MONTANA STATE UNIVERSITY

LH and prolactin levels in postpartum beef cows and prolactin levels in cyclic beef cows subjected to various mating stimuli

by David Kendal Han

A thesis submitted to the Graduate Faculty in partial fulfullment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University
© Copyright by David Kendal Han (1973)

Abstract:
Specific bovine radioimmunoassays (RIA) were utilized for quanitat-ing blood levels of LH and prolactin in beef cattle. Radioiodination damage was a problem with the prolactin assay and was characterized by the formation of molecular aggregates of prolactin-I 131. These aggregates exhibited abnormal binding affinity with the prolactin antibody and were not displaced from the antibody in a direct relationship with in-creasing concentrations of unlabeled prolactin. By using milder reaction conditions for preparation of the prolactin-I 131 and a double purification system on Sephadex G-75 columns, the prolactin-I-131 aggregates could be minimized. Following the second purification, the remaining prolactin-I 131 was suitable for use in the assay system and produced very desirable standard curves.

Four postpartum Hereford cows were randomly divided into a non-suckled and suckled group. Daily blood samples were collected through indwelling jugular cannulas for subsequent plasma LH and prolactin measurement. Calves from the non-suckled groups were removed from their dams on the day of parturition. Data were analyzed by the method of least-squares. Least-squares prolactin means were significantly different ($P<.01$) between non-suckled (94.9+13.6 ng/ml) and suckled (176.3+10.7 ng/ml) groups. A significant negative linear regression ($b= -6.795+0.985$) of prolactin on the day postpartum was observed in a combined group analysis. No significant differences in LH were detected between groups. The within subclass correlation coefficient ($r= -.29$) between LH and prolactin was significant ($P<.06$) for the combined group analysis.

Serum prolactin was measured in 16 mature estrous synchronized beef cows from the onset of estrus until ovulation. Blood samples were collected through indwelling jugular cannulas at hourly intervals for the first 24 hrs and at 2 hr intervals thereafter until ovulation.

Cannulas were inserted at the onset of estrus. All cows were divided into two treatment groups. The control groups (NS) received no clitoral stimulation while the treated group (S) received manual clitoral stimulation or natural mating to a surgically sterilized bull. Data were analyzed by the method of least-squares. LH data from this group of cows was previously reported by Randel et al. (1973) . The data was grouped and analyzed with the onset of estrus and the LH peak as distinct physiological events. A significant group X sample time interaction ($P<.01$) was observed when the data was aligned on the onset of estrus. When the data was regrouped and centered on the LH peak for the individual cows, a significant group difference ($P<.01$) was observed during the interval prior to the LH peak. The least-squares prolactin means for the interval before the LH peak were 39.7+3.6 ng/ml and 16.7+2.6 ng/ml, respectively, for the NS and S groups.

In presenting this thesis in partial fulfillment of the requirements for an advanced degree of Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature David Han

Date 9/18/73

LH AND PROLACTIN LEVELS IN POSTPARTUM BEEF COWS AND PROLACTIN LEVELS IN CYCLIC BEEF COWS SUBJECTED TO VARIOUS MATING STIMULI

by

DAVID KENDAL HAN

A thesis submitted to the Graduate Faculty in partial fulfullment of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

Approved:

R. V. Blackwell
Head, Major Department

Edward Moody
Chairman, Examining Committee

K. Greening
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1973

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. E. L. Moody for his assistance and encouragement throughout my graduate program. Special thanks are also given to Drs. P. J. Burfening, J. Catlin and S. Rogers for their assistance and cooperation as committee members and to R. D. Randel for supplying the blood samples for the second study.

Further appreciation is extended to Mr. V. A. LaVoie for his assistance with jugular cannulations and to Mrs. Cindy Himelspach for technical laboratory assistance.

I would also like to thank Dr. E. P. Smith, Dr. P. J. Burfening and Mr. Robert Friedrich for assistance and advice in statistical analyses of these data.

Very special appreciation is extended to Mrs. Frankie Larson for typing the manuscript.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGEMENTS	iii
INDEX TO TABLES	vii
INDEX TO FIGURES	viii
INDEX TO APPENDIX	x
ABSTRACT	xii
INTRODUCTION	1
LITERATURE REVIEW	2
Postpartum Interval	2
Calving Season	2
Disease	2
Nutrition	3
Lactional Status	4
Endocrine Physiology of the Postpartum and Cycling Female	4
Bovine FSH, LH and Prolactin Levels During the Postpartum Interval	5
Exogenous Hormone Treatment of Postpartum cows	7
Bovine LH and Prolactin Levels During the Estrous Cycle as Determined by RIA	8
Hormonal Response to Mating Stimuli	9
Half-Life and Metabolic Clearance Rate of Prolactin and Gonadotropins	10
Ovulation and Luteal Function	11
Extrinsic Factors Affecting Prolactin Release	16
Pharmaceutical Agents Affecting Prolactin Release	18
Prolactin Release in Response to Milking	19
Hypothalmic Control of Gonadotropins and Prolactin	21
Hypophysitropic Hormones	21
Autofeedback Mechanism	24
Hypothalmic Areas Controlling Ovulation and Pseudogregnancy	26
Sex Differentiation of the Hypolalmus	27

Page

Evidence for Antogonism Between Prolactin and Gonadotropin Production. 33

Assay Procedures for Reproductive Hormones 39
HCG Augmentation Assay for FSH 39
Ovarian Ascorbic Acid Depletion Assay (OAAD) for LH. . . . 39
Uptake of Radioactive Phosphorus by the Chick Testis Assay for LH 40
Prolactin Bioassays. 40
Pigeon Crop Sac Assay for Prolactin. 40
Lactogenic Assay for Prolactin 41
Luteotrophic Assay for Prolactin 42
Radioimmunoassays. 43

MATERIAL AND METHODS 45

Postpartum Cows. 45

MAP Estrous Synchronized Cows Subjected to Various Mating Stimuli 47

LH Assay 49

Prolactin Assay. 52

Data Analysis. 56

RESULTS. 58

LH Assay 58

Prolactin Assay. 58

LH and Prolactin Levels for Postpartum Cows. 60

General Comments on Postpartum Cows. 61

Prolactin Levels in MAP Estrous Synchronized Cows Subjected to Various Mating Stimuli. 62

DISCUSSION 66

Prolactin Assay. 66

Page

LH and Prolactin Levels in Postpartum Cows 70

Rectal Temperatures of Postpartum Cows 76

Temperament Evaluation of Postpartum Cows. 77

Prolactin Levels in MAP Estrous Synchronized Cows Subjected to Various Mating Stimuli. 77

Comparison of Blood Sampling Techniques. 80

SUMMARY. 96

APPENDIX 99

LITERATURE CITED 116

INDEX TO TABLES

TABLE		Page
1	TEMPORAL RELATIONSHIPS AMONG THE ONSET OF ESTRUS, THE LH SURGE AND OVULATION IN THE COW	8
2	THE EFFECT OF POSSIBLE NEUROTRANSMITTERS ON THE PLASMA LEVELS OF FSH, LH AND PROLACTIN	38
3	LEAST SQUARES ANALYSIS OF VARIANCE FOR COMBINED NON-SUCKLED AND SUCKLED GROUPS	60
4	TEMPERAMENT EVALUATION DURING BLOOD SAMPLING OF POSTPARTUM COWS	61
5	LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS IN NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FROM THE ONSET OF ESTRUS TO OVULATION	63
6	LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INTERVAL FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS AND THE INTERVAL FROM THE LH PEAK TO OVULATION	64
7	LEAST SQUARES PROLACTIN MEANS AND STANDARD ERRORS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INTERVAL FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS AND THE INTERVAL FROM THE LH PEAK TO OVULATION	64
8	LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INDIVIDUAL SAMPLE TIMES FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS	65
9	LEAST SQUARES PROLACTIN MEANS AND STANDARD ERRORS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INDIVIDUAL SAMPLE TIMES FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS	65

INDEX TO FIGURES

Figure		Page
1	Elution pattern of radioiodinated LH (Bio-Gel P-60 column)	82
2	Elution pattern of radioiodinated prolactin from first purification (Sephadex G-75 column)	83
3	Elution pattern of radioiodinated prolactin from second purification (Sephadex G-75 column)	84
4	LH standard curve (NIH-LH-B7)	85
5	Comparison of prolactin standard curves from peaks A and B (Fig. 2) following single purification on Sephadex G-75 (NIH-P-B3)	86
6	Prolactin standard curve following second purfication (Fig. 3) on Sephadex G-75 column (NIH-P-B3)	87
7	LH and prolactin levels for cow 603 from parturition until 21 days postpartum (non-suckled group)	88
8	LH and prolactin levels for cow 621 from 3 days postpartum until 17 days postpartum (non-suckled group)	89
9	LH and prolactin levels for cow 613 from 4 days prepartum until 26 days postpartum (suckled group)	90
10	LH and prolactin levels for cow 673 from parturition until 27 days postpartum (suckled group)	91
11	Least squares periparturient LH and prolactin means for combined suckled and non-suckled groups (Day 0 = parturition)	92

Figure Page

12 Least squares periparturient prolactin means for suckled and non-suckled groups (Day 0 = parturition) 93

13 Least square prolactin means for non-stimulated and stimulated estrus synchronized cows from the onset of estrus to ovulation (Estrus = 0). 94

14 Least squares prolactin means for non-stimulated and stimulated groups before and after the LH peak (LH peak = 0) 95

INDEX TO APPENDIX

Figure		Page
I	Prolactin levels for cow 7939 from estrus until ovulation (Group I)	100
II	Prolactin levels for cow 9704 from estrus until ovulation (Group I)	101
III	Prolactin levels for cow 9107 from estrus until ovulation (Group I)	102
IV	Prolactin levels for cow 9041 from estrus until ovulation (Group I)	103
V	Prolactin levels for cow 9771 from estrus until ovulation (Group II).	104
VI	Prolactin levels for cow 9379 from estrus until ovulation (Group II).	105
VII	Prolactin levels for cow 8343 from estrus until ovulation (Group II).	106
VIII	Prolactin levels for cow 8056 from estrus until ovulation (Group II).	107
IX	Prolactin levels for cow 9117 from estrus until ovulation (Group III)	108
X	Prolactin levels for cow 9834 from estrus until ovulation (Group III)	109
XI	Prolactin levels for cow 9656 from estrus until ovulation (Group III)	110
XII	Prolactin levels for cow 9871 from estrus until ovulation (Group IV).	111
XIII	Prolactin levels for cow 7720 from estrus until ovulation (Group IV).	112

Figure		Page
XIV	Prolactin levels for cow 9809 from estrus until ovulation (Group V)	113
XV	Prolactin levels for cow 7566 from estrus until ovulation (Group V)	114
XVI	Prolactin levels for cow 5386 from estrus until ovulation (Group V)	115

ABSTRACT

Specific bovine radioimmunoassays (RIA) were utilized for quanitating blood levels of LH and prolactin in beef cattle. Radioiodination damage was a problem with the prolactin assay and was characterized by the formation of molecular aggregates of prolactin-I^{131}. These aggregates exhibited abnormal binding affinity with the prolactin antibody and were not displaced from the antibody in a direct relationship with increasing concentrations of unlabeled prolactin. By using milder reaction conditions for preparation of the prolactin-I^{131} and a double purification system on Sephadex G-75 columns, the prolactin-I^{131} aggregates could be minimized. Following the second purification, the remaining prolactin-I^{131} was suitable for use in the assay system and produced very desirable standard curves.

Four postpartum Hereford cows were randomly divided into a non-suckled and suckled group. Daily blood samples were collected through indwelling jugular cannulas for subsequent plasma LH and prolactin measurement. Calves from the non-suckled groups were removed from their dams on the day of parturition. Data were analyzed by the method of least-squares. Least-squares prolactin means were significantly different ($P<.01$) between non-suckled (94.9 ± 13.6 ng/ml) and suckled (176.3 ± 10.7 ng/ml) groups. A significant negative linear regression ($b= -6.795\pm0.985$) of prolactin on the day postpartum was observed in a combined group analysis. No significant differences in LH were detected between groups. The within subclass correlation coefficient ($r= -.29$) between LH and prolactin was significant ($P<.06$) for the combined group analysis.

Serum prolactin was measured in 16 mature estrous synchronized beef cows from the onset of estrus until ovulation. Blood samples were collected through indwelling jugular cannulas at hourly intervals for the first 24 hrs and at 2 hr intervals thereafter until ovulation. Cannulas were inserted at the onset of estrus. All cows were divided into two treatment groups. The control groups (NS) received no clitoral stimulation while the treated group (S) received manual clitoral stimulation or natural mating to a surgically sterilized bull. Data were analyzed by the method of least-squares. LH data from this group of cows was previously reported by Randel *et al*. (1973). The data was grouped and analyzed with the onset of estrus and the LH peak as distinct physiological events. A significant group X sample time interaction ($P<.01$) was observed when the data was aligned on the onset of estrus. When the data was regrouped and centered on the LH peak for the individual cows, a significant group difference ($P<.01$) was observed during the interval prior to the LH peak. The least-squares prolactin means for the interval before the LH peak were 39.7 ± 3.6 ng/ml and 16.7 ± 2.6 ng/ml, respectively, for the NS and S groups.

INTRODUCTION

The annual loss of income to U. S. beef producers, resulting from prolonged postpartum anestrus and infertility, has been conservatively estimated to be $621,000,000. This loss of income has been attributed to the reduced reproductive performance of cows that are not maintained on a 12-month calving interval. Calving intervals with a duration greater than 12 months, lead to extended breeding and calving seasons which produce economic losses due to increased labor requirements, reduction in management efficiency and lighter weight calves at weaning time from late calving cows. The actual physiological problem associated with postpartum anestrus and infertility seems to have increased in recent years due to selection practices designed to increase milk production. Studies designed to determine the relationship between milk production and fertility indicate that these events are negatively correlated. In the following study relating to postpartum anestrus and infertility, an attempt was made to assess some of the differences in endocrine physiology between non-suckled and suckled postpartum cows.

The economic benefits of artificial insemination (A.I.) in the beef industry are clearly established, however, A.I. conception rates have been shown to be lower in beef cattle than in dairy cattle. Increased conception rates resulting from improved A.I. techniques would be advantageous to both the beef and dairy industry. For this reason, a second study was designed to determine differences in the endocrine physiology of cows receiving various degrees of manual and natural mating stimulation.

LITERATURE REVIEW

Postpartum Interval

Reports in the literature indicate extreme variation in postpartum intervals of beef and dairy breeds ranging from 18 to 104 days (Casida, 1968; Wagner and Oxenreider, 1971). The following review will relate to the majority of factors contributing to the wide range in postpartum intervals.

