2 to 3 days before ovulation and then increased. Follicular growth was observed during the

first week postpartum. More cows ($P<.05$) which ovulated before day 20 postpartum had follicles ≥ 10 mm during the second week after calving than cows which ovulated after day 20 postpartum. These results suggest that follicular growth which occurred during this period may have been reflected in variable estradiol secretion. Low concentrations of estrogen are known to cause increases in LH production and may have accounted for early ovulation in those cows where LH was higher during the early postpartum period.

Progesterone remained less than 0.2 ng/ml following parturition until development of the first postpartum corpus luteum (CL). Following ovulation, progesterone increased ($P<.01$) above pre-ovulation concentrations after 2 to 3 days. The first estrous cycle averaged 17.7 days and was shorter ($P<.05$) in duration than the second estrous cycle of 20.1 days. The first estrous cycle appeared to be shorter due to decreased ($P<.05$) CL diameter and reduced ($P<.01$) peak progesterone concentrations in young cows compared to old cows. However, no difference in CL size was observed overall during the first two postpartum ovarian cycles, while peak progesterone concentrations increased during the second cycle ($P<.01$). Negative feedback of progesterone on LH could account for increased LH concentrations observed during the first estrous cycle when progesterone is lower than during the second cycle when LH is decreased and progesterone is increased in peripheral concentrations.

Interval to first ovulation was extended due to increased milk production. Cows which ovulated before day 15 produced less ($P<.05$) milk during the first 40 days postpartum than cows which ovulated at later postpartum intervals, and cows which ovulated after day 20 postpartum produced the most milk ($P<.05$). Levels of genetic ability for

milk production did not appear to be different between interval groups, however all cows which ovulated after day 20 were cows which had above average level of genetic ability for milk production. These data suggest that while increased milk yield extended the interval to first ovulation, the effect of milk production was probably secondary to hormonal regulation and control.

Calculated energy balance based on weekly body weight measurements indicated that all animals were in positive balance during the first six weeks postpartum, while week to week analysis showed that pluriparous cows were at zero to slightly negative energy balance during the first two weeks postpartum. This was reflected in cumulative loss of body weight which was greater ($P<.05$) during the first two weeks and then decreased. Most animals had regained body weight equal to that lost during the first two weeks by the end of the fourth week postpartum. Overall, energy demands for body maintenance and lactation did not appear to inhibit postpartum ovarian activity, since most cows apparently had adequate body stores in addition to ration nutrients to maintain positive energy balance.

Blood was collected at frequent intervals on day 7 and day 14 postpartum to study the pattern of postpartum LH secretion. Overall, cows which ovulated before day 15 postpartum had higher ($P<.01$) LH concentrations, increased ($P<.01$) episodic LH peaks and increased ($P<.05$) magnitude of the largest LH peak compared to those which ovulated after day 14 postpartum. While average LH concentration was not higher for cows in interval II, they had more ($P<.05$) episodic LH peaks than cows which ovulated during interval III. Onset and timing of episodic LH peaks in addition to higher peripheral LH concentrations appeared

to initiate early ovulation. These data suggest that developing follicles may require regular LH stimulation to result in follicular maturation and ovulation. Pituitary-ovarian interaction is possibly involved in this process since multiple regression analysis of variance showed that estradiol and the interaction between estradiol and LH on day 7 were significant variables to account for variation in days to first postpartum ovulation. LH was negatively correlated ($r = -.40$, $P<.05$) while estradiol was positive related ($r = +.38$, $P<.05$) with the interval to first postpartum ovulation. While no such relationship was observed on day 14, average LH concentrations had increased ($P<.001$) in all groups.

Serum glucocorticoid concentrations measured in blood collected at frequent intervals on day 7 and day 14 postpartum revealed no significant relationships with respect to time of ovulation. However, significant ($P<.0005$) variation in sample to sample concentrations indicated that cows may have responded to exteroceptive stimuli by increasing glucocorticoid secretion. Initial decreases in concentration at each 30 minute sampling period may have resulted from handling of cows while consistent increases during the second and third hour of sampling in glucocorticoid concentrations on day 14 by most cows cannot be similarly explained. Perhaps, increased secretion may be in response to metabolic status. Multiple regression analysis of variance on day 14 hormone concentrations and energy balance revealed that glucocorticoid concentrations interacted with factors involved in energy balance to account for 35% of the variation in days to first ovulation. Results from analyses of data on day 7 and day 14 indicated the possibility of glucocorticoid control of some energy processes related to energy balance. Cows which had not ovulated by day 14 postpartum had greater nutrient demand for increased milk production

compared to cows which ovulated earlier. Perhaps, the importance of glucocorticoid secretion during the postpartum interval is related to the negative effects of milk production on the interval to first postpartum ovulation. Further study of the endocrine state of the dairy cow is necessary to understand the role of glucocorticoids and its control or response to body metabolism and energy requirements.

Additional study of postpartum pituitary-ovarian relationships is warranted to complete information concerning initiation of postpartum ovarian activity. Imbalances in this endocrine milieu are probably responsible for anestrus and increased calving intervals in some cows. However, since all cows in this study began ovarian cycles by day 35 postpartum, anestrus in the dairy cow does not appear to be limiting more efficient reproduction. The problem of identifying animals in estrus following postpartum initiation of estrous cycles remains the greatest practical problem to solve in order to reduce days open and maintain optimal calving intervals of 12 months or less.

APPENDICES A THROUGH E

APPENDIX A

EXTRACTION AND RADIOIMMUNOASSAY OF PROGESTERONE

(Convey et al., 1977, J. Anim. Sci. 35:(in press)).

Extraction

1. Duplicate aliquants of serum (200 µl) were pipetted into disposable culture tubes (16 X 100 mm).

2. To account for procedural loss, a third aliquant from a representative number of unknowns (10 per assay) was added to a culture tube (16 X 100 mm) which contained 2500 cpm ^{3}H-1, 2, 3, 7-progesterone (New England Nuclear; 80 to 100 Ci/mM; repurified by column chromatography) in methanol. Twenty-five hundred cpm ^{3}H-1, 2, 6, 7-progesterone was also added to scintillation vials (4 to 6 per assay) for total counts (100% recovery).

3. Tubes containing serum and ^{3}H-progesterone were vortexed (10 seconds); labeled and endogenous progesterone were allowed to equilibrate for 30 minutes.

4. Serum was extracted with 3 ml benzene:hexane (1:2) by vortexing for 30 seconds and was then stored at -20° C for at least one hour to freeze the aqueous phase.

   Solvent extracts destined for assay were decanted into disposable culture tubes (12 X 75 mm).

   Extracts from aliquants for procedural loss were decanted into scintillation vials. Recovered radioactivity was averaged to determine a single correction factor to account for procedural losses for all samples.

5. For comparison among assays, standard sera with high and low progesterone were assayed in triplicate in each assay.

Radioimmunoassay

1. Progesterone (Sigma Chemical Company) for standards (.05, .10, .15, .20, .25, .30, .40, .60, .80, and 1.0 ng) was pipetted into disposable culture tubes (12 X 75 mm) from a stock solution of 10 ng/ml in absolute methanol and included in each assay.

2. Standards and serum extractions were evaportaed dry in a vacuum oven (28-30 inches vacuum) at a temperature less than 50° C.

3. 1st Antibody[a] (200 µl) diluted 1:3000 in 0.1% Knox PBS[b] containing 1:100 NRS (normal rabbit sera) was added to each tube, vortexed briefly (2 sec.) and incubated at room temperature for 2 hours.

4. Then, 200 µl containing 5000 cpm ^{3}H-1, 2, 6, 7-progesterone (New England Nuclear; 104 Ci/mM; repurified by column chromatography) diluted in 0.1% Knox PBS[b] was added to each tube, vortexed briefly (2 sec.), and stored at 4° C for 18 to 24 hours.