The first factor to be discussed stems from the definition of the term postpartum interval. Postpartum interval refers to the period of time beginning with parturition and ending with a designated event such as first ovulation, first estrus, first breeding, conception or complete involution of the uterus (Casida, 1968). The lack of a standardized event marking the end of the postpartum interval has introduced much of the variation observed between individual studies in the literature.

The following factors have been reported to effect the duration of the postpartum interval to first ovulatory estrus in cattle.

Calving Season. Buch, Woehling and Casida (1955) observed that winter calving was accompanied by the longest postpartum intervals, while summer calving resulted in the shortest. Spring and fall calving were found to be intermediate between the two extremes. No differences were observed due to age or parity of the dams.

Disease. Morrow (1971) concluded that periparturient (pre- and post-partum) diseases consisting of abortion, dystocia, retained fetal membranes, metritis, milk fever, active mastitis, ketosis, displaced abomassum or other debilitating diseases, significantly retarded the

first postpartum ovulation. The differences being that ovulation occurred on the average of 18 days postpartum in normal dairy cows compared to 42 days postpartum in abnormal dairy cows.

Nutrition. Reduced energy levels prior to calving have been reported to increased postpartum intervals to first estrus (Wiltbank et al. 1962 and Dunn et al. 1969), while other reports indicate that increasing energy intake following parturition will decrease postpartum intervals to first estrus. Wiltbank et al. 1964 and Dunn et al. 1969). Wagner and Oxenreider (1971) found significant differences in the interval required to attain a 10 mm follicle, between high and low energy groups of postpartum cows (133% N.R.C. requirement vs. 66% N.R.C. requirement). The difference being a 10 mm follicle observed at day 10 postpartum in the high energy group and day 16 in the low energy group. Reduction of blood glucose levels were demonstrated in the low energy group and suggestions were made that hypoglycemia resulting from inadequate nutrition could be a primary factor affecting postpartum interval.

In the rat, underfeeding or starvation has been reported to decrease anterior pituitary and target organ function (as reviewed by Meites, 1970). Hypothalmic content of luteinizing hormone releasing hormone (LH-RH) in rats fed 50% of the normal food intake, showed a significant reduction to 25% of the level measured in the control ad libitum fed rats. Pituitary LH concentration was also significantly reduced, however, pituitary FSH did not change significantly (Meites, 1970).

Lactational Status. Several reports indicate that suckled cows have longer postpartum intervals than cows which are milked or non-suckled (Graves et al. 1968; Oxenreider, 1968; Saiduddin et al. 1968; Wagner and Oxenreider, 1971). Other evidence indicates that suckling retards the growth of early postpartum follicles, although it is suggested that follicles large enough to mature and ovulate are present in lactating cows by 1 to 2 weeks postpartum (Wagner and Oxenreider, 1971). Short et al. (1972) observed significant differences in the postpartum interval between groups of cows having different lactational status. The postpartum interval to first estrus for suckled, non-suckled and mastectomized cows was 65, 25 and 12 days, respectively. These effects were observed after the nutrient intake of the individual groups was adjusted for lactational status to minimized postpartum weight changes. Saiduddin et al. (1968) reported that the postpartum interval of dairy cows was 9 days longer for genetically high milk producers as compared to genetically low producers, regardless of the level of concentrate feeding. Menge et al. (1962) found a positive correlation ($r = .84$) between milk production and calving interval between sires lines for the first 90 days of lactation. This evidence suggests direct relationship between milk production and interval to first postpartum estrus.

Endocrine Physiology of the Postpartum and Cycling Female

In the following review, major emphasis will be placed on the

endocrine physiology of the cow, however, studies utilizing the female rat, rabbit, and ewe will also be included in areas where basic information is lacking for the cow.

Bovine FSH, LH and Prolactin Levels During the Postpartum Interval. Assessment of pituitary FSH during the postpartum interval, utilizing the HCG augmentation assay, indicates that FSH is high in parturition and declines as the postpartum interval progresses (Labhsetwar et al. 1964 and Saiduddin et al. 1968).

Several studies utilizing the ovarian ascorbic acid depletion assay (OAAD), have indicated that bovine pituitary LH exhibits an inverse relationship to FSH during the postpartum interval, being low at parturition and increasing gradually as the interval progresses. Saiduddin and Foote (1964) reported an increase in bovine pituitary LH from day 5 to day 30 postpartum in suckled cows. Similar results were observed by Saiduddin et al. (1966) indicating that pituitary LH increased progressively in suckled cows from days 10, 20 and 30 postpartum when compared to levels at parturition. Labhsetwar et al. (1964) observed an increase in pituitary LH from parturition to 20 days postpartum in lactating cows, while Sawhney (1966) reported that pituitary LH decreased during the 10-day interval before parturition and then increased until first ovulation. Wagner, Saatman and Hansel (1969) indicated that the rise in bovine pituitary LH was nonsignificant from day 7 to day 30 postpartum when LH was measured by the uptake of P^{32}

in chick testis.

Riesen et al. (1968) using the systemic pigeon crop sac assay, measured pituitary prolactin in suckled and non-suckled dairy cows and found significantly higher levels in the non-suckled group at 10, 20 and 30 days postpartum.

Arije, Wiltbank and Hopwood (1971) measured serum LH and prolactin by radioimmunoassay (RIA) in three multiparous suckled cows from 3 to 4 weeks prepartum until second estrus postpartum. Blood samples were collected through indwelling jugular cannulas. During the postpartum interval LH levels remained between 1 and 1.5 ng/ml with periodic peaks reaching 3 ng/ml after two weeks postpartum. During late gestation, prolactin levels remained below 50 ng/ml, increasing to 300 ng/ml two days before parturition until 20 days postpartum. Throughout the remainder of the postpartum interval, prolactin levels remained between 100 and 200 ng/ml.

In a similar study conducted by Ingalls, Hafs and Oxender (1971), serum LH and prolactin were measured in 32 heifers, from 30 days prepartum until first estrus postpartum. Blood samples were collected through indwelling jugular cannulas and analyzed by RIA. LH levels from 30 days prepartum until first estrus postpartum did not change significantly. Prepartum prolactin levels ranged from 50 to 100 ng/ml until two days before parturition when concentrations exceeded 200 ng/ml. By 60 hours postpartum, the levels had fallen to 50 ng/ml

and ranged between 50 and 100 ng/ml throughout the remainder of the postpartum interval.

Kaprowski, Tucker and Convey (1972) were able to detect a circadian pattern of prolactin secretion in lactating dairy cows. Serum prolactin averaged 28 ng/ml between 7 and 10 A.M. and increased to 58 ng/ml at 4 P.M., declining irregularly to 28 ng/ml by 4 A.M.

As seen from the previous studies, relating to postpartum pituitary and plasma hormone levels, prolactin tends to be the dominant pituitary secretion. For this reason, prolactin will be given special attention in a later section of this review.

Exogenous Hormone Treatment of Postpartum Cows. Modification of the postpartum interval by exogenous hormone therapy has been attempted in several studies. Results have shown that significant alterations can be achieved, however, a highly reliable method for shortening postpartum intervals to conception has not been demonstrated.

Foote and Hunter (1964) treated postpartum beef cows with ovarian steroids and found that a combined treatment of progesterone and estrogen resulted in a reduction in the interval to conception.

Foote (1971) injected 10 mg estradiol 17-β (I.V.) into beef cows early in their postpartum period (9 to 15 days postpartum). Average interval to first ovulation and first estrus was significantly ($P<0.05$) shorter in treated than untreated animals.

Brown, Peterson and Foote (1972) reported that exogenous hormone

therapy was most effective when treatment was initiated early in the postpartum period (5 to 25 days postpartum). Combination progesterone and estrogen treatments proved to be the most effective for initiation of estrus, ovulation and conception.

Bovine LH and Prolactin Levels During the Estrous Cycle as Determined by RIA. Circulating levels of LH throughout the bovine estrous cycle range between .4 and 4 ng/ml with preovulatory peaks of 3 to 100 ng/ml, at approximately the time of estrus (Schams and Karg, 1969; Hendricks, Dickey and Niswender, 1970; Arije, Wiltbank and Hopwood, 1971; Snook, Saatman and Hansel, 1971; Sprague et al. 1971). Other LH peaks occurring on day 8 or 9 of the cycle (Schams and Karg, 1969) and from 4 to 7 days before ovulation have been reported (Schams and Karg, 1969; Snook, Saatman and Hansel, 1971).

Geschwind (1972) summarized the observations of several investigators in regard to the time intervals recorded from the onset of estrus to the LH surge, from the LH surge to ovulation, and the duration of the LH surge in the cow (table 1).

Sinha and Tucker (1969) reported large increases in pituitary prolactin 3 days before ovulation, with minimum levels being detected at the time of ovulation in cycling heifers. A surge in plasma prolactin prior to or at the time of estrus has been reported in heifers (Hafs and Morrow, 1970) and cows (Raud, Kiddy and Odell, 1971). Raud, Kiddy and Odell (1971) found that diestrus serum prolactin

levels ranged between 31 and 64 ng/ml with a proestrus surge over 200 ng/ml in four cycling cow samples by jugular cannulas.

TABLE 1. TEMPORAL RELATIONSHIPS AMONG THE ONSET OF ESTRUS, THE LH SURGE AND OVULATION IN THE COW*

	Onset of estrus to LH surge (hr)	LH peak to ovulation (hr)	Duration of LH surge (hr)	Reference
1.	10	15-22	$\leq$6;8	Schams and Karg (1969a,b)
2.	3-6	22-26	8-10	Henricks, Dickey and Niswender (1970a)
3.	-1.2±2.5	31±6	$\sim$6	Swanson and Hafs (1970)
4.	-4- +12		4-8	Garverick et al. (1971)
5.	9.2±8.2	24.0±2.5	12.4±1.6	Christensen, Wiltbank and Hopwood (1971)
6.	0-12		6-<12 .	I.I. Geschwind, P.T. Cupps and R. D. Dewey (Unpublished data)

* From Geschwind (1972)

Hormonal Response to Mating Stimuli. VanDemark and Hays (1952) measured uterine activity in reponse to mating stimuli in mature cows. Increase in uterine tone and contraction rate were observed due to various mating stimuli. Additional bovine studies demonstrated that stimulation of the vulva and cervix initiates the release of an oxytocin-like substance capable of increasing mammary pressure (Hays and VanDemark, 1953), while rectal massage of the cervix and vagina produced a short-term release of prolactin (Schams and Bohm, 1972). Natural mating to a bull has also been reported to elicit a small immediate increase in

plasma prolactin following each service (Cummins et al. 1973).

Half-Life and Metabolic Clearance Rate of Prolactin and Gonadotropins. Bryant, Greenwood and Linzell (1968) reported that the half-life of ovine prolactin-I^{131} injected i.v. into a single goat was 19 min. as determined by RIA.

Half-life determinations of prolactin in the rat have been extremely variable. Gay, Midgley and Niswender (1970) and Watson and Danhoff (1971) reported the half-life to be approximately 13 min., while Koch, Chow and Meites (1971) observed a half-life of approximately 5 min. The observed difference has been explained in terms of the interval between blood collections for the individual studies. In this situation, a greater intensity sampling scheme results in a more accurate estimate of the half-life, favoring the estimated 5 min. half-life calculated by Koch et al.(1971).

Half-life determinations of LH in the rat have been reported to range between 20 and 32 min., while FSH ranges between 107 and 149 min. (as reviewed by Geschwind, 1972).

Metabolic clearance rate (MCR), defined as the volume of blood cleared of hormone per unit time, has been estimated by single injection and constant infusion of labeled prolactin. Both techniques have been shown to yield comparable results. In the rat, MCR of prolactin has been calculated to be 1.26 ± 0.08 ml/min. (Koch, Chow and Meites, 1971), while in sheep, MCR was shown to be significantly higher in lactating

ewes (6.09±0.41) than in lambs (4.45±0.38). The MCR in ovariectomized ewes 2.93±0.13) was significantly lower than all other groups (Davis and Borger, 1973).

Metabolic clearance rate for LH has been measured in premenopausal women by constant infusion techniques and calculated to be 24.4±1.8 ml/min. (Kohler, Ross and Odell, 1968), while premenopausal MCR of FSH has been reported to be 14.2±1.1 ml/min. (Coble et al. 1969).

Ovulation and Luteal Function. The effect of FSH on the ovary is to initiate growth of graffian follicles to a point near maturity. FSH does not bring follicles to complete maturity, induce the formation of thecal or luteal tissue, or induce estrogen secretion. With a background of FSH activity, LH promotes complete maturation of follicles and thecal tissue, while initiating estrogen secretion. Following a preovulatory surge of gonadotropins, the follicle is ovulated and a functional C.L. develops secreting progestrone (as reviewed by Harris and Campbell, 1966).

Depletion of pituitary FSH stores has been reported to be associated with ovulation in the rat (Caligaris, Astrada and Teleisnik, 1967) and sheep (Santolucito, Clegg and Cole, 1960; and Robertson and Hutchinson, 1962). Recent studies indicate a synergistic role between LH and FSH in the induction of ovulation. These studies support the idea that ovulating hormone consists of more than one hormonal substance. Labhsetwar (1970) blocked ovulation in 4-day cyclic rats by

administration of a potent antiestrogen during diestrus. On the day of proestrus, treatment with sub-threshold doses of LH or FSH were only marginally active in restoring ovulation, while combination of the gonadotropins resulted in the incidence of ovulation being higher than the sum of the two groups receiving LH or FSH individually. Labhsetwar (1972) induced partial ovulations on the day of proestrus in 4-day cyclic rats by administration of either 4 ug LH or 15 ug FSH. When identifical doses of LH and FSH were administered in combination, normal ovulation occurred in 93% of the rats. Ovine prolactin alone, or in combination with either LH or FSH did not exert synergistic effects on ovulation. Similar evidence supports the synergistic concept of LH and FSH for the induction of ovulation in the rabbit (Jones and Nalbandov, 1972).

Blood levels of FSH have been shown to fluctuate in a similar pattern with blood levels of LH, throughout the estrous cycle of the rat. FSH begins to increase during proestrus, reaches a peak on the afternoon or evening of proestrus, and declines slowly for 3 days following proestrus. The magnitude of the FSH peak being considerably less than the LH peak, while the duration of the peak is much longer (Gay, Midgley and Niswender, 1970; Daane and Parlow, 1971). The differences in the duration of the LH and FSH peak are most likely attributed to the differences in the half-life of the two hormones (as reviewed by Geschwind, 1972).