5. 2nd Antibody[c] (400 µl) diluted 1:15 in 0.1% Knox PBS[b] was added to each tube, vortexed briefly (2 sec.), and incubated at 4° C for 48 hours.

6. All assay tubes were centrifuged at 2500 g for 15 min. at 4° C and a 0.5 ml aliquant of supernatant was diluted with 5 ml scintillation fluid (3a70B Preblend; Research Products International Corp., Elk Grove Village, Illinois) for quantification of radioactivity in a liquid scintillation spectrophotometer.

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[a] 1st Ab - MSU #74 anti-progesterone was prepared in rabbits against 20-progesterone-oxime-human serum albumen.

[b] Knox Gelatin, Inc., Johnston, N.Y.; PBS-phosphate (.01 M) buffered (pH 7.4) saline (0.85%).

[c] 2nd Ab - MSU castrated sheep anti-rabbit gamma globulin.

APPENDIX B

EXTRACTION AND RADIOIMMUNOASSAY OF ESTRADIOL

(Stellflug et al., 1977, Biol. Reprod. 77:(in press) and Oxender et al., 1977, Amer. J. Vet. Res. 38:203)

Extraction

1. Triplicate aliquants of serum (.5 ml) were pipetted into disposable culture tubes (16 X 125 mm).

2. To account for procedural loss, a fourth aliquant from a representative number of unknowns (8 to 12 per assay) was added to a culture tube (16 X 125 mm) which contained 2500 cpm ^{3}H-2, 4, 6, 7-estradiol (New England Nuclear; 100 Ci/mM; repurified by column chromatography) in benzene. Twenty-five hundred cpm ^{3}H-2, 4, 6, 7-estradiol was also added to scintillation vials (4 to 6 per assay) for total counts (100% recovery).

3. Tubes containing serum and ^{3}H-estradiol were vortexed (10 sec.); and labeled and endogenous hormone were allowed to equilibrate for 30 minutes.

4. Serum was extracted with 5 ml benezene by vortexing for 60 seconds.

5. Extracted serum was then frozen over dry ice in methanol and solvent extract decanted into a culture tube (12 X 75 mm) for radioimmunoassay.

6. Solvent extracts from aliquants for procedural losses were decanted into scintillation vials. Radioactivity recovered in these extracts was averaged to determine a single correction factor to allow for procedural losses in all serum samples.

7. For comparison among assays, standard sera with high or low estradiol were assayed in triplicate in each assay.

Radioimmunoassay

1. Estradiol-17β (Sigma Chemical Company) for standards (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 pg) was pipetted into 12 X 75 mm culture tubes from a stock solution of 100 pg/ml in absolute methanol and incorporated into each assay.

2. Standards and serum extracts were evaporated dry in a vacuum over (28 to 30 inches vacuum) at a temperature of less than 50° C.

3. 1st Antibody[a] (200 µl) diluted 1:10,000 in 0.1% Knox PBS[b] containing 1:100 NRS (normal rabbit sera) was added to each tube, vortexed briefly (2 sec.), and incubated at room temperature for hours.

4. Then, 200 µl containing ∿10,000 cpm ^{3}H-2, 4, 6, 7-estradiol (New England Nuclear; 100 Ci/mM; repurified by column chromatography) diluted in 0.1% Knox PBS was added to each tube, vortexed briefly (2 sec.), and incubated at 4° C 18 to 24 hours.

5. 2nd Antibody[c] (400 µl) diluted 1:30 in 0.1% Knox PBS[b] was added to each tube, vortexed briefly (2 sec.), and incubated at 4° C for 48 hours.

6. All assay tubes were centrifuged at 2500 g for 30 min. and 0.5 ml aliquant of the supernatant was diluted with 5 ml scintillation fluid (3a70B Preblend; Research Products International Corp., Elk Grove Village, Illinois) for quantification of radioactivity in a liquid scintillation spectrophotometer.

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[a]1st Ab - MSU #74 anti-estradiol was prepared in rabbits against estradiol -6-oxime-human serum albumen.

[b]Knox Gelatin, Inc., Johnston, N.Y.; PBS - Phosphate (.01 M) buffered (pH 7.4) saline (0.85%).

[c]2nd Ab - MSU castrated sheep anti-rabbit gamma globulin.

APPENDIX C

RADIOIMMUNOASSAY FOR BOVINE LUTEINIZING HORMONE (LH)

(Convey et al., 1976. Proc. Exp. Biol. Med. 151:84)

The assay is as follows: Dilution duplicates of serum, 300 µl, diluted to 500 µl with PBS-0.1% Knox Gelatin are incubated at 4° C with first antibody (guinea pig antibovine LH) for 24 hours. Then ^{125}I-LH is added and incubation continued for another 24 hours. The second antibody (sheep anti-guinea pig gamma globulin) was then added. After an additional 72 hours, 3 ml of PBS was added and the samples were centrifuged at 2,500 g for 30 minutes. The supernatant fluid was decanted and ^{125}I in the precipitate was counted in a gamma counter.

1. Incubation with 1st Antibody. Each unknown was assayed in dilution duplicate. 300 µl of each unknown was added to separate disposable glass culture tubes (12 X 75 mm with a Micromedic automatic pipette. A total volume of 500 µl is obtained in each tube by adding PBS-0.1% Knox. Three sets of 12 tubes containing 0.015, 0.125, 0.175, 0.25, 0.35, 0.50, 0.75, 1.00, 1.50, 2.0, 4.0, or 5.0 ng of standard LH (NIH-LH-B8) in 500 µl of PBS-0.1% Knox was placed in each assay. Two hundred µl of LH antibody (1st antibody) was added at a dilution of 1:1,000,000 to each of the culture tubes and the tubes are incubated at 4° C for 24 hours. Each tube was vortexed gently (2 sec.) and covered during incubation at 4° C for 24 hours.

2. ^{125}I-LH. Methods for radioiodination of purified bovine LH (LER-1072-2) were essentially those of Niswender et al., 1969. Endo. 84:1166; except that ^{125}I was used and the column of Bio-Gel P-60 was coated with egg white albumen. The stock solution of ^{125}I-LH is diluted with PBS-0.1% Knox so that 100 µl contained about 20,000 cpm. One hundred µl of ^{125}I-LH solution is then added to each tube Incubation is continued at 4° C for 24 hours.

3. 2nd Antibody. Sheep anti-guinea pig gamma globulin (SAGPGG) referred to as 2nd antibody, diluted to a titer which would optimally precipitate the gamma globulin was used to form an antigen-antibody-antibody complex large enough to be precipated by centrifugation (1:15). Two hundred µl of SAGPGG is added to each tube and incubation was continued for 72 hours

4. Precipitation. Following final incubation, 3 ml of cold PBS is added to each tube to dilute the unbound ^{125}I-LH. Centrifugation at 2,500 g for 30 minutes in a refrigerated centrifuge was used to precipitate the bound ^{125}I-LH. The supernatant fluid was decanted and the tubes were allowed to drain for 30 minutes. Any fluid adherent to the neck and lip of the tube was removed with absorbent

tissue. The bound ^{125}I-LH of the precipitate was then quantified in an automatic gamma counter. Samples were counted for 10 minutes or to a total of 4,000 counts, whichever occurred first.

5. Control tubes. Control tubes are included in each assay to determine background radioactivity (2 tubes containing 1:400 control guinea pig serum in place of first antibody), total counts added (2 tubes containing only ^{125}I-LH) and counts in the precipitate (2 tubes containing no unknown or standard).

6. For comparison among assays, standard sera of high and low LH were included in triplicate in each assay.

APPENDIX D

EXTRACTION AND COMPETITIVE PROTEIN BINDING RADIOASSAY OF TOTAL GLUCOCORTICOIDS

(Smith et al., 1972. J. Dairy Sci. 55:1170;
Smith et al., 1973. J. Anim. Sci. 36:391)

Extraction

1. Duplicate aliquants of serum (200 µl) were pipetted into disposable culture tubes (16 X 100 mm).

2. To account for recovery, a third aliquant from representative unknowns (8 to 12 per assay) was added to a culture tube (16 X 100 mm) which contained 200 cpm ^{3}H-1, 2, 6, 7-cortisol (New England Nuclear; 91 Ci/mM; repurified by column chromatography) in methanol. Twenty-five hundred cpm ^{3}H-1, 2, 6, 7-cortisol was also added to scintillation vials (4 to 6 per assay) for total counts (100% recovery).

3. Tubes containing serum and ^{3}H-cortisol were vortexed (10 sec.) and allowed to equilibrate for 30 minutes at room temperature.

4. Serum was extracted and vortexed with 2 ml Iso-octane (2, 2, 4-trimethyl pentane) for 1 minute, then stored at -20° C for at least 1 hour to freeze the aqueous phase. Supernatant was poured off and discarded. Samples were allowed to thaw.

5. Thawed aqueous phase was then extracted and vortexed with 2 ml Methylene chloride for 1 minute and stored at -20° C for at least 1 hour to freeze aqueous phase. Solvent extracts were then decanted into disposable assay culture tubes (12 X 75 mm). Extracts from aliquants for recovery loss were decanted into scintillation vials. Recovered radioactivity was averaged to determine a single correction factor to account for recovery.

6. For comparison among assays, standard sera with high and low cortisol were included in triplicate in each assay.

Protein Binding Assay

1. Cortisol (Sigma Chemical Company) for sets (3 per assay) of standards (.05, .10, .15, .20, .25, .50, 1.00, 1.50, 2.00, 2.50 ng) was pipetted into disposable culture tubes (12 X 75 mm) from a stock solution of 10 ng/ml in absolute methanol and included in each assay. Thus, unknown glucocorticoid is calculated in terms of cortisol.

2. Standards and serum extracts were evaporated dry in a vacuum oven (28-30 inches vacuum) at a temperature less than 50° C.

3. Then, 1.0 ml 1.25% dog plasma[a] with approximately 11,000-12,000 cpm/0.5 ml 1, 2, 6, 7 ^{3}H-cortisol is added to each tube. The tubes are vortexed briefly (2 sec.) and stored at 4° C for 12 to 18 hours.

4. The assay tubes are placed in an ice bath and allowed to equilibrate for 10-15 minutes. To separate the bound from the free glucocorticoids, 0.5 ml of 1% dextran T70 (Pharmacia, Uppsula, Sweden) and 0.5% carbon decolorizing neutral norit (Fisher Scientifica Co.) in glass distilled water is added to each tube.

   Contents are rapidly added and allowed to incubate in an ice bath for 5 minutes, then centrifuged at 2,000 g for 15 minutes at 5° C. A 0.5 ml aliquant of the supernatant fluid is diluted with 5 ml scintillation fluid (3a70B Preblend, Research Products International Corp., Elk Grove Village, Illinois) for quantification of radioactivity in a liquid scintillation spectophotometer.

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[a]Dog plasma (Colorado Serum Co.) was diluted to 2.5% in 500 ml glass distilled water and mixed with 60 g Florisil (80 mesh; Matheson, Coleman & Bell) for 3 hours to strip endogenous steriods. The suspension was centrifuged at 2,800 rpm for 15 minutes and the supernatant fluid volume is doubled with glass distilled water to give 1.25% plasma, ^{3}H-1, 2, 6, 7-cortisol was added to the 1.25% plasma to give about 11,000-12,000 cpm/0.5 ml and stored at 5° C for up to one month.

APPENDIX E

EQUATIONS FOR DETERMINING ENERGY BALANCE

(N.R.C., 1971)

1. Net energy → NE (Mcals)
   - Intake (I) → NE_I (Mcals/kg feed intake)
   - Maintainance (M) → NE_M (Mcals/kg body weight)
   - Lactation (L) → NE_L (Mcals/kg milk)

2. $NE_I = NE_{CS} + NE_H + NE_{HL} + NE_G$
   - NE_{CS} ↓ corn silage
   - NE_H ↓ alfalfa hay
   - NE_{HL} ↓ haylage
   - NE_G ↓ grain

3. $NE_M = .085 \times (BW)^{.75}$
   - BW ↓ Body Weight

4. $NE_L = NE_L$ / kg milk
   $= .3512 + (.09619 \times \text{Fat \%})$

5. $N.R.C._{req} = .085 \times (BW)^{.75} + .3512 + (.09619 \times \text{Fat \%}) \times \text{kg milk}$
   - req ↓ requirement

6. $\text{Energy Balance} = \frac{NE_I}{N.R.C._{req}} \times 100\%$

If Energy Balance is:

- < 100% : negative balance
- = 100% : zero balance
- > 100% : positive balance

APPENDIX F

APPENDIX F

Table 7. -- Serum LH concentrations on days 7 and 14 postpartum.

Period	LH[a]	No. Episodic LH Peaks	Duration of LH Peak[b]	Magnitude of Largest LH Peak[a]
I. Day 7	1.05 ± 0.2[c]	1.4 ± 0.1[e]	27 ± 3.0	2.7 ± 0.3[g]
II. Day 14	1.57 ± 0.2[d]	1.8 ± 0.1[f]	32 ± 3.0	3.7 ± 0.3[h]

[a] ng/ml

[b] minutes

[c,d] Means with dissimilar letters with category are different (P<.001).

[e,f] Means with dissimilar letters with category are different (P<.08).

[g,h] Means with dissimilar letters with category are different (P<.10).

Table 8. -- Effect of interval to first postpartum ovulation on serum glucocorticoid concentrations.

Days to Ovulation	Glucocorticoids[a]
I. 9 - 14	3.6 ± 0.7
II. 15 - 20	2.2 ± 0.4
III. 21 - 34	3.1 ± 0.7

[a] ng/ml (average of samples collected on days 7 and 14).

Table 9. -- Serum glucocorticoid concentrations on day 7 and day 14 postpartum

Period	Glucocorticoids[a]
Day 7	3.3 ± 0.2
Day 14	3.7 ± 0.2

[a] ng/ml

Table 11. -- Distribution of cows within intervals to first ovulation by level of genetic ability for milk production

Days to Ovulation	Best	Worst
I. 9 - 14 (n = 7)	4	3
II. 15 - 20 (n = 13)	7	6
III. 21 - 34 (n = 5)	5	0

$\chi^2 = 3.54$, 2 df, ($P>.10$)

Table 12. -- Changes in body weight during the first six weeks postpartum as affected by parity, week of lactation and interval to first ovulation.

Parity	% Change in BW	Cumulative BW Change (kg)
Primiparious (n = 12)	-0.16 ± 0.8	-1.7 ± 4.4
Pluriparous (n = 15)	-1.46 ± 0.8	-9.0 ± 4.0
Week		
1	-1.95 ± 0.5[a]	-11.0 ± 2.7[a]
2	-2.34 ± 0.5[a]	-13.2 ± 2.7[a]
3	-0.98 ± 0.5[b]	- 5.9 ± 2.1[b]
4	-0.44 ± 0.5[b]	- 3.3 ± 2.1[b]
5	1.01 ± 0.5[c]	4.1 ± 2.1[c]
6	1.56 ± 0.5[c]	6.9 ± 2.1[c]
Days to Ovulation		
I. 9 - 14 (n = 8)	-0.27 ± 1.0	- 3.4 ± 5.5
II. 15 - 20 (n = 14)	-1.84 ± 0.8	-10.8 ± 4.2
III. 21 - 34 (n = 5)	-1.30 ± 1.3	- 8.6 ± 6.9

[a,b,c]Means with dissimilar letters are different (P<.05)

Table 13. -- Changes in energy balance during the first six weeks postpartum as affected by week of lactation and interval to first ovulation.