The luteotropic substance which maintains progesterone secretion during the luteal phase of the cycle varies between species. LH is believed to be luteotropic in most species, while prolactin is generally accepted as being part of the luteotropic hormone complex in the rat (as reviewed by Harris and Campbell, 1966). Synergistic effects on luteal function have been reported for LH and prolactin in the rat by Armstrong and Greep (1962).

Malven and Sawyer (1966) reported that in the rat, prolactin exerts a luteotropic action on newly formed corpora lutea and a luteolytic effect on inactive corpora lutea from the previous cycle. A luteolytic effect on previously formed corpora lutea has been associated with the proestrus prolactin surge in the rat (Wuttke and Meites, 1971) and mouse (Grandison and Meites, 1972). Malven and Hoge (1971) reported that ergocornine acted at the pituitary level to suppress prolactin secretion in rats. Their work demonstrated that pituitary tissue transplanted to the kidney capsule released sufficient prolactin to initiate structural luteolysis of nonfunctional luteal tissue, however, continuous ergocornine treatment inhibited structural luteolysis. This evidence indicates that structural luteolysis occurs from tonic prolactin secretion rather than from the proestrus prolactin surge suggested by Wuttke and Meites (1971) and Grandison and Meites (1972).

A similar study has demonstrated that an ergot derivative

injected intramuscularly in cycling ewes depresses prolactin release and abolishes the proestrus prolactin surge without altering the proestrus LH and FSH surge. Estrous cycles of normal length and normal regression of corpora lutea (marked with carbon) were observed in both control and treated animals. These results have been interpreted to indicate that in sheep the proestrus prolactin surge is not required for normal cyclic behavior or for the regression of the previous crop of corpora lutea (Niswender, 1972). Blockage of the proestrus surge of prolactin with ergocornine does not interfere with ovulation or induce alterations in the estrous cycle of rats (Wuttke, Cassell and Meites, 1971).

Several studies have reported luteotropic properties of LH in cattle. Mason, Marsh and Savard (1962) were the first to demonstrate that purified preparations of both LH and FSH were capable of stimulating in vitro progesterone biosynthesis in bovine luteal tissue. The authors suggested that contamination of the FSH preparation with LH could have been responsible for the luteotropic action of FSH. Donaldson and Hansel (1965) injected pituitary extract and purified bovine LH into separate groups of cows and observed an extension of the estrous cycle from 20 days in control animals to 31 and 36 days, respectively, in the treated groups. Rectal palpatation and laparotomy revealed nonregressing corpora lutea in treated animals. Armstrong and Black (1966) demonstrated that LH would stimulate progesterone

biosynthesis in in vitro slices of bovine luteal tissue, if the C.L. was obtained before day 19 of the estrous cycle.

In contrast to earlier reports, prolactin has been reported to have luteotropic properties in both the ewe and cow. In the ewe, pituitary stalk section allows for the maintenance of progesterone secretion, which has been attributed to the continued secretion of prolactin from the pituitary (Denamur, Martinet and Short, 1966; and Bryant et al. 1971). In the cow, both prolactin and LH enhance the secretion of progesterone from bovine ovaries containing luteal tissue and perfused in vitro, while only LH stimulates progesterone secretion in the contralateral ovary containing only follicles (Bartosik et al. 1967). Under conditions of constant infusion of labeled acetate, increased progesterone secretion was induced by LH and accompanied by an increase in specific activity, while prolactin failed to increase the specific activity of progesterone. These results were interpreted to indicate that prolactin and LH operate through different mechanisms to exert their effect on progesterone biosynthesis.

Snook et al. (1969) injected antibovine LH into intact and hysterectomized heifers throughout the estrous cycle. Treated heifers exhibited decrease in C.L. weight, total progesterone, total progestins and 20-β-ol concentration; however, the progesterone concentration per unit weight of C. L. tissue was not affected. The suggestion was made that the inability of the antibovine LH to significantly reduce

progesterone concentration might indicate additional factor(s) involved in C.L. maintenance in the bovine.

Extrinsic Factors Affecting Prolactin Release. Suckling was first shown to reduce pituitary prolactin levels in the rat by Reece and Turner (1937). Ratner and Meites (1964) reported that suckling stimulus reduces the content of prolactin inhibiting factor (PIF) in the hypothalmi of lactating rats, resulting in increased prolactin release from anterior pituitary cultures. Grosvenor (1965a) observed that hypothalmic extracts of rat, bovine and ovine sources would block the suckling-induced release of prolactin from the anterior pituitary of lactating rats. Grosvenor, Mena and Schaefgen (1967) observed that 2 minutes of suckling reduced pituitary stores the same extent as 30 minutes of suckling in lactating rats. Shino, Rennels and Williams (1971) used the electron microscope to observe the ultrastructure of pituitary prolactin cells in rats. The absence of suckling during lactation produced an accumulation of secretory granules, while acute suckling resulted in their discharge.

Various types of stress in the rat have been reported to deplete pituitary prolactin stores (Grosvenor, 1965a; Grosvenor, McCann and Nallar, 1965) and increase serum prolactin levels (Neill, 1970). Exterioceptive stimuli arising from an interrelationship between the mother and the hungry young has also been reported to initiate a discharge of prolactin in lactating rats (Grosvenor, 1965b;

Grosvenor et al. 1967).

Raud, Kiddy and Odell (1971) evaluated the effect of stress on bovine serum prolactin levels by RIA. Detailed tests were conducted on 15 cycling dairy cows, 4 to 5 years of age. Nine animals were sampled by jugular puncture and four by indwelling cannulas. Mean diestrus prolactin concentrations of cows sampled by jugular puncture ranged from 113 to 485 ng/ml, while cows sampled by indwelling cannulas ranged between 31 and 64 ng/ml. Included in this study was an investigation to determine the effect of noise and restraint on serum prolactin levels. An increase from 64 to 206 ng/ml was observed within 30 minutes when a single cannulated cow as subjected to noise and restraint for a 10 minute period. Neither technique of bleeding (jugular puncture vs. indwelling cannula) had any apparent effect on LH concentration in any of the cows studied. Conclusion by the authors indicated that physiological studies measuring bovine prolactin should be conducted under carefully controlled environmental conditions.

Johke (1970) studied the effects of various stimuli on plasma prolactin levels in Holstein cows. Rapid increases in plasma prolactin were detected by RIA in cannulated animals arising from stimuli associated with needle puncture (pain, forced restraint, emotional disturbances), while no significant increases were detected during continuous sampling of cannulated animals receiving no external stress stimuli. Washing the udder with warm water, followed by machine milking, induced

prompt increase in plasma prolactin resulting in peaks occurring from 4 to 20 minutes after stimulation. Prolactin peaks resulting from milking were highest in the early stage of lactation and decreased throughout lactation. Lowest plasma prolactin levels were obtained 2 to 3 hours after milking.

Koprowski, Convey and Tucker (1971) reported that washing the brisket as well as washing the udder, stimulated significant increases in plasma prolactin in unbred heifers, dry cows and lactating cows.

Butler, Willett and Malven (1971) studied patterns of prolactin release in sheep and concluded that heat, surgical and psychological stress cause a release of pituitary prolactin,

Pharmaceutical Agents Effecting Prolactin Release. Several tranquilizing drugs are know to increase prolactin release. In the rat, reserpine, chloropromazine, meprobamate (as reviewed by Meites, Nicoll and Talwalker, 1963) and perphenazine (Ben-David et al. 1971), stimulate prolactin release while acepromazine (Bryant, Connan and Greenwood, 1968) and perphenazine (McNeilly and Lamming, 1971) are potent stimulators in the sheep.

Shelesnyak (1958) reported that the ergot drug ergotoxine could prevent prolactin secretion in the rat. Wuttke, Cassell and Meites (1971) demonstrated that ergocornine would completely block the proestrus surge of prolactin in the rat and suppress all fluctuations in serum prolactin during the estrus cycle without inhibiting ovulation

and normal cycling. Similar results were noted in the ewe (Niswender, 1972).

Prolactin Release in Response to Milking. Tucker (1971) devised a series of experiments to test the patterns of prolactin release associated with milking procedures. Blood samples were obtained through indwelling cannulas and analyzed for prolactin by RIA. Baseline prolactin levels obtained 2 minutes before udder washing from four first-calf heifers averaged 18 ng/ml. Stimulation of the udder by washing, followed by 4 minutes of machine milking, increased plasma prolactin to 39 ng/ml. Five minutes after removal of the milking machine, plasma levels reached 44 ng/ml and decreased to 23 ng/ml by 6 hours post-milking.

A second experiment, using four multiparous cows, was designed to measure the time interval necessary for plasma prolactin levels to return to baseline after milking. Average baseline levels from samples obtained at 10 and 4 minute intervals prior to washing were 45 and 38 ng/ml, respectively. Five minutes after washing the levels increased slightly to 52 ng/ml. At this time, the milking machine was attached and followed by a 5 minute milking interval. Plasma prolactin levels obtained immediately after the removal of the milking machine averaged 119 ng/ml and decreased to 51 ng/ml by 30 minutes post-milking. Both experiments 1 and 2 demonstrated extreme biological variation between animals in the degree of response to the milking stimulus.

A third experiment was conducted to determine if continuous stimulation of the udder by machine milking could maintain elevated prolactin levels. Tests were carried out on two cows during the latter stage of lactation by 40 minute machine milkings. Prolonged milking failed to maintain elevated prolactin levels with baseline values being detected 20 minutes after the initiation of milking.

Koprowski and Tucker (1973) measured serum prolactin by RIA in postpartum Holstein cows at 4-week intervals throughout lactation. Samples were collected by venipuncture of the tail vein 2 to 4 hours before the PM milking, 5 minutes after the removal of the milking machine and 1 hour after the PM milking. The magnitude of the serum prolactin release in response to milking and the milk production were greatest during the 8th week of lactation. As lactation advanced past the 12th week, serum prolactin release in response to milking declined until the 32nd week when prolactin was no longer released in response to milking. Serum prolactin levels 2 to 4 hours before milking and 1 hour after milking were greater for nonpregnant cows than pregnant cows. In contrast, serum prolactin levels 5 minutes after milking did not differ between nonpregnant and pregnant cows.

Fell et al. (1973) measured serum prolactin in response to milking in dairy cattle subject to different time intervals between milkings. Blood samples were collected by a continuous sampling device inserted into the jugular vein. Sampling began immediately before milking and

continued for a 30-minute period. Deletion of one or two consecutive afternoon milkings produced a significant decrease in the amount of prolactin released in response to the following milking.

In a second experiment, a significant drop was noted in serum prolactin released in response to milking when cows were milked at 30-hour intervals as compared to 22-hour intervals. A 30 to 50% drop in milk yield was observed during the 30-hour interval.

Hypothalmic Control of Gonadotropins and Prolactin

Recent advances in neuroendocrinology have clearly established the concept of hypothalmic control over the pituitary gland.

Hypophysitropic Hormones. Luetinizing hormone releasing hormone (LH-RH) has recently been isolated from porcine hypothalmi and physiological studies indicate that it stimulates the release of both LH and FSH (Schally et al. 1971a). Artificial synthesis of LH-RH has been accomplished and both in vivo and in vitro studies indicate it has equal biological potency with nuatural LH-RH of porcine origin (Schally et al. 1972). From these studies, it has been suggested that a single hypothalmic hormone designated as gonadotropin releasing hormone (Gn-RH) is responsible for the secretion and release of both LH and FSH from the anterior pituitary in several species (Schally et al. 1971b; Schally, et al. 1972).

Observations from several studies have developed the concept that hypothalmic control of prolactin secretion and release is due to an

inhibiting factor rather than a stimulating factor associated with the secretion and release of other anterior pituitary hormones. Supportive evidence for this idea stems from the following studies. Increased prolactin secretion accompanied by decrease secretion of other anterior pituitary hormones results from the following experimental procedures: (1) transection of pituitary stalk, (2) transplantation of the anterior pituitary to noncranial sites, (3) administration of central nervous system depressants, (4) properly oriented lesions in the hypothamlus, (5) and in vitro cultures of anterior pituitary glands (Meites, Nicoll and Talwalker, 1963). The increase in prolactin secretion and release observed in these type of experiments has established the presence of an unidentified prolactin inhibiting factor (PIF).

A second hypothalmic factor controlling prolactin secretion and release has been suggested in the following studies. This factor, tentatively known as prolactin releasing factor (PRF), has been shown to be present in hypothalmic extracts of lactating rats.

Meites, Talwalker and Nicoll (1960) reported that hypothalmic extracts from postpartum rats would stimulate mammary secretion in estrogen primed mature rats.

In a similar experiment, Mishkinsky, Khazen and Sulman (1968) observed that either hypothalmic or anterior pituitary extracts from postpartum lactating rats would increase lactogenic activity in estrogen primed virgin female rats. Their results indicate that

hypothalmic extracts obtained on the 10th day postpartum and anterior pituitary extracts obtained on the 14th day postpartum, stimulated peak lactogenic activity in virgin estrogen primed rats.

Desclin and Flament-Durand (1969) tested the effects of reserpine (stimulates prolactin release) on the morphology of pituitaries in situ and of pituitaries grafted into the hypothalmus and to the kidney capsule of rats. Pituitary grafts located in a definite region of the hypothalmus including the anterior hypothalmic area up to the level of the paraventricular nuclei, the ventromedial nuclei and the arcuate nuclei were strikingly different from grafts located in other areas of the hypothalmus or in the kidney capsule. In the designated areas of the hypothalmus, pituitary grafts showed numerous prolactin secreting cells with a high degree of stimulation resembling prolactin cells seen during lactation in the in situ pituitary. Pituitary grafts located outside the designated areas contained small oval cells comparable to untreated controls. These results were interpreted to indicate the presence of a prolactin stimulating factor located in the previously designated hypothalmic areas.

Mishkinsky and Sulman, as reviewed by Sulman (1970), demonstrated that hypothalmic extracts from suckled rats would enhance the lactogenic effect observed by perphenazine suppression of PIF in nonsuckled estrogen primed rats. The enhanced lactogenic effect was suggested to be the result of a prolactin releasing factor (PRF).

Valverde-R, Chieffo and Reichlin (1972) extracted porcine and rat hypothalmic tissue with methanol and obtained a factor that stimulated prolactin release in estrogen-progesterone primed male rats. Identical treatment of cerebral cortex tissue failed to initiate a measureable response. In the experimental rats, plasma prolactin rose significantly from the pre-injection level of 11.8 ng/ml to 106 ng/ml within 10 minutes. Baseline values were re-established by 20 minute post-injection. Similar effects were observed in estrogen primed rats, while progesterone primed rats showed no response.