Days to Ovulation	% N.R.C.[a]
I. 9 - 14 (n = 8)	131 ± 6
II. 15 - 20 (n = 14)	126 ± 5
III. 21 - 34 (n = 5)	114 ± 8
Week	
1	127 ± 3.4[b]
2	124 ± 3.4[b]
3	120 ± 3.4[b]
4	108 ± 3.4[c]
5	122 ± 3.4[b]
6	130 ± 3.4[b]

[a](Intake ÷ Requirement) X 100%

[b,c]Means with dissimilar letters are different (P<.01).

Table 15. -- Changes in energy balance during the first six weeks postpartum as affected by parity by week of lactation interaction.

	Week of Lactation[a]						
Parity	1	2	3	4	5	6	S.E.
Primiparous (n = 12)	147[b]	126[b]	130[b]	132[b]	137[b]	134[b]	±4.8
Pluriparous (n = 16)	103[c,d]	97[c]	113[d]	115[d]	110[c,d]	125[b,d]	±4.3

[a](Intake ÷ Requirements) X 100%

[b,c,d]Means with dissimilar letters are different (P<.05) between parity groups and within week of lactation.

APPENDIX G

INDIVIDUAL PROFILES OF LH AND GLUCOCORTICOID CONCENTRATIONS MEASURED IN BLOOD COLLECTED ON DAY 7 AND DAY 14 POSTPARTUM

Figure 16. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 7 postpartum from cow 1289 (Interval I).

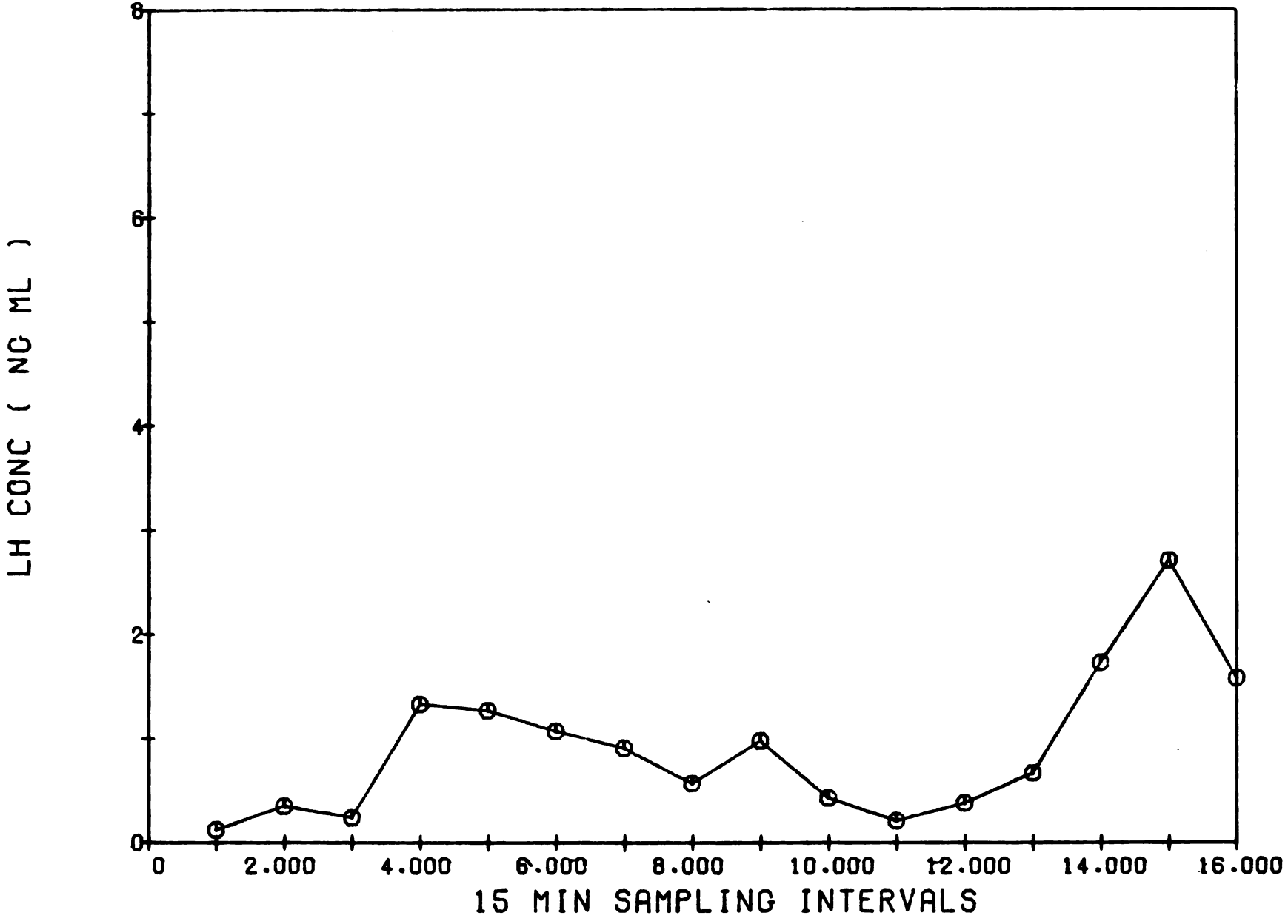
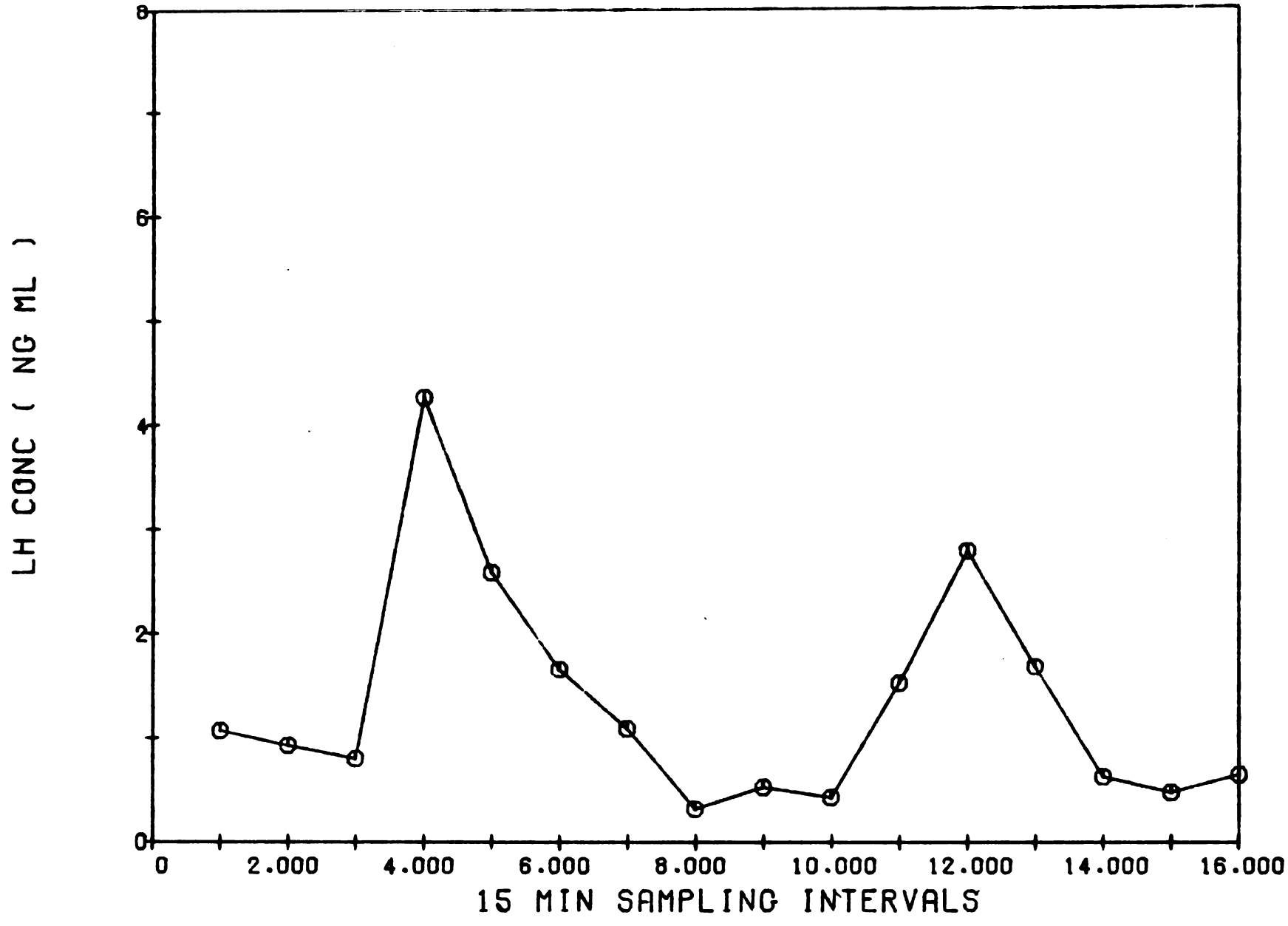