Synthetic thyrotropin-releasing hormone (pyro-glutamyl-histidyl-proline) has been shown to initiate a release of prolactin in cattle (Convey, et al. 1973). Single i.v. infusion via jugular cannulae, produced a significant peak in plasma prolactin occurring from 8 to 10 minutes post-infusion. The authors concluded that synthetic TRH is not the proposed prolactin releasing factor because in vivo studies failed to show a dose-related response to TRH and in vitro studies failed to initiate a significant release of prolactin.

Autofeedback Mechanism: Torok (1964) observed the existence of a two-directional blood flow in the hypophysial portal system from the hypothalmus to the pituitary and vice versa. This observation was conducted in live dogs and cats and uncovered an anatomical pathway by which pituitary hormones may pass directly from the pituitary to the hypothalmus and regulate their own production. Regulation of this

type is referred to as internal, direct, short or autofeedback (Flerkó, 1966; Sulman, 1970).

The existence of an autofeedback mechanism for LH (Corbin and Cohen, 1966) and FSH (Corbin and Storey, 1967) have been reported in the rat. Median emminence implants of LH and FSH were found to significantly reduce the pituitary content of the corresponding gonadotropin.

A similar autofeedback mechanism for prolactin regulation has also been demonstrated in the rat (Clemens and Meites, 1968). Median eminence implants of prolactin significantly reduced prolactin secretion, mammary growth and luteal function. Additional studies by Clemens, Sar and Meites (1968, 1969) indicate that median eminence implants of prolactin will inhibit lactation and luteal function in rats.

Chen, Minaguchi and Meites (1967) measured pituitary prolactin by the pigeon crop sac method in ovariectomized-adrenalectomized rats bearing pituitary tumors capable of prolactin and GH secretion. Anterior pituitary weight and prolactin content of tumor-bearing rats were significantly lower than control rats. Measurement of hypothalmic PIF content showed a significant increase in tumor-bearing rats over controls. Intense lobuloalveolar development of the mammary gland was also noted in tumor-bearing rats.

Chen, Voogt and Meites (1968) demonstrated that prolactin implants in the median eminence of rats on day 1, 4 or 8 of pseudopregnancy,

significantly shortened the duration of pseudopregnancy, while implants of FSH and LH had no significant effect.

Hypothalmic Areas Controlling Ovulation and Pseudopregnancy. Differentiation of the hypothalmic areas controlling ovulation and pseudopregnancy have been investigated in several studies by sterotaxic lesions and electrical or electrochemical stimulation.

Lesions placed rostral to the paraventricular nuclei, medial in the plane of the paraventricular nuclei, or in the ventromedial nucleus, produce periods of prolonged diestrus indicative of pseudopregnancy in some experimental rats (as reviewed by Everret, 1966). Lesions in the thalmohypothalmic border initiate prolonged periods of diestrus and hyperluteinized ovaries indicative of prolactin secretion (as reviewed by Flerkó, 1966).

Electrical stimulation of the preoptic area (POA) induces ovulation while stimulation of the basal medial hypothalmus (BMH) results in ovulation accompanied by pseudopregnancy (Everret and Quinn, 1966). Measurement of plasma LH and prolactin before and after electrical stimulation of the POA in rats, results in increased plasma LH and decreased prolactin, while stimulation of the BMH results in increased LH and prolactin (Clemens et al. 1971a).

Clemens et al. (1970) measured plasma LH and FSH in female rats before and after electrochemical stimulation of the preoptic area (POA). A significant increase in LH was detected within 30 minutes

post-stimulation while FSH remained constant. In a similar study by Clemens et al. (1971a), electrochemical stimulation of the medial septum, preoptic area (POA), anterior hypothalmic area (AHA) and median eminence arcuate complex (MEAC), was applied to determine the effect on plasma LH and FSH. Approximately 80% of the stimulation sites in the POA, AHA and MEAC which were found to be stimulatory to LH, were also stimulatory to FSH secretion. Plasma LH peaked .5 hour after stimulation while FSH did not increase until 3 hours post-stimulation.

Sex Differentiation of the Hypothalmus. Early work in 1936 by Pfeiffer (as reviewed by Harris and Campbell, 1966 and Barraclough, 1967) has contributed greatly to the present understanding of the hypothalmic control mechanisms regulating gonadotropins in both male and female rats. In a series of experiments, Pfeiffer observed that male rats castrated at birth and implanted with an ovary when adult show normal follicular and corpora lutea development, while females ovariectomized at birth and implanted with an ovary when adult show normal vaginal cycles accompanied by follicular growth and corpora lutea development. In contrast, when testis were implanted into intact females at birth, the ovaries of the adult females show only follicular activity with no C. L. development accompanied by persistent vaginal cornification after puberty. From these observations, Pfeiffer concluded that the mechanisms regulating gonadotropin secretion are undifferentiated

in both males and females at birth and that gonadal steriods secreted early in life initiate hypothalmic differentiation.

Barraclough (1961) observed an androgen sensitive period in neonatal female rats from birth to the 10th day of age. Treatment of female rats with 1.25 mg testostoerone propionate at 2 to 5 days of age initiates permanent sterility, while treatment after ten days has no affect on reproductive processes.

Flerkó and Bardos (as reviewed by Barraclough and Gorski, 1961) observed that small specific lesions in the suprachiasmatic nucleus of the hypothalmus produce an anovulatory condition in normal rats similar to that observed in androgen sterilized rats.

Lesions placed in the anterior hypothalmic-preoptic region, located topographically dorsal and adjacent to the suprachiasmatic nucleus, induce an anovulatory syndrome in normal female rats (Van Dyke et al. 1957; Taleisnik and McCann, 1961).

Flerkó and Bardos reported that stimulation of the suprachiasmatic nucleus in normal rats initiates ovulation, while similar stimulation in androgen sterilized rats has no observable effects (as reviewed by Barraclough and Gorski, 1961).

Segal and Johnson (as reviewed by Gorski, 1966) transplanted pituitaries of androgen sterilized females into normal hypophysectomized females and observed normal cyclic activity in the recipient rats.

Barraclough and Gorski (1961) electrically stimulated anterior

hypothalmic areas in progesterone primed androgen sterilized rats causing sufficient release of gonadotropins to initiate ovulation.

With the information obtained from several previous studies, Barraclough and Gorski (1961) postulated a model with dual function for the regulation of hypothalmic gonadotropins in the female rat. In their proposed scheme, the first area of gonadotropin control, located in the arcuate-ventromedial nuclei of the median eminence, regulates tonic gonadotropin secretion in sufficient quantity to maintain estrogen production. Electrical stimulation of this area in androgen sterilized females initiates gonadotropin secretion while lesions result in inhibition of estrogen production, ovarian atrophy and anestrus (Flerkó and Bardos, 1959; as reviewed by Barraclough and Gorski, 1961). The second area of gonadotropin control located in the anterior hypothalmus (anterior hypothalmic-preoptic-suprachiasmatic region) initiates rhythmic secretion necessary for ovulation. This area is believed to be undifferentiated at birth and will differentiate normally in the female to form the second area of control, however, if subjected to androgen treatment before differentiation occurs, as in the case of the androgen sterilized neonatal female and normal male, the area becomes refractory to both intrinsic and extrinsic stimuli. With the absence of the second area of control, only baseline gonadotropin levels prevail which results in anovulatory sterility in females due to the lack of cyclic gonadotropin surges.

A similar hypothalmic control mechanism for regulating prolactin has been postulated by Neil (1972). This mechanism is identical with the scheme proposed by Barraclough and Gorski (1961) for gonadotropin regulation. The first area of control regulates tonic prolactin release and is located in the arcuate-ventromedial nuclei, while the second area of control regulates rhythmic prolactin release and is located in the anterior hypothalmus (anterior hypothalmic-preoptic-suprachiasmatic region). The following studies have provided the basis for the proposed dual mechanism of hypothalmic control over prolactin secretion and release.

Several investigators observed a major prolactin peak during proestrus in the absence of extrinsic stimuli (Kwa and Verhofstad, 1967; Gay, Midgley and Niswender, 1970; Wuttke and Meites, 1970). The obervation that spontaneous prolactin release occurs during proestrus, introduced the idea that some unknown intrinsic factor could elicit a major prolactin surge similar to the rhythmic discharge of gonadotropins previously discussed.

Additional studies have demonstrated that a single intrinsic factor is responsible for the initiation of the rhythmic peak of gonadotropins and prolactin. Ferin et al. (1969) reported that 17β-estradiol secretion during diestrus is a prerequisite for the preovulatory LH surge. Administration of a specific antibody to 17β-estradiol on diestrus-2 abolished the LH surge and ovulation in female rats.

In a similar study by Neill, Freeman and Tillson (1971) injection of a specific antibody to 17β- estradiol blocked both the LH and prolactin surge.

Chen and Meites (1970) injected ovariectomized rats with 1, 1, and 5 ug of estradiol bonzoate daily for six days. Serum prolactin concentrations increased 2, 3 and 10-fold, respectively, over ovariectomized controls.

Sharr and Clemens (1971) measured plasma prolactin levels before and after estrogen infusion in the rat and concluded that estrogen is necessary for the activation of neurons which either inhibit the secretion of PIF or stimulate an unidentified prolactin releasing factor.

Several investigators have reported that the anterior hypothalmic-preoptic-suprachiasmatic region concentrates estradiol (Kato and Villee, 1967; Pfaff, 1968), while other evidence indicates that intramuscular injections of estrogen alter the electrical activity of this area when subjected to extrinsic stimuli (Lincoln and Cross, 1967).

In a study conducted by Neill (1972), estrogen was injected into female rats ovariectomized when adult, adult neonatally castrated males, androgen sterilized females and males castrated when adult. On the third day of the estrogen treatment, blood samples were collected through arotic catheters at two-hour intervals from 1100-0100 hours on the following morning for LH and prolactin analysis. Female rats ovariectomized when adult and adult neonatally castrated male rats

showed surges of LH and prolactin on the afternoon and evening of the third day. Androgen sterilized females and males castrated when adult showed no surge of LH or prolactin in response to the estrogen treatment. In an additional experiment, surgical cuts were made in the hypothalmus of females ovariectomized when adult and treated with estrogen. The surgical cuts were made directly behind the optic chiasma (retrochiasmatic) with a modified Halasz knife. The retrochiasmatic cut inhibited the surge of LH and prolactin, while sham operated animals showed the characteristic surge of LH and prolactin. These results were interpreted to indicate fundamental differences between male and female rats in the hypothalmic regulation of LH and prolactin. The differences resulting from neonatal steriod feedback from the gonads, which stimulate the hypothalmic surge center to differentiate into male or female components.

Evidence For Antagonism Between Prolactin and Gonadotropin Production

Several studies have attempted to demonstrate that antagonism exists between prolactin and gonadotropin production at the hypothalmic level. Compete understanding of the control mechanisms in this area would tentatively aid in understanding the mechanism responsible for postpartum anestrus and associated infertility in the bovine species, however, most of the available information in this particular area stems from studies conducted primarily with the laboratory rat.

Studies with ovariectomized rats indicate that the removal of gonadal steroids results in increased gonadotropin secretion (as reviewed by Harris and Campbell, 1966) and decreased prolactin secretion (Amenomori, Chen and Meites, 1970). Administration of 5 ug of estradiol benzoate to ovariectomized rats, significantly increases pituitary and serum prolactin (Amenomori, Chen and Meites, 1970). Estrogen has been reported to promote prolactin secretion in the rat by direct action on the pituitary (Nicoll and Meites, 1964) and by reducing the PIF content of the hypothalmus (Ratner and Meites, 1964). Estrogen implants into the pituitary or median eminence of rats, have resulted in reduced gonadotropin (Ramirez, Abrams and McCann, 1964) and increased prolactin secretion (Ramirez and McCann, 1964).

Ajika et al. (1972) measured plasma and pituitary FSH, LH and prolactin after subcutaneous injection of varying doses of estradiol benzoate (.2-5 ug) in ovariectomized rats. Single injection of all

doses of estradiol produced a significant decrease in plasma LH and FSH at 48 hrs post-treatment, while plasma prolactin increased significantly. At the 5 ug doses of estradiol, pituitary content of LH, FSH and prolactin increased significantly by 48 hrs post-treatment.

As previously stated, Clemens and Meites (1968), demonstrated that median eminance implants of prolactin into mature intact and ovariectomized rats, resulted in increased hypothalmic content of PIF and reduced anterior pituitary prolactin content. These findings have been utilized to establish the existence of an autofeedback loop for prolactin regulation.

Clemens, Sar, and Meites (1968) reported that prolactin implants in the median eminence of postpartum lactating rats caused regression of the corpora lutea of lactation and resumption of estrous cycles. Voogt, Clemens and Meites (1969) reported that implants of prolactin into the median eminence of immature female rats stimulated the release of pituitary FSH, as indicated by a fall in pituitary FSH and growth of follicles. These observations indicate that when endogenous prolactin secretion is suppressed by median eminence implants of prolactin through autofeedback mechanisms, the pituitary responds by increasing gonadotropin secretion.

Sinha and Tucker (1968) observed that exogenous prolactin administration or additional pituitary's transplanted to the kidney capsule, decrease anterior pituitary prolactin contents in mature

virgin female rats and increase the content of mammary DNA and RNA.

Rothchild (1960) summarized his studies on the postpartum lactating rat, reporting that follicular quiescence during lactation results from the suppression of folliculotrophins (LH and FSH) and that the factor responsible for folliculotrophin suppression is also responsible for increased prolactin secretion. The factor being the intensity of the suckling stimulus from the nursing young. The more intense suckling stimulus having a greater suppressing effect on folliculotrophin production than a less intense suckling stimulus. In this study, the intensity of the suckling stimulus was dependent only upon the number of suckling young, not on the frequency of nursing. Rats nursing small litters (1 to 2 pups) were capable of conceiving during lactation while rats nursing large litters (6 and over) did not conceive until after weaning of the previous litter.

Ben-David, Danon and Sulman (1971) devised an experiment to test the possibility of antagonism between prolactin and gonadotropin secretion in rats. Their techniques utilized perphenazine, a compound that promotes prolactin secretion and release, and methallibure, a non-steroidal gonadotropin suppressor. Throughout a series of experiments utilizing perphanzaine and/or methallibure, the authors reported evidence that when the pituitary is secreting elevated levels of gonadotropin, as is the case in ovarectomized rats, its ability to release prolactin in response to perphenazine stimulation is severely

reduced. However, when subthreshold dose of methallibure were administered in combination with perphenazine to ovariectomized animals, serum prolactin increased to levels comparable with those measured in perphenazine treated intact rats. Conclusion; by the authors suggest that when gonadotropin levels in the pituitary are high, the ability of the pituitary to produce prolactin is reduced.