LH CONC (NG ML)
8
6
4
2
0
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

Figure 17. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 14 postpartum from cow 1289 (Interval I).


LH CONC (NG ML)
8
6
4
2
0
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

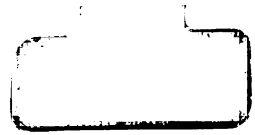
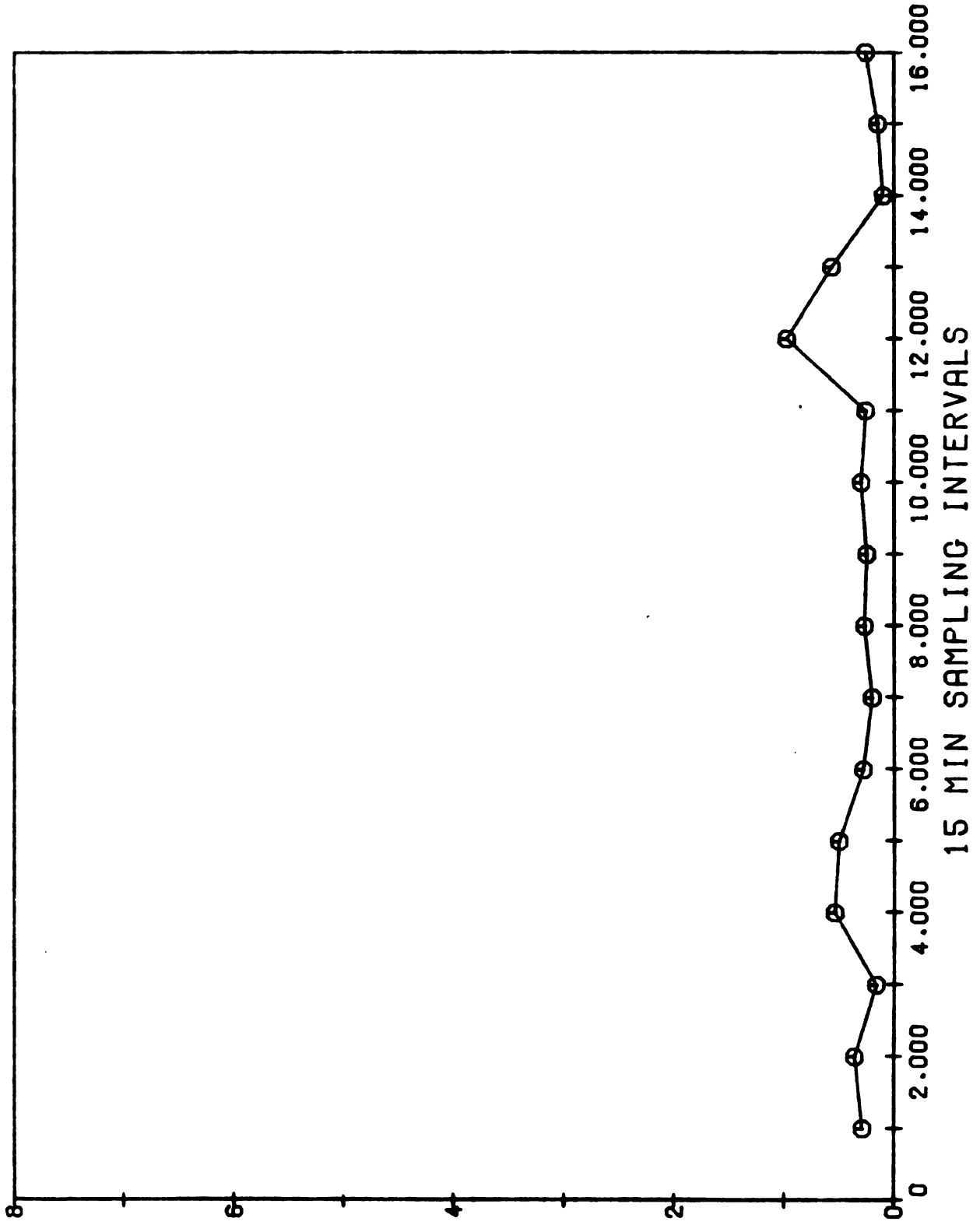


Figure 18. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 7 from cow 1416 (Interval II).


LH CONC (NG ML)
0
2
4
6
8
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

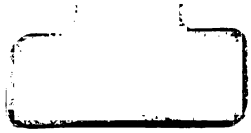


Figure 19. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 14 postpartum from cow 1416 (Interval II).

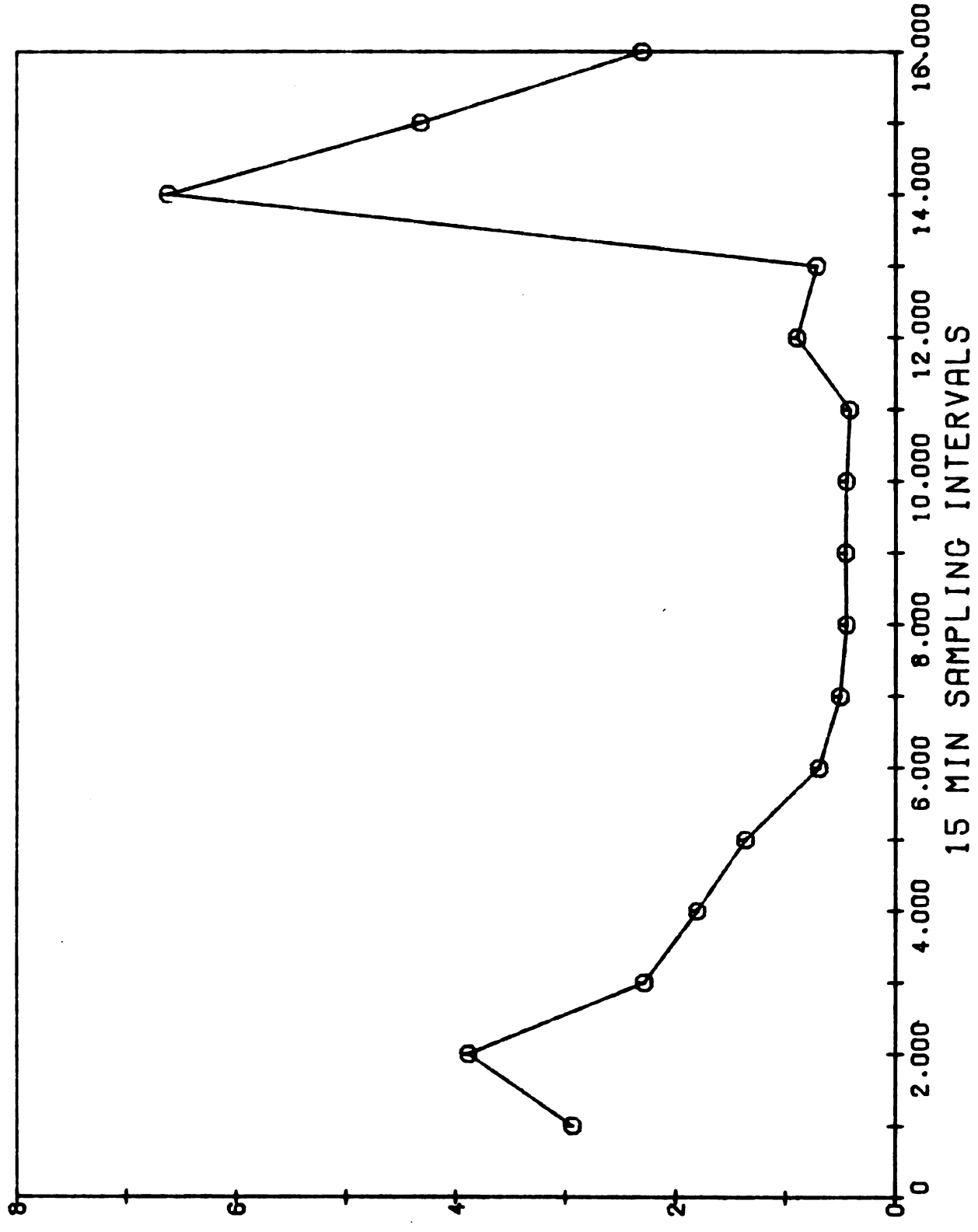
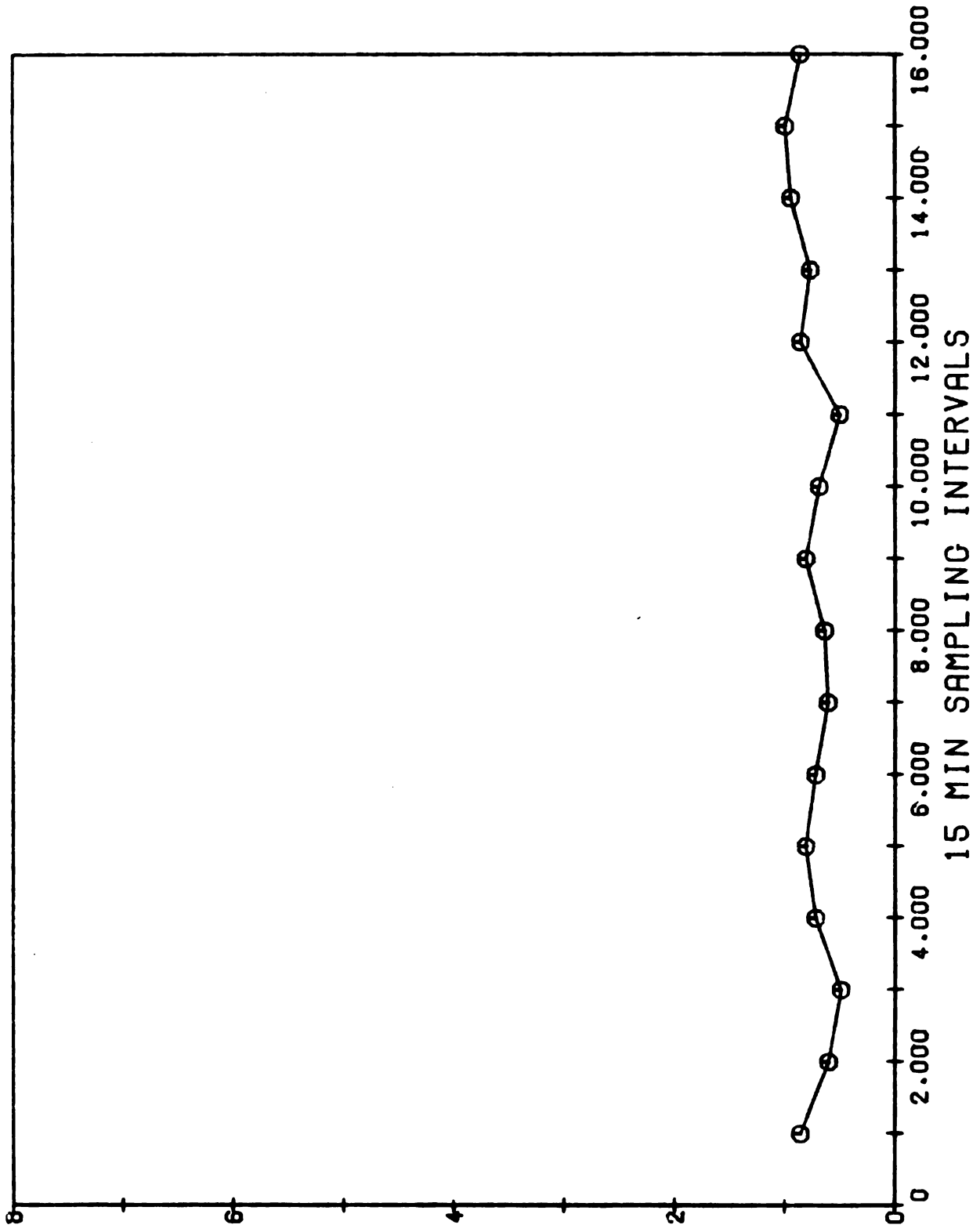

LH CONC (NG ML)
0
2
4
6
8
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

Figure 20. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 7 postpartum from cow 1359 (Interval III).


LH CONC (NG ML)
0
2
4
6
8
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

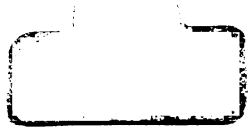


Figure 21. -- Serum LH concentrations in blood samples collected at 15 minute intervals for 4 hours on day 14 postpartum from cow 1359 (Interval III).