Veomett and Daniel (1971) studied the effect of the degree of suckling on the ability of lactating rats to maintain pregnancy. Reduction of the litter size to 2 or 3 pups at parturition, resulted in resumption of cyclic ovarian activity, mating and implantation in the majority of rats by 9 days postpartum. Implantation was diagnosed by laparotomy in both control and experimental animals. Following implantation, the litter size of the experimental animals was increased to 11 to 14 pups while controls remained constant. The increased suckling stimulus following implantation resulted in termination of pregnancy in all experimental animals, while 90% of the controls gave birth to normal young.

Hammons, Velasco and Rothchild (1973) devised a study to test the effect of suckling stimulus on serum LH levels in intact and ovariectomized postpartum rats. Their results deomonstrated that in ovariectomized females from day 6 to 18 postpartum, 9 pups had a greater suppressing effect on serum LH. than a suckling stimulus from 2 pups. No observable differences in LH were detected between intact females

nursing 2 or 9 pups. Additional studies determined that removal of the 9 pup litters from ovariectomized females on day 6 or 11 postpartum, induced prompt increases in serum LH. In contrast, increasing the litter size from 2 to 9 pups on days 6 or 11 postpartum, initiated only minor decreases in serum LH. The results were interpreted to reinforce previous finds that suckling supresses and castration stimulates gonadotropin secretion. Failure of the intact females to respond in a similar fashion to ovariectomized females has been attributed to the ovarian activity characteristic of pseudopregnancy, resulting from functional corpora lutea of lactation.

Nakayama and Nickerson (1973) observed the pituitary histology of female rats implanted with the MtT-WIO pituitary tumors capable of prolactin and growth hormone secretion. Tumor-bearing animals showed significant reductions in anterior pituitary weight in comparison to non-tumor-bearing controls. Pituitary mammotrophs in tumor-bearing animals exibited a gradual reduction in diameter of the secretory granules throughout a five week interval, while pituitary somatotrophs in tumor-bearing animals demonstrated a remarkable reduction in cell size, diameter and number of secretory granules. Pituitary gonadotrophs of tumor-bearing animals progressively increased in size until they became the largest cell in the anterior pituitary. During enlargement of the gonadotrophs, there was a progressive arrangement of cytoplasmic organelles characteristic of

a highly secretory state.

Schwartz and McCormack (1972) have summarized the work of Kamberi, Schneider and McCann, dealing with the potential neurotransmitters and associated anterior pituitary function in the rat. Table 2 is taken directly from their review and illustrates an interesting point, i.e., that all of the potential neurotransmitters when injected into the third ventricle of the brain at one dose or the other, produce inverse effects on the secretion of gonadotropins (LH and FSH) and prolactin.

TABLE 2. THE EFFECT OF POSSIBLE NEUROTRANSMITTERS ON THE PLASMA LEVELS OF FSH, LH AND PROLACTIN* **

Substance	Dose in µg	LH	FSH	Prolactin
Dopamine HCl (DA)	1.25, 2.5	Inc(103)	Inc(106)	Dec(107)
	100	NC (103)	NC (106)	NC (107)
Norepinephrine (NE)	2.5, 5.0	NC (103)	NC (106)	NC (107)
	100	Inc(103)	Inc(106)	Dec(107)
Epinephrine (E) bitartrate	2.5,5.0	NC (103)	NC (106)	NC (107)
	100	Inc(103)	Inc(106)	Dec(107)
Serotonin creatinine sulfate (5HT)	1.0,5.0	Dec(103)	Dec(108)	Inc(108)
	50	---	Dec(108)	Inc(108)
Melatonin (M)	1,0,5.0	Dec(103	Dec(108)	Inc(108)
	50	Dec(103)	Dec(108)	Inc(108)

Inc=Significant increase
Dec=Significant decrease
NC = No significant change
* Test animals were adult male Sprague Dawley rats; volume injected into third vetricle was 2.5 or 5 µl; FSH, LH and prolactin were measured by specific radioimmunoassay procedures.
**From Schwartz and McCormack (1972).

Assay Procedures for Reproductive Hormones

HCG Augmentation Assay for FSH. Steelman and Pohley (1953) described a technique for measuring folicle stimulating hormone (FSH) activity in pituitary tissue. The method is based on the observation that human chorionic gonadotropic (HCG) will augment the action of FSH on the ovary of the intact immature female rat. Relatively high doses of HCG (10-50 IU) make the ovary extremely sensitive to exogenous FSH, and a linear relationship exists between ovarian weight and the amount of FSH administered. The techniques of the HCG augmentation assay have been utilized to measure pituitary FSH contents of several species, although the sensitivity is relatively low.

Ovarian Ascorbic Acid Depletion Assay (OAAD) for LH. Parlow (1961), as reviewed by Apostolakis and Doraine (1967), described a procedure for measuring luteinizing hormone (LH) activity in pituitary tissue, based on the ability of LH to deplete the ascorbic acid content in the ovary of the intact immature female rat. Pretreatment with pregnant mare serum (PMS) and human chorionic gonadotropin (HCG) prepared the ovaries for the bioassay which is conducted 5 to 9 days later. Prior to bioassay, a control group is selected while LH standards and unknowns are administered intravenously to the remaining animals. Three hours after administration of the LH standards and unknowns, a single ovary is removed from LH treated and control rats and analyzed for ascorbic acid content. A linear negative regression in ascorbic acid content

exists in rats treated with LH standards and serves to quantitate the unknowns. Advantages of assay are its increased sensitivity and specificity, while disadvantages are its inability to measure LH in some strains of rats.

Uptake of Radioactive Phosphorus by the Chick Testis Assay for LH. Florsheim, Velcoff and Bodfish (1959) developed an assay for measuring total gonadotropins based on the uptake of p^{32} by the chick testis. Sensitivity of the assay was very high compared to the OAAD, however, specificity for individual gonadotropins was lacking, making it difficult to accurately measure single entities.

Prolactin Bioassays. Bioassay techniques for prolactin have utilized the lactation response in several species and the luteotrophic response in rats and mice as an indirect measurement of prolactin. The lactation response in pigeons and doves is characterized by the development of crop sac glands while the lactation response in mammals is characterized by the development of mammary tissue. The luteotrophic response in rats and mice stems from the ability of prolactin to enhance progesterone secretion from the corpora lutea causing prolonged maintenance of the corpora lutea or psyeudopregnancy.

Pigeon Crop Sac Assay for Prolactin: In 1933, Riddle, Bates and Dykshorn outlined procedures for a bioassay of prolactin, utilizing the crop sac response of immature doves and pigeons to local prolactin administration (as reviewed by Bates,Garrison and Cornfield, 1963).

Lyons and Page (1935) reported that prolactin could be detected in the urine of pregnant women by single intradermal injection of prolactin into the crop sac of immature pigeons. Purification procedures were necessary to combat occasional inflammatory processes occurring from untreated samples. Bates et al. (1963) obtained increased precision in the crop sac assay by increasing the duration of prolactin administration and using mature birds in place of immature birds. Their technique consisted of intramuscular injections of prolactin into the pectoral muscle once daily for 4 to 7 days. Approximately 24 hours after the last injection the pigeons were sacrificed, crop sacs were removed, freed of crop milk and weighed. Sensitivity of the assay was decreased when prolactin was administered systemically as compared to the local method of administration employed in earlier studies.

Lactogenic Assay for Prolactin: Bradley and Clark (1956) outlined a procedure for measuring prolactin in crude extracts, utilizing its lactogenic properties. Techniques included intraductal injections of prolactin into the mammary glands of pseudopregnant rabbits, followed by prolactin measurements based on weight per unit area of responding sectors of the gland. Precision of the assay was extremely low because of little variation in the response between high and low doses. Chadwick (1963), using similar techniques, obtained a response with .25 IU of prolactin having an index of precision ranging from 0.09 to 0.13; however, the routine application of the assay was hindered by

inherent variability among rabbits and laborious techniques for measuring lactose during the prolactin quantitation step.

Prop (1961), as reviewed by Forsyth (1967), observed that addition of prolactin in concentrations of 10^{-2} to 10^{-4} IU/ml would induce lobular alveolar development in in vitro cultures of mammary glands from virgin mice pretreated with insulin, progesterone and cortisol. Prop (1962) used this method for quantitative measurements of prolactin and reported that considerable variation in sensitivity was observed between individual mice, however, only small differences in sensitivity were found between mammary glands of each individual. LH, FSH, GH and ACTH were tested in the assay with no apparent effects being observed.

Luteotrophic Assay for Prolactin. The luteotrophic action of prolactin has been demonstrated in the hypophysectomized rat by the ability of prolactin injected animals to maintain pregnancy (Cutuly, 1941). In order for prolactin to exert its luteotrophic action, treatment must begin very soon after hypophysectomy (Evans et al. 1941; Astwood, 1941), Kovačić (1962) developed a technique for detecting prolactin, utilizing its luteotrophic response on the corpora lutea of the intact mouse. Administration of prolactin on the first day of diestrus resulted in prolongation of diestrus and was an "all or none" response. The method could not be used for precise quantitative measurements and was not specific because several factors were known to prolong diestrus in the mouse.

Other techniques for prolactin detection, based on its luteotrophic response in the mouse, have been summarized by Forsyth (1967). Measurements being based on either an indirect response of prolactin to support deciduomata formation in the hypophysectomized mouse (Kovačić, 1962), and in the intact mouse (Kovačić, 1965), or a direct response of prolactin, inducing hyperaemia of the corpora lutea in the mouse (Kovačić, 1963).

Wolthius (1963) devised a prolactin bioassay utilizing its luteotrophic properties to stimulate an increase in luteal cell size in hypophysectomized immature female rats, pretreated with PMS and HCG. Measurements were based on the number of luteal cell nuclei per unit area, by histological examination. The assay being sufficiently sensitive to measure prolactin in blood samples was hindered by being slow and tedious.

Radioimmunoassays. Berson et al. (1956) demonstrated the presence of an insulin antibody in the plasma of human subjects, capable of binding insulin-I^{131}. This observation stimulated an interest among researchers to apply this principle to the quantitative measurement of hormones. Since that time, radioimmunoassay (RIA techniques have been developed for the measurement of several protein and steriod hormones. The principle of RIA depends on the competition between radioactively-labeled and unlabeled hormone for the specific binding sites on the antibody. The greater the concentration of unlabeled

hormone, the less radioactively-labeled hormone will be bound to the antibody and vice versa. After separating the bound hormone (antigen-antibody complex) from the unbound hormone, quantitation can be achieved by measuring the amount of radioactivity in the antigen-antibody complex and comparing it to standard hormone preparations reacted under identical conditions (Wright and Taylor, 1967). The potential advantages of RIA over bioassay techniques, include an increase in sensitivity, specificit and application to assaying large numbers of samples with a reduction in time required for complete analysis. Criticism of the RIA stems from the fact that immunological and biological binding sites may reside at completely different sites on the hormone molecule. If the hormone remains intact, immunological and biological measurements should give identical results; however, if metabolic degradation of the hormone occurs, immunological and biological measurements may exhibit extreme variation (Berson and Yalow, 1964).

MATERIALS AND METHODS

Postpartum Cows. Six 6-year-old cows were chosen at random and cannulated approximately one week before their expected calving dates. Cannulation procedures consisted of jugular punctures with a 12-gauge thin-walled, hypodermic needle. Silastic medical-grade tubing (O.D. 0.085, I.D. 0.040) was inserted into the jugular vein through the 12-gauge hypodermic needle. Female adapters, prepared from 16-gauge hypodermic needles, were inserted into the silastic tubing and sutured to the skin. Cannulas were heparinized and capped for later collections.

The cannulated cows were confined in pairs at the Montana State University dairy center in small lots with adjacent shelter. The interval from cannulation until parturition served as a training period to allow the cows to become accustomed to the blood sampling procedure. During this time period, the cannulas were heparinized daily to avoid serious clotting. The training period was conducted to minimize the amount of stress during later blood collections. Two cows were terminated during the training period: one because of undesirable temperament and one due to a lost cannula.

Blood collections were scheduled to begin 3 days before the expected day of parturition; however, two cows (673 and 603) calved early and no samples were obtained for these animals before parturition.

The animals were divided into two groups--suckled and non-suckled. Calves from the non-suckled group were removed from their dams on the day of parturition after one nursing.

Daily blood sampling began 3 days prior to the expected day of parturition. The sampling period for the suckled group continued until 25 days postpartum, while the sampling period for the non-suckled group continued until after estrus had been observed.

Following the daily blood collection, rectal temperature of each cow was measured and recorded as a means of evaluating the overall health of the animal. Records were kept to evaluate the temperament of the animals during the blood collection period. This period also included the time involved in moving the animals from their confinement area to the bleeding stanchion. This record was based on four classes of temperament and scored on a 1 to 4 scale by the technician (Table 4). A score of 1 was recorded if the cow was moved from the confinement pen to the bleeding stanchion with a minimum of coaxing and the blood sample was collected with no apparent stress to the animal. A score of 4 was recorded when the animal was moved from the confinement pen to the bleeding stanchion with difficulty and the blood sample was collected while the animal was struggling. A score of 2 or 3 was recorded when the animal's temperament fell between the two extremes (1 and 4) and was dependent on the evaluation of the technician. To minimize the error of the temperament scores and decrease the stress associated with strangers, all the blood samples were taken by the same technician throughout the entire study. Approximately 25 ml of blood was collected daily in heparinized tubes. After collection, the samples were

refrigerated until it was convenient for transport to the lab where they were centrifuged. The plasma was collected and stored in Whirl-packs at -10°F for later analysis of LH and prolactin.

Heparin, used as an anticoagulent in the cannulas after blood collections, was prepared in aseptic 100 ml vials. Two ml of Terramycin injectable liquid was added to each 100 ml of heparin to reduce bacterial growth in both the heparin vial and the cannula tubing.

Heat detection was carried out by two daily observations with the assistance of an epididymectomized bull, housed adjacent to the test animals.

Rectal palpation of the suckled cows, after termination of the study, was utilized to determine the condition of the reproductive tract. Non-suckled cows were palpated periodically after estrus to detect the presence of newly forming corpora lutea and to determine the condition of the uterus.