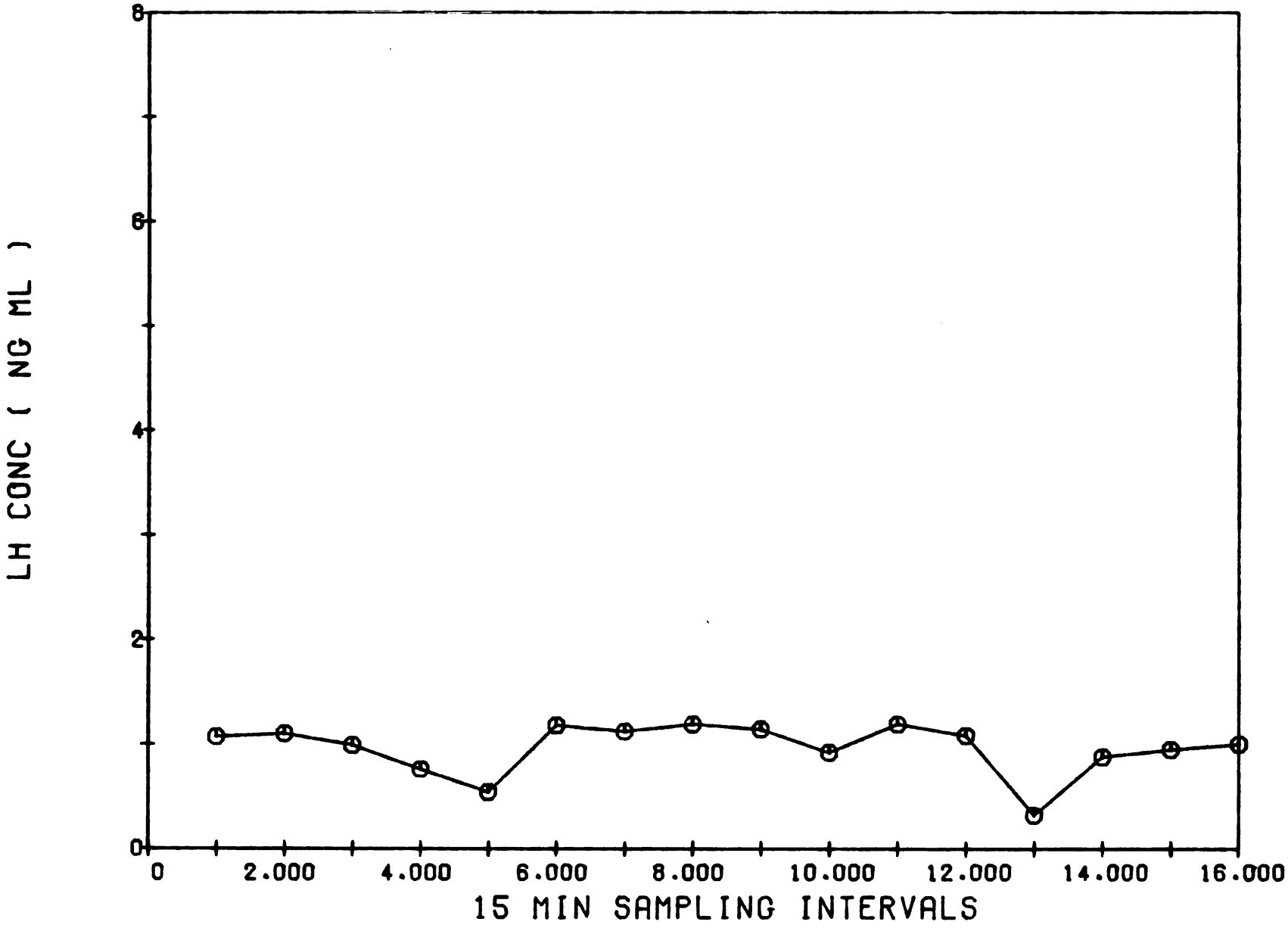

LH CONC (NG ML)
8
6
4
2
0
0
2.000
4.000
6.000
8.000
10.000
12.000
14.000
16.000
15 MIN SAMPLING INTERVALS

Figure 22. -- Serum glucocorticoid concentrations in blood samples collected at 30 minute intervals for 4 hours on day 7 postpartum from cow 1435.

GLUCOCORTICOID CONC (NG ML)
12
8
4
0
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

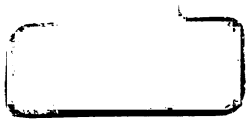
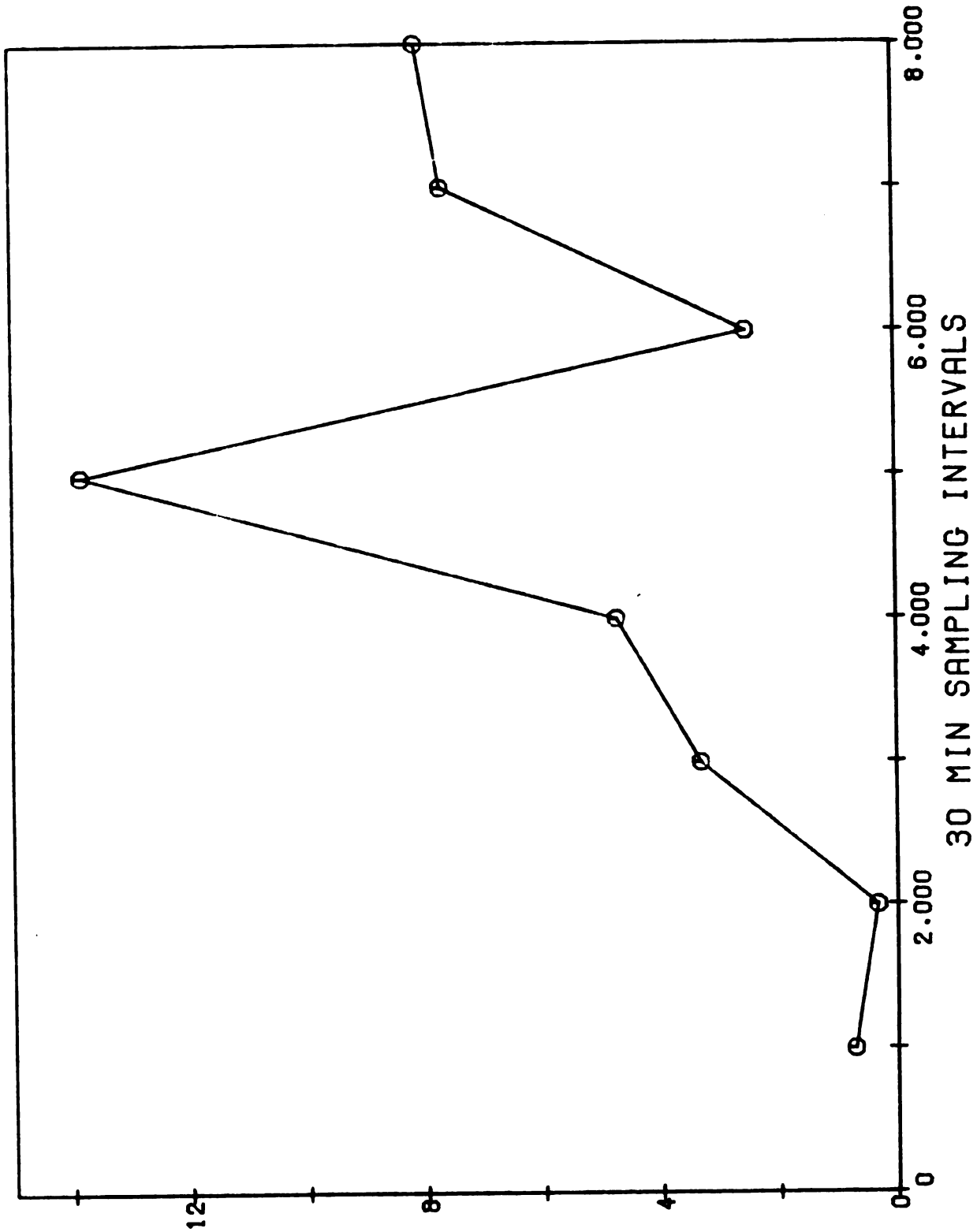


Figure 23. -- Serum glucocorticoid concentrations in blood samples collected at 30 minute intervals for 4 hours on day 14 postpartum from cow 1435.


GLUCOCORTICOID CONC (NG ML)
12
8
4
0
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

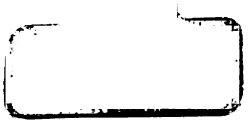
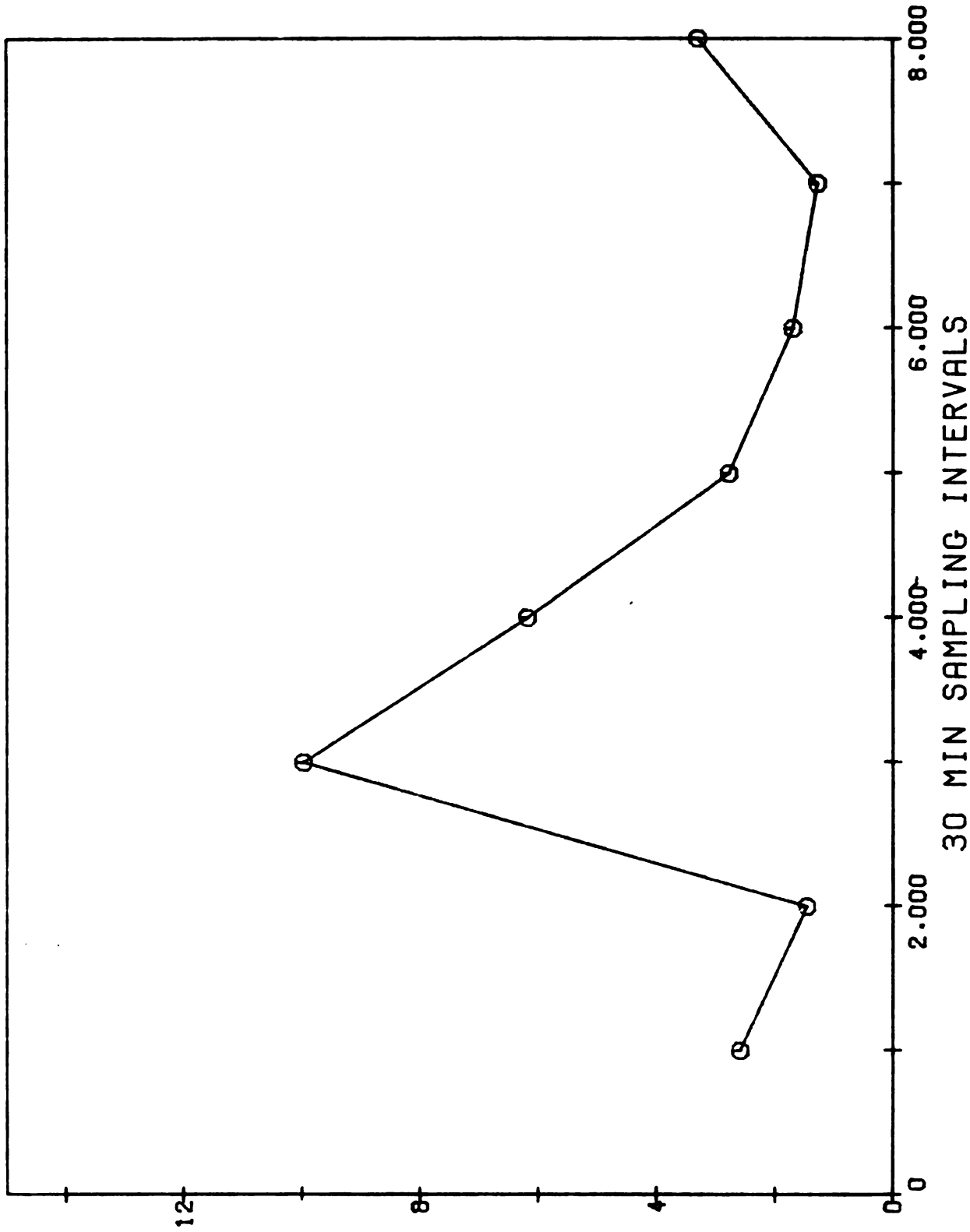


Figure 24. -- Serum glucocorticoid concentrations in blood samples collected at 30 minute intervals for 4 hours on day 7 postpartum from cow 1364.


GLUCOCORTICOID CONC (NG ML)
0
4
8
12
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

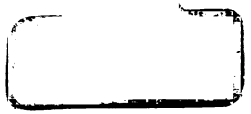
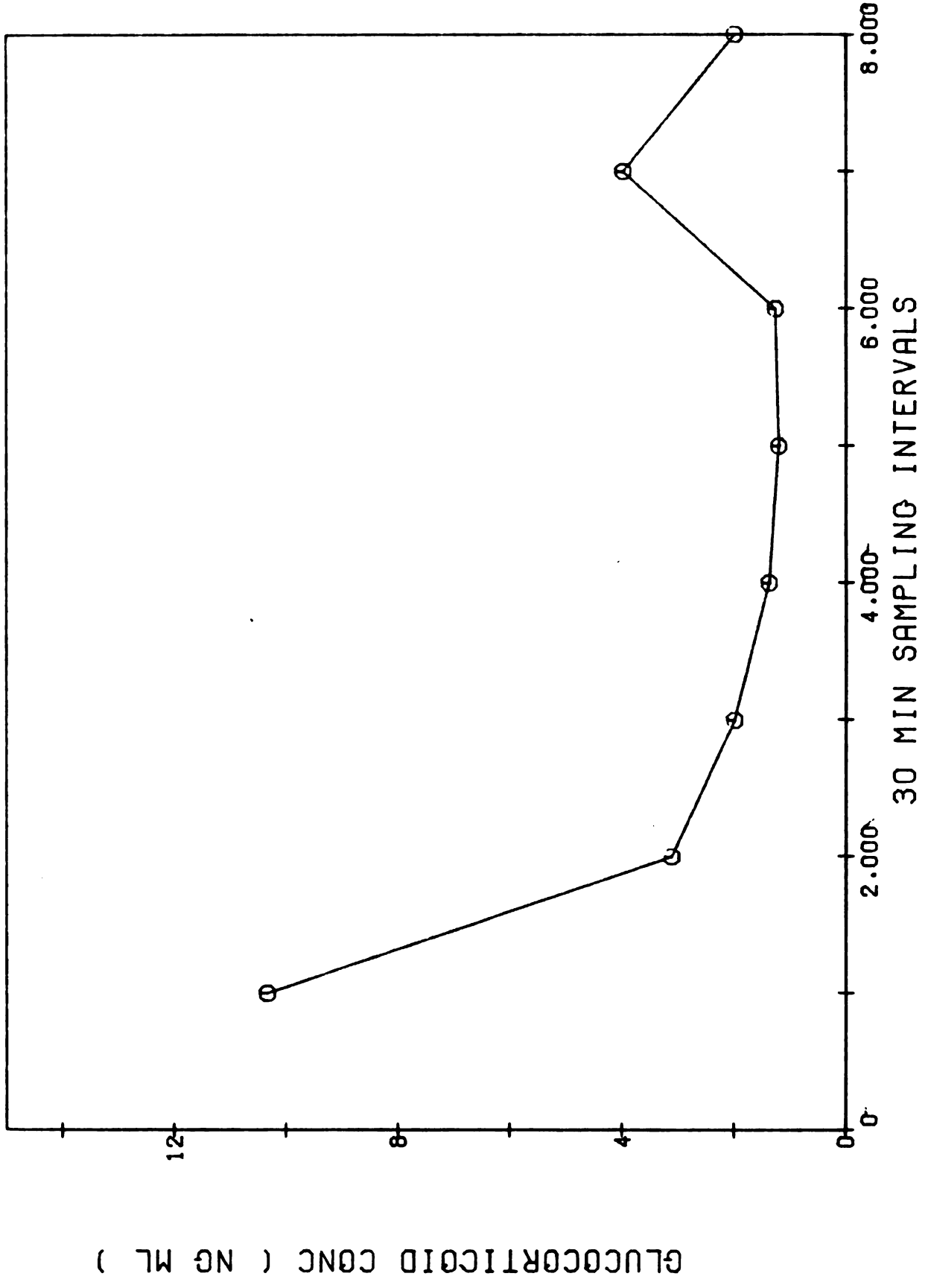


Figure 25. -- Serum glucocorticoid concentrations in blood samples collected at 30 minute intervals for 4 hours on day 14 postpartum from cow 1364.


GLUCOCORTICOID CONC (NG ML)
12
8
4
0
0
2.000
4.000
6.000
8.000
30 MIN SAMPLING INTERVALS

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