MAP Estrous Synchronized Cows Subjected to Various Mating Stimuli

In a second study (conducted at the U. S. Range Livestock Experiment Station, ARS, Miles City, Montana) estrus was synchronized in mature cycling cows by feeding 180 mg medroxyprogesterone acetate (MAP) per head per day for 11 days and injecting 5 mg estradiol benzoate on day 2 of MAP feeding. At the postsynchronization estrus, the cows were assigned to 5 groups differing in intensity of experimentally administered mating stimulation. The number of experimental animals in Groups I

through V were 4, 4, 3, 2, 3, respectively. Cows in Groups I, II, III, IV were confined with estrogenized cows and observed continuously until detection of standing estrus. Cows in Group V were confined with an epididymectomized bull and observed continuously for standing estrus, followed by a single service from the bull. At the detection of standing estrus all cows were removed from their confinement area, cannulated (jugular vein) and exposed to the following stimuli. Cows in Group I received no further stimulus, cows in Group II received artificial insemination with no clitoral stimulation, cows in Group II received artificial insemination accompanied with a 10 second manual stimulation of the clitoris, cows in Group IV were immediately bred three times in succession by a bull and cows in Group V received no further stimulation. Immediately following the appropriate mating stimulus, the first blood sample was collected. Blood samples were collected hourly for 24 hours from the onset of estrus and at two-hour intervals thereafter until ovulation was determined by rectal palpation. Rectal palpations began 24 hours after standing estrus. Blood samples were collected by crowding several animals into a narrow runway so that samples could be taken without further methods of restraint. This was done to minimize stress associated with blood sampling. Jugular cannulas were extended to the dorsal part of the neck and secured with several layers of adhesive tape wrapped around the neck. This procedure allowed the technician to collect the blood sample while working above and behind

the animal's line of vision.

Blood samples in this study were analyzed for LH at the U. S. Range Livestock Experiment Station and for prolactin at Montana State University. No LH levels will be reported for the cows in this study; however, reference will be made to the exact time of their LH peaks. Specific LH data for these cows has previously been reported by Randel et al. (1973).

LH Assay

Quantitative measurement of LH was achieved by a double antibody RIA described by Niswender et al. (1969) and personal communication. The basis of this assay resides in the competition between labeled and unlabeled hormone for binding sites on the primary antibody. A secondary antibody was used to separate the primary antibody-hormone complex from the unbound hormone in the system.

The primary antibody (rabbit antiserum, Lot No.225), developed in laboratory rabbits by injection of purified bovine LH, was donated by Dr. G. Niswender. The primary antibody was diluted 1:400 with .05 M EDTA-PBS (pH7), containing 1:10,000 merthiolate, and frozen. Further dilutions to obtain a working concentration of 1:50,000 were made with normal rabbit serum (NRS), diluted 1:400 with the previous buffer.

The secondary antibody (sheep anti-rabbit gamma globulin) was prepared by injecting sheep subcutaneously with rabbit gamma globulin (Pentex Fraction II) in complete Freund's adjuvant. The secondary

antibody (anti-RGG) was diluted 1:3 with .05 M EDTA-PBS containing 1:10,000 merthiolate, and stored at -10°C in amounts required for a single assay.

Standard solutions corresponding to .1, .2, .4, .8, 1.6, 3.2, 6.4 and 12.8 ng LH (NIH-LH-B7) per ml PBS-1% egg white (EW) were prepared and frozen in 2 ml aliquots by submersion in dry ice and pentane and stored at -10°C.

Purified LH (LER-1072-2) suitable for radioiodination was obtained from Dr. L. Reichert. The iodination procedure was modified from that of Greenwood, Hunter and Glover (1963). Ten microliters of sodium phosphate buffer (pH 7.5) were added to 1 mc I^{131} (NaI^{131} in 0.1 N NaOH, New England Nuclear, Boston, Mass.) and transferred to the reaction vial containing 2.5 ug LH. The iodination reaction was initiated by the addition of 30 ug chloramine-T (2 ug CH-T/ul .05 M NaPhos., pH 7.5). Following a 2 minute reaction time, the reaction was stopped by the addition of 50 ul sodium metabisulfite (2.5 ug sodium metabisulfite/ul .05 M PO_4, pH 7.5). Separation of LH-I^{131} was carried out on a 1 x 22 cm Bio-Gel P-60 column chromatography apparatus (Gio-Gel P-60, 50-100 mesh, Bio-Rad Laboratories, Richmond, Calif.), previously coated with 1.5 ml PBS-5% EW. Transfer of LH-I^{131} and free I^{131} was accomplished by the addition of 100 ul 16% sucrose to the reaction vial, followed by transfer of the contents to the surface of a Bio-Gel P-60 column. The iodination vial was rinsed twice with 70 ul 8% sucrose

and transferred to the column to increase the recovery of the LH-I^{131}. Fractions were collected in 1 ml aliquots in tubes containing 1 ml PBS-5% EW. Aliquots (0.1 ml) were removed from each collection tube and counted for 0.1 minutes on an automated gamma counter (Automatic Gamma County System, Model 4233, Manufactured by Nuclear Chicago Co.), to determine the peak tube (figure 1). The contents of the peak tube were diluted in PBS-1% EW so that the 0.1 ml emitted 50-60,000 cpm ("IX" solution). The "IX" solution was placed in small vials and stored in a lead tub at -10°C.

The assay was conducted in disposable glass culture tubes (12x75 mm) with duplicate samples of standard points, total count, normal rabbit serum controls and unknowns. Sequential addition of PBS-1% EW, standards or unknowns and anti-LH, were made on the first day of the assay, followed by the addition of LH-I^{131} and anti-RGG at 24 and 48 hours, respectively. Following a 72-hour incubation period, beginning with the addition of the anti-RGG, 3 ml cold PBS were added to each culture tube (excluding the total count tubes), and centrifuged at 2,000 rpm for 30 minutes: (Model PR-2 centrifuge, International Equipment Co., Boston, Mass.). The supernatant was discarded and the remaining precipitate was counted on the automated gamma counter after a 2-hour interval which allowed the residual supernatant in the tubes to settle to the bottom. The percent bound $\left(\frac{\text{std.pt. conc. (cpm)}}{\text{zero pt. conc.(cpm)}}\right)$ versus the standard point concentration was plotted on semi-logarithmic paper and

served as the standard curve. Values of the unknowns were calculated directly from the standard curve and adjusted to a ng/ml basis according to the dilution of plasma (200 ul) in the culture tubes containing the unknowns. All plasma samples for an individual cow were measured within a single assay.

Prolactin Assay

Quantitative measurement of prolactin was accomplished with a double antibody RIA system described by Dr. P. V. Malven (Annual Report of Regional Research Project NE-72, 1970). The primary antibody (donated by Dr. Malven, Lot #401-6) was developed in rabbits by multiple injections of ovine prolactin (NIH-S-7) in complete Freund's adjuvant. The initial dilution of the primary antibody was 1:400 with .05M EDTA-.01M-PBS (pH 7.5), while further dilution to a working concentration of 1:5,200 was carried out with 1:400 normal rabbit serum (NRS) in EDTA-PBS. Bovine prolactin (NIH-B-3) was shown to cross react completely in the assay system with ovine prolactin, thus making the antibody applicable for use in measuring both ovine and bovine prolactin.

The secondary antibody (sheep anti-RGG) for the prolactin assay was the same as that used in the LH assay.

Standard solutions corresponding to .1, .2, .4, .8, 1.6, 3.2, 6.4 and 12.8 ng prolactin (NIH-P-B3) per ml PBS-0.1% gelatin were prepared frozen and stored with the same procedure used for making the LH standards.

Purified prolactin (ovine, NIH-P-S10) suitable for radioiodination was donated by NIH. The iodination procedure for prolactin was similar to that discussed previously for LH, with slight modifications. Addition of 30 ul 0.5 M sodium phosphate buffer to the iodination vial containing 2.5 ug prolactin was necessary to buffer the protein against the affects of NaOH, the carrier substance for the I^{131} (Na-Iodide^{131}). Addition of 20 ul 0.5 M sodium phosphate buffer to the V vial containing 1 mCi of I^{131} was carried out to increase the recovery of I^{131}. The iodination reaction was initated by the addition of 20 ug chloramine-T (1 ug Ch-T/ul .05 M NaPhos., pH 7.5). The reaction was stopped by the addition of 60 ug of sodium metabisulfite (2.0 ug sodium metabisulfite/ul .05 M NaPhos., pH 7.5), following a 20 to 30 second reaction. Transfer of the contents of the iodination vial to the column chromatography apparatus was identical to that described for LH. Separation of I^{131}-prolactin from free I^{131} was achieved by use of 1 x 28 cm Sephadex G-75 column chromatography apparatus (Sephadex G-75, 40-120 particle size, Pharmacia Fine Chemicals, Piscataway, N. J.). Ten drop aliquots were collected from the Sephadex G-75 column into 12 x 75 mm disposable culture tubes containing .25 ml PBS-.01% gelatin. Aliquots (0.1 ml) were taken from each collection tube and counted for 0.1 minutes to determine the shape of the iodination curve (figure 2). Several tubes from peak B of the iodination curve (figure 2) were covered with parafilm and frozen for later use.

Before the prolactin-I^{131} could be used in the assay system, a second purification of each tube saved from the original iodination was necessary. This purification step was carried out on a Sephadex G-75 column identical to that used in the first purification step. The collection procedures were also identical, the only exception being that 15 collection tubes were sufficient for the second purification. Following the second purification, the total contents of each tube (approximately .75 ml) were counted for 0.1 minutes to determine the shape of the purification curve. Figure 3 illustrates the major peaks of radioactivity detected after the second purification. Several tubes from peak B were selected for preparing the working solution of prolactin-I^{131}. These tubes are designated by an asterisk below the corresponding tube number in figure 3. The tubes selected from peak B were pooled and diluted with PBS-0.1% gelatin so that the final working concentration ("IX" Solution) emitted 25-30,000 cpm/0.1 ml.

The prolactin assay was run with duplicate samples similar to the LH assay, with minor adjustments; the adjustments being the addition of the labeled hormone on day 1 for the prolactin assay, followed 24 hours later by the secondary antibody (sheep anti-RGG), followed by a 72-hour incubation. This procedure resulted in a 5-day assay interval for prolactin as compared to a 6-day interval for LH. The standard amount of plasma used in the prolactin assay was 50 ul. All plasma samples for an individual cow were measured within a single

assay. In the second experiment or the mating stimulation study, Groups I and II were combined into a non-stimulated group (receiving no clitoral stimulation) while Groups III, IV and V were combined into a stimulated group (receiving manual or natural clitoral stimulation). Prolactin assays for the non-stimulated and stimulated groups were paired so that a cow from each group was assayed on the same day to reduce day to day assay variation in mean prolactin levels between groups. Technican variation was reduced by altering the two technicians between the non-stimulated and stimulated groups.

Laboratory plasma standards were included in several prolactin assays to evaluate the between assay and within assay variation. Plasma from the laboratory standard was assayed at two dilutions; whole plasma (1:1) and plasma plus PBS (1:2). The mean prolactin values from the duplicate samples of the 1:1 and 1:2 dilutions, were used to calculate two individual estimates of the between assay variation. Prolactin values from each individual duplicate sample were used to calculate a within assay variation for the 1:1 and the 1:2 dilutions.

Specific activities of the prolactin -I^{131} preparations were calculated in the following manner:

1. Count total V vial containing one uCi I^{131} for on minute with altered geometry (sample is placed in a lead pig over the counting well on the automated gamma counter).

2. Count the reaction vial, transfer syringes, empty V vial and Sephadex G-75 column individually for one min with altered geometry, after completing the reaction and the first purficiation.

3. Substract total counts in step 2 from total counts in step 1 and compute the total loss of radioactivity (percent loss).

4. Substract the total percent loss from 100% to calculate the percent recovery (100% = 1 uCi or some value appropriately adjusted for radioactive decay according to the assay date of the I^{131} preparation).

5. Adjust the total percent recovery to a uCi basis by multiplying the percent recovery (step 4) by the known value for uCi (step 1).

6. Calculate the percent radioactivity in the protein portion of the iodination curve (100% = total counts collected from the Sephadex G-75 column, counted with normal geometry).

7. Multiply the uCi value recovered from the iodination reaction (step 5), by the percent radioactivity in the protein portion of the iodination curve (step 6).

8. Divide the value in step 7 by the weight of the hormone in the reaction vial (2.5 ug).

9. The value from step 8 is an estimation of the specific activity of the preparation, calculated in uCi/ug.

Data Analysis

All data were analyzed by the least-squares method (Harvey, 1968) at the MSU Computing Center. The data from the postpartum study was subjected to combined group and between group analysis for LH and prolactin levels. Two between group analyses of prolaction were conducted for the mating stimulation study using two separate groupings of the cows. The first analysis utilized Groups I to V

and tested for differences associated with the degree of mating stimulation. The second analysis pooled Groups I and II into a single group receiving no clitoral stimulation (NS) and Groups III, IV, and V into a single group receiving clitoral stimulation (S). The NS and S groups were analyzed with the onset of estrus and the LH peak as two common physiological groupings, to detect differences in clitoral vs no clitoral stimulation.

RESULTS

LH Assay

Illustrated in figure 1 is a typical elution pattern for LH-I^{131} and free unbound I^{131} eluted from a Bio-Gel P-60 column. The peak tube in the first radioactive peak represents the material most suitable for use in the LH assay.

Figure 4 illustrates the shape of the standard curve for the LH assay. The sensitivity of the curve being in the range of 0.1-0.2 ng, making the minimum level of detectable plasma LH between 0.5 and 1.0 ng/ml.

Prolactin Assay

In the developmental stages of the prolactin assay, the reaction conditions for the preparation of prolactin-I^{131} varied between individual preparations. The addition of 20 ug CH-T, followed by a 20 sec. reaction stopped with the addition of 60 ug sodium metabisulfide, resulted in the most desirable preparation. The specific activity of these preparations was approximately 155 uCi/ug. Increasing the amount of CH-T or the reaction time resulted in a slight increase in the specific activity and a substantial increase in the amount of iodination damage, as determined by an increase in the magnitude of peak A (figure 2), following purification on Sepadex G-75.

As seen from the shape of the iodination curve for prolactin (figure 2), a series of radioactive peaks were presented. Peaks A and B were capable of binding with the primary antibody and therefore

considered to be two different forms of prolactin-I^{131}. Standard curves from peak A and B (figure 2) lacked sufficient slope to yield a sensitive assay system, as illustrated in figure 5. Further purification of the material contained in peak B (figure 2) resulted in an elution pattern (figure 3) similar to that observed in the first purification (figure 2). During both purification steps, two radioactive peaks were eluted from the Sephadex G-75 column prior to collection tube #12. Following the second purification step (figure 3), several tubes in peak B were utilized to prepare a working solution of prolactin-I^{131} suitable for use in the assay. These tubes are designated by an asterisk in figure 3. A standard curve from prolactin-I^{131} prepared in this manner is illustrated in figure 6. Standard curves of this type were acceptable for prolactin quanitation.

Peak C (figure 2) represents smaller molecular weight material considered to be free unbound I^{131}.

The percent prolactin-I^{131} bound by the anti-prolactin (200 ul, 1:5200 dilution) in the absence of standard or unknown amounts of prolactin, averaged 25.3%.

Between assay variation for prolactin assays averaged 50% at the 1:1 and 41% at the 1:2 dilution of the laboratory plasma standard. Within assay variation averaged 19.4% and 25.4%, respectively, for the 1:1 and 1:2 dilutions.

LH and Prolactin Levels for Postpartum Cows

LH and prolactin levels for individual postpartum cows are illustrated in figures 7 to 10. Least squares LH and prolactin means, for daily samples across groups, are illustrated in figure 11. Least square means $\pm$ S.E. for periparturient prolactin levels between the non-suckled (94.9$\pm$1.36 ng/ml) and the suckled (176.3$\pm$10.7 ng/ml) groups (figure 12) were significantly different ($P<0.01$, table 3). Combined group analysis revealed a significant difference ($P<0.05$, table 3) in prolactin levels for day postpartum. A significant negative linear regression ($P<0.01$, table 3) of prolactin on day postpartum was also detected. The regression coefficient was b= -6.795$\pm$0.985. No significant differences were observed between groups for LH. The combined group analysis revealed a significant negative correlation ($P<0.06$) between LH and prolactin. The within subclass correlation coefficient was r= -.29.

TABLE 3. LEAST SQUARES ANALYSIS OF VARIANCE FOR COMBINED NON-SUCKLED AND SUCKLED GROUPS

Source of Variation	Degrees of freedom	Mean Squares	
		LH	Prolactin
Total	97		
Days	31	.62	12354.9*
Linear	1	--	276537.3**
Residual	30	--	3549.9
Group	1	2.05	129477.1**
Day x Group	24	.71	2708.5
Error	41	1.32	5812.7

* $P<0.05$
** $P<0.01$

General Comments on Postpartum Cows

Table 4 illustrates temperament evaluation scores for all postpartum cows throughout the experiment.

TABLE 4. TEMPERAMENT EVALUATION DURING BLOOD SAMPLING OF POSTPARTUM COWS

	Suckled		Non-Suckled	
Cow Number	673	613	603	621
Day Postpartum				
0	1	1	1	2
1	3	2	2	2
2	4	4	2	2
3	1	1	2	3
4	4	4	2	2
5	1	1	2	2
6	1	1	3	1
7	1	1	3	1
8	1	1	1	3
9	2	2	1	1
10	2	2	1	2
11	1	1	2	1
12	1	1	1	1
13	1	1	1	1
14	1	1	1	2
15	1	1	1	1
16	1	1	1	1
17	1	1	1	
18	1	1	1	
19	1	1	1	
20	1	1	1	
21	1	1	1	
22	1	1		
23	2	2		
24	1	1		
25	1	1		
26	1	1		

1 - quiet
2 - semiquiet
3 - semihyper
4 - hyper

The average daily rectal temperatures for the non-suckled and

suckled groups were 101.5°F and 102.2°F, respectively, throughout the experiment.

All cows had normal deliveries and the calves were strong and vigorus. No retained placenta's were observed.

Prolactin Levels in MAP estrous Synchronized Cows Subjected to Various Mating Stimuli

Prolactin levels for individual cows are illustrated in appendix figures I through XVI. No significant differences were observed between groups (I-V) receiving different degrees of mating stimulation. Due to the extremely small number of animals in groups I through V (4, 4, 3, 2 and 3, respectively), it was necessary to regroup the animals into two treatments (8 animals per group) utilizing the criteria of no clitoral stimulation (NS) versus clitoral stimulation (S). Analyses of the NS and S groups were conducted utilizing the onset of estrus and the LH peak as distinct physiological events.

The least squares prolactin means for the appropriate sampling times from the onset of estrus to ovulation are illustrated in figure 13. Analysis of the NS and S groups from the onset of estrus to ovulation, revealed a significant ($P<0.01$, table 5) interaction of group on sample time.

Analysis of the NS and S groups, aligned on the specific LH peaks for the individual cows, resulted in a significant group difference ($P<0.01$, table 6) between the overall least squares prolactin means $\pm$ S.E. (23.4 ± 1.2 ng/ml and 18.1 ± 1.2 ng/ml, respectively, for the NS

and S Groups, table 7). The least square prolactin means ± S.E. for the time intervals from the LH peak back to the onset of estrus (23.2±1.4 ng/ml, table 7) and from the LH peak to ovulation (18.3±0.9 ng/ml, table 7) were significantly different (P<0.01, table 6) across groups. The group by time interval interaction was also significant (P 0.01, table 6).

The least squares prolactin means for the designated sample times from the LH peak back to the onset of estrus and from the LH peak to ovulation, are illustrated for the NS and S groups in figure 14. A significant group difference (P<0.01, table 8) was detected from the LH peak back to the onset of estrus. The overall least squares prolactin means ± S.E. for the NS and S groups from the onset of estrus to the LH peak, were 39.7±3.6 ng/ml and 16.7±2.6 ng/ml (table 9), respectively. The interaction of group on sample time before the LH peak was significant (P<0.01, table 8).

TABLE 5. LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS IN NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FROM THE ONSET OF ESTRUS TO OVULATION.

Source of variation	Degrees of freedom	Mean squares
Total	429	
Group	1	627.1
Sample time	28	428.6
Group x sample time	25	564.8**
Error	375	247.2

** P<0.01

TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INTERVAL FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS AND THE INTERVAL FROM THE LH PEAK TO OVULATION

Source of Variation	Degree of freedom	Mean squares
Total	429	
Group	1	2507.8**
Time interval	1	2182.5**
Group x time interval	1	6235.4**
Error	426	257.6

** $P<0.01$

TABLE 7. LEAST SQUARES PROLACTIN MEANS AND STANDARD ERRORS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INTERVAL FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS AND THE INTERVAL FROM THE LH PEAK TO OVULATION

	Group					
	Non-stimulated		Stimulated		Mean	
LH Peak	n	Prolactin ng/ml	n	Prolactin ng/ml	n	Prolactin ng/ml
Before	61	30.0±2.0	70	16.4±1.9	131	23.2±1.4
After	166	16.8±1.2	133	19.8±1.4	299	18.3±0.9
Mean	227	23.4±1.2	203	18.1±1.2	430	20.8±0.8

TABLE 8. LEAST SQUARES ANALYSIS OF VARIANCE OF PROLACTIN LEVELS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INDIVIDUAL SAMPLE TIMES FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS

Source of Variation	Degree of freedom	Mean squares
Total	130	
Group	1	11687.6**
Sample time	14	490.6
Group x sample time	11	1013.6**
Error	104	320.6

** P 0.01

TABLE 9. LEAST SQUARES PROLACTIN MEANS AND STANDARD ERRORS FOR NON-STIMULATED AND STIMULATED ESTROUS SYNCHRONIZED COWS FOR THE INDIVIDUAL SAMPLE TIMES FROM THE LH PEAK BACK TO THE ONSET OF ESTRUS

Group					
Non-stimulated		Stimulated		Mean	
n	Prolactin ng/ml	n	Prolactin ng/ml	n	Prolactin ng/ml
61	39.7±3.6	70	16.7±2.6	131	28.2±2.5

DISCUSSION

Prolactin Assay

The results of the two purifications of prolactin-I^{131} on the Sephadex G-75 column suggest that peak A in figures 2 and 3, corresponds primarily to prolactin-I^{131} that was damaged during the iodination reaction. The damaged prolactin-I^{131} in this case is characterized as molecular aggregates which pass rapidly through the Sephadex column. The aggregates of prolactin-I^{131} contained in peak A seem to exhibit abnormal binding affinity with the prolactin antibody (anti-prolactin). This is illustrated in figure 5, where the standard curve from peak A exhibits a substantial decrease in the slope of the curve. The decrease in slope of the standard curve results in a reduction in the ability to distinguish between different levels of hormone. One may speculate that aggregates of prolactin-I^{131} are not displaced from the anti-prolactin in direct relationship to the increasing concentrations of unlabeled prolactin. The apparent inability of unlabeled prolactin to displace the aggregates of prolactin-I^{131} would account for the lack of slope in the standard curves seen in figure 5. Removal of the remaining aggregates in peak B (figure 2) by a second purification step resulted in the acceptable standard curve illustrated in figure 6.

Hunter (1969) discusses the problems of radioiodination damage to protein hormones and indicates that damage can be assessd by: (1) a shift in the standard curve from left to right, resulting in a loss of sensitivity; (2) a decrease in the slope of the standard curve; and (3) a decrease in the percentage of labeled hormone bound to the

primary antibody in the presence of excess antibody. The standard curves illustrated in figure 5 from peak A and B are in full agreement with the changes associated with radioiodination damage from criterion 1 and 2 above. These standard curves demonstrate a shift in the standard curve from left to right and a decrease in the slope of the curve, when compared to the standard curve in figure 6. No attempt was made in this study to evaluate the binding affinity of the damaged prolactin-I^{131} in the presence of excess antibody; however, previous discussion in this paper points out that the binding affinity of prolactin-I^{131} aggregates in the presence of limited antibody seemed to be greater than the binding affinity of undamaged prolactin-I^{131}.

Davis, Reichert and Niswender (1971) reported similar problems associated with radioiodination damage of prolactin as those discussed by Hunter (1969). They also indicate that three radioimmunoassayable peaks of prolactin were present in male (ovine) serum and pituitary extracts corresponding with molecular weights ranging from 24,000-28,000, 40,000-48,000 and 100,000-120,000, as determined by gel filtration. The major peak of radioimmunoassayable prolactin was associated with the 24,000-28,000 molecular weight range. Heating the samples to 37°C for 30 minutes created a shift in the radioimmunoassayable peaks, so that 60% of the material was contained in the peak associated with the 100,000-120,000 molecular weight. The change in the radioimmunoassayable peaks was suggested to be the result of the formation of prolactin-I^{131} aggregates.

Similar results were observed in our lab resulting from iodination damage. These results are best explained by observing the elution pattern of prolactin-I^{131} from the Sephadex G-75 column (figure 2). The magnitude of peaks A and B varied accordingly with the amount of chloramine-T (CH-T) and the reaction time. By increasing the amount of CH-T and the reaction time, the magnitude of peak A increased while peak B decreased. This procedure resulted in a large portion of the prolactin-I^{131} molecules in peak A corresponding with the damaged aggregates not suitable for use in the assay. Reduction in the amount of CH-T and the reaction time produced a shift in the two peaks in the opposite direction so that peak B became the major peak. By continuous step by step reduction in the amount of CH-T and the reaction time, peak A could be reduced to a very minor peak; however, in this case, the specific activity of the prolactin-I^{131} in peak B was reduced considerably (63 uCi/ug). At the 1:5200 dilution of the anti-prolactin, standard curves using the low specific activity prolactin-I^{131} (63 uCi/ug) were suitable for prolactin quantitation; however, the amount of usable prolactin-I^{131} was very low. In our system, we found 20 ug CH-T and a 20 second reaction time produced two peaks of comparable size (peaks A and B, figure 2), with an overall specific activity of approximately 155 uCi/ug. Further purification of peak B (figure 2) removed additional aggregates of prolactin-I^{131} that were not removed during the first purification (figure 3). The prolactin-I^{131} recovered in peak B (figure 3) after the second purification produced desirable

standard curves (figure 6) at an antibody concentration of 1:5200. The amount of usable material recovered after the second purification was enough for approximately 500 culture tubes.

Between assay variation for the prolactin assay was calculated to be 50±10.3% at the 1:1 dilution of the lab plasma standard and 41±3.7% at the 1:2 dilution. This estimate is in full agreement with the value of 50% reported by Fell et al. (1971). A large between assay variation was not considered to be detrimental in our situation because all samples for an individual cow were analyzed within a single assay. Duplicate assays were also conducted simultaneously including a cow from each of the two major groups in the particular study. This procedure should have minimized the error variation in mean prolactin level between major groups. The slightly larger between assay variation associated with the 1:1 dilution of the lab standard (50%) as compared to the 1:2 dilution (41%), may indicate that the lower end of the standard curve, where larger values of prolactin are recorded, is less reliable for prolactin quanitation. This would be expected because of the loss of slope at the lower end of the sigmoid shaped standard curve.

Within assay variation was calculated to be 19.4% and 25.4%, respectively, for the 1:1 and 1:2 dilutions. These values are considably higher than the value of 10% reported by Fell et al. (1971). The magnitude of the within assay variation in the present study may be partially explained in terms of the technique used for pipetting the

plasma samples. Duplicate plasma samples (50 ul) were measured by filling a 200 ul pipette with plasma and draining off 50 ul aliquots into two separate culture tubes. Errors produced while pipetting the first duplicate were compensated for in the second duplicate, so that the total of the two duplicates equaled 100 ul. This procedure produced a slight variation in the total amount of plasma between duplicate samples. The slight difference in the amount of plasma between duplicate samples expanded the within assay variation because the within assay variation was calculated from the difference in the values recorded for each individual duplicate sample. The actual value reported for the particular plasma samples was the average of the two duplicate samples and therefore compensate for pipetting errors. The primary reason for pipetting errors of this type relates to the difficulty in measuring 50 ul aliquots with the 200 ul pipettes available in our lab.

LH and Prolactin Levels in Postpartum Cows

The significant difference ($P<0.01$) in least squares periparturient prolactin means between non-suckled and suckled groups may be attributed to three factors. The first factor which may have contributed to the observed difference, results from a difference in the environmental stress during the blood collection procedure. Both groups were handled in a similar fashion during sampling to minimize prolactin release associated with stress; however, the cows in the

suckled group were forced to abandon their calves daily when the blood sample was being collected in the clinic adjacent to the housing quarters. The additional stress imposed on the suckled cows from this situation could have resulted in increased plasma prolactin levels. Johke (1970) listed emotional disturbances associated with venipuncture as one type of stress situation capable of initiating significant prolactin release in lactating Holstein cows. In the present study, a difference in the degree of emotional disturbance between groups could have been responsible for part of the observed difference in mean prolactin levels.

The second factor which may have contributed to the observed difference in least squares prolactin means between the non-suckled and suckled groups relates to a difference in the physiological state of the two groups. In this case, the lack of suckling stimulus in the non-suckled group, as compared to the recurring suckling stimulus in the suckled group, accounts for the two distinctly separate environmental situations capable of effecting prolactin secretion. Several investigators have reported that stimulation of the udder by milking elicits a rapid short-term release of prolactin in dairy cattle (Tucker, 1971; Kaprowski and Tucker, 1973; Fell et al. 1973). These reports indicate that the prolactin secretion pattern in lactating dairy cows is composed of a series of post-milking peaks followed by a rapid return to baseline levels. If this same type of secretion

pattern prevails in lactating beef cattle, the possibility of collecting a blood sample during a post-suckling increase in plasma prolactin, could account for part of the observed difference in plasma prolactin between groups. To clearly establish if an actual biological difference exists in the secretion pattern of prolactin between non-suckled and suckled cows, a more intense blood sampling scheme is necessary to depict post-suckling prolactin surges from tonic baseline secretion.

A third possibility for the difference in least squares prolactin means between groups may relate to an actual difference in the baseline prolactin level. An increase in baseline prolactin level in the suckled group, resulting from frequent suckling induced prolactin releases, may constitute a valid argument for the difference in least squares prolactin means between groups.

The significant negative linear regression of days on prolactin level for all postpartum cows may have resulted from the following point of discussion. Throughout the course of this experiment, the stress associated with the procedure of collecting blood may have been progressively reduced by cows becoming more accustomed to the experimental situation. In this case, a continual reduction in stress could account for a reduction in prolactin levels throughout the experiment.

In the non-suckled group, a negative regression of days of prolactin level would be expected due to the lack of suckling stimuli.

The stress situation associated with parturition may also play

an important role in the elevation of prolactin levels around the time of parturition. As a cow recovers from the dramatic stress of parturition, a decrease in plasma prolactin would be expected due to the declining stress situation. Ingalls, Hafs and Oxender (1971) observed a substantial drop in serum prolactin 60 hrs after parturition.

No significant difference in LH levels were detected between groups. The pattern of LH secretion observed in all postpartum cows was characterized by periodic spikes ranging from .5 to 5.5 ng/ml. No substantial increases in LH were observed during estrus in the non-suckled group, which may be attributed to the low frequency blood sampling employed in this experiment.

The negative correlation between LH and prolactin may be interpreted as indicating that the secretion of LH and prolactin, generally do not occur simultaneously in the cow. Considerable evidence has been compiled in the rat which supports the concept that antagonism exists between LH and prolactin secretion. If this concept is carried over to the cow, the factors responsible for prolactin secretion would then be antognisitic to LH secretion. In this case, the suckling stimulus accompanied by various other stressful situations would initiate severe increases in prolactin secretion that should act to depress LH secretion. If this argument holds true in the cow, one may speculate that postpartum anestrus in cattle may occur from insufficient gonadotropin secretion, resulting in ovarian inactivity similar to that

observed in the postpartum rat by Rothchild (1960). The present data does not show a significant depression of LH in the suckled group and therefore does support the theory entirely. A possible explanation of these results in relation to the proposed theory would suggest that the factors responsible for prolactin secretion may suppress the LH surge center necessary for ovulation, rather than the area associated with tonic LH secretion. Wagner and Oxenreider (1971) suggested that follicles large enough to mature and ovulate are present in suckled and milked cows by 1 to 2 weeks postpartum; however, first postpartum ovulations were not detected until 52 ± 2.9 and 45 ± 4.2 days, respectively, for these groups. The observation that follicular growth and maturation were occurring early in the postpartum interval presents evidence to support the theory of a suckling or milking induced supression of the hypothalmic surge center controlling the ovulatory quota of gonadotropins. In this situation, the frequency of the prolactin surge associated with milk removal (suckling or milking) would be the primary factor determining the degree of supression on the hypothalmic surge center. Evidence to support this statement stems from the observations that postpartum intervals increase accordingly between mastectomized, non-suckled and suckled groups (Short et al. 1972) and between non-suckled, milked and suckled groups (Wagner and Oxenreider, 1971). The primary difference between groups in these studies relates to a difference in the frequency of milk removal. As the frequency of milk

removal increases, a greater number of post-milk removal prolactin spikes are initiated, resulting in greater supression of the hypothalmic surge center controlling the ovulatory quota of gonadotropins.

Recalling the work of Kamberi, Schneider and McCann (as reviewed by Schwartz and McCormack, 1972), the potential neurotransmitters, dopamine, norepinephrine, epinephrine, serotonin and melatonin, produced inverse effects on the secretion of prolactin and gonadotropins when infused into the third ventricle of the rat brain at a particular dose level. If similar conditions exist in the bovine, one would expect that the neurotransmitter(s) associated with prolactin release would result in a reduction in the amount of gonadotropins released. Therefore, increasing the frequency of milk removal would stimulated a more frequent neurosecretion of the appropriate neurotransmitter associated with prolactin secretion. This in turn would result in a supressing effect on gonadotropin secretion which may be the controlling factor responsible for postpartum anestrus. Whether the gonadotropic supression occurs at the hypothalmic surge center or at the baseline control center may be species specific and account for minor differences between the rat and cow. Another alternative proposal would suggest that the supression of gonadotropins occurs at both levle of hypothalmic control and that a more intense system of blood sampling would demonstrate short-term inverse fluctuations in prolactin and gonadotropin secretion.

Rectal Temperatures of Postpartum Cows

Evaluation of the rectal temperature of postpartum cows revealed that the mean temperature of the suckled group (102.2°F) was slightly higher than the mean of the non-suckled (101.5°F) group. This difference was not tested for significance, however, it may reflect a difference in the metabolic rate between lactating and non-lactating cows.

Cow #613 had an elevated rectal temperature of 103.6°F and 103.0°F on postpartum days 15 and 25, respectively. This is above the acceptable range of 101.4 - 102.8°F reported by Ensminger (1971). Temperatures above the acceptable level suggest bacterial infection capable of altering the normal postpartum physiology. No antibiotic treatment was initiated because the rectal temperature did not remain above the acceptable level for two consecutive days. Previous unpublished observations of jugular cannulated cattle at MSU indicated that long-term cannulation may result in serious bacterial infections leading to a high death loss in experimental animals. For this reason, additional aseptic techniques were employed to reduce bacterial innoculation through the cannulas. In this case, the procedures outlined in the Materials and Methods section of this paper proved to be effective in eliminating death loss due to bacterial infections, however, a low grade systemic infection was still apparent in one experimental animal.

Localized areas of infection encompassing the cannula tubing were apparent in the suckled group at 19 and 26 days post-cannulation for cow 613 and 673, respectively. Drainage from these areas was minor, however, inflammation persisted until termination of the study. No localized areas of infection were present in the non-suckled cows. The difference in the degree of localized infection between groups could have accounted for the difference in the average daily rectal temperature between groups.

Temperament Evaluation of Postpartum Cows

The records of temperament evaluation (table 4) completed during the daily blood sampling procedure could not be consistently linked to prolactin level in the corresponding sample. In this study, the temperament records served no useful purpose, but were included to emphasize the fact that records of this type may prove to be useful in subsequent studies for evaluating severe fluctuations in circulating prolactin levels.

Prolactin Levels in MAP Estrous Synchronized Cows Subjected to Various Mating Stimuli

Grouping the NS and S Groups in relation to the LH peak (figure 14), revealed an elevated prolactin level prior to the LH peak in the NS group. The least squares prolactin mean for the NS group during the interval from the LH peak back to the onset of estrous (30.0 ± 2.0 ng/ml), was significantly greater ($P < 0.01$, table 6) than the least squares

prolactin mean during the interval from the LH peak to ovulation (16.8 ± 1.2 ng/ml). This observation is in agreement with other studies that report a substantial increase in prolactin near estrus (Hafs and Morrow, 1970; Raud, Kiddy and Odell, 1971). In the present study, cannulation was carried out immediately before the first sample was collected and no training period was conducted to familiarize the test animals with the blood collecting procedure. Therefore, stress associated with the first and possibly several subsequent blood samples may have been responsible for the significantly higher prolactin level observed during the interval from the LH peak back to the onset of estrus.

The least squares prolactin means during the interval from the LH peak back to the onset of estrus was significantly different ($P<0.01$, table 8) between the NS and S groups (39.7 ± 3.6 ng/ml and 16.7 ± 2.6 ng/ml, table 9, respectively). Interpretation of the group difference is difficult because of a lack of the supportive evidence in this area of the literature. Assuming that stress associated with cannulation and blood sampling was constant between the NS and S groups throughout the experiment, the group difference from the LH peak back to the onset of estrus should reflect a true treatment difference. In view of the individual prolactin levels for all cows in the study (appendix figures I through XVI), it is apparent that extremely high prolactin values in certain samples for individual cows could have had

a substantial effect on the mean for the group. Therefore, the assumption that stress was constant may be invalid. Being that no blood sampling records or temperament evaluations were taken during this study, it was impossible to exclude stressed samples from the analysis. From the limited data presented in this study, it appears that mating stimulation (either manual or natural) intiates a suppressive effect on prolactin levels during the interval from the onset of estrus until the LH peak. Whether the reduction in prolactin resulted from a decreased secretion rate or an increased metabolic clearance is unknown. Davis and Borger (1973) indicate that the MCR of prolactin in the ewe may vary between different physiological conditions.

Cummins et al. (1973) reported that natural service initiates a small immediate increase in plasma prolactin. In the present study, an immediate prolactin response assocated with the particular mating stimulation could not be separated from the stress associated with cannulation because cannulation occurred immediately before the first blood collection.

No significant group difference was detected during the interval from the LH peak to ovulation. Stress associated with blood collection during this interval should have been minimal due to the gradual adjustment of the cows to the sampling procedure. The least squares prolactin means during this interval for the NS and S groups (16.8 ± 1.2 ng/ml and 19.8 ± 1.4 ng/ml, respectively, are considerably lower than the range of values reported by Raud, Kiddy and Odell (1971) for diestrus prolactin

levels in non-lactating dairy cows (31-64 ng/ml). This difference may be attributed to either assay differences or various other factors associated with stress or physiological condition of the animals.

An important criticism of the present study relates to the fact that it was not orginally designed for prolactin analysis. The major criticism arises from the environmental stress associated with jugular cannulation immediately prior to the first blood collection. In several animals, this was presumably the factor responsible for elevated prolactin levels in first and possible subsequent samples.

Comparison of Blood Sampling Techniques

In view of the prolactin levels in all postpartum cows, the question arises as to the effectiveness of the blood sampling technique utilized for minimizing the stress associated with blood collection. In this study, blood samples were collected through jugular cannulas with the technician standing directly in front of the animal. This situation probably resulted in a stress of variable degree between animals. The fact that all cows in this study were 6-year-old range cows also contributed to the possibility that a substantial stress may have resulted from working near the head of the animal.

In the second study dealing with the estrous synchronized cows, blood samples were collected through jugular cannulas extended to the dorsal region of the neck. This allowed the technician to collect the sample while standing above and behind the line of vision of the animal.

levels reported to Raud, Kiddy and Odell (1971).

Koprowski and Tucker (1973) utilized venipuncture of the tail vein for collecting blood and reported it to be a satisfactory method of collection for reliable prolactin analysis in dairy cattle. Preliminary results in our lab indicate that tail vein samples may be an acceptable substitute for jugular cannulas in reducing stress associated with blood collection in beef cattle.

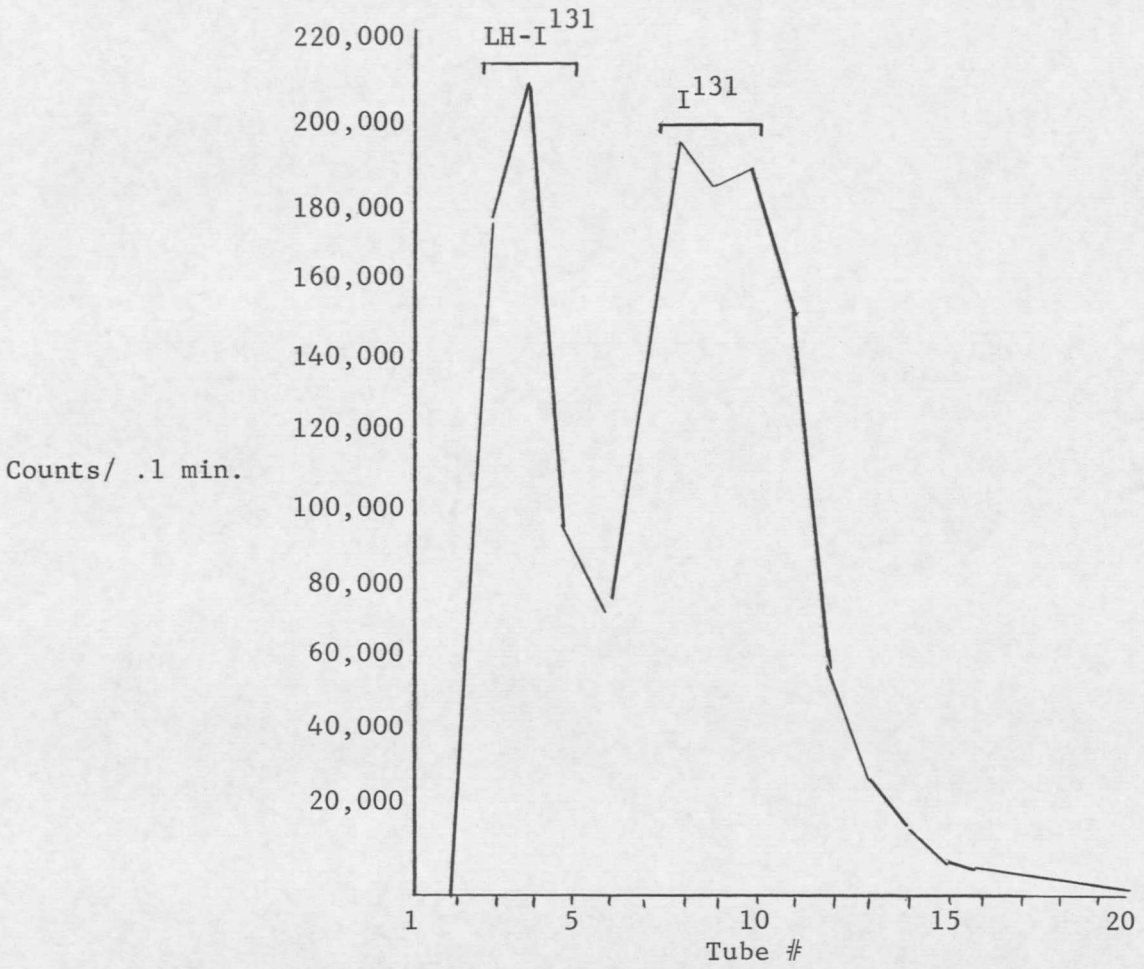


Figure 1. Elution pattern of radioiodinated LH (Bio-Gel P-60 column).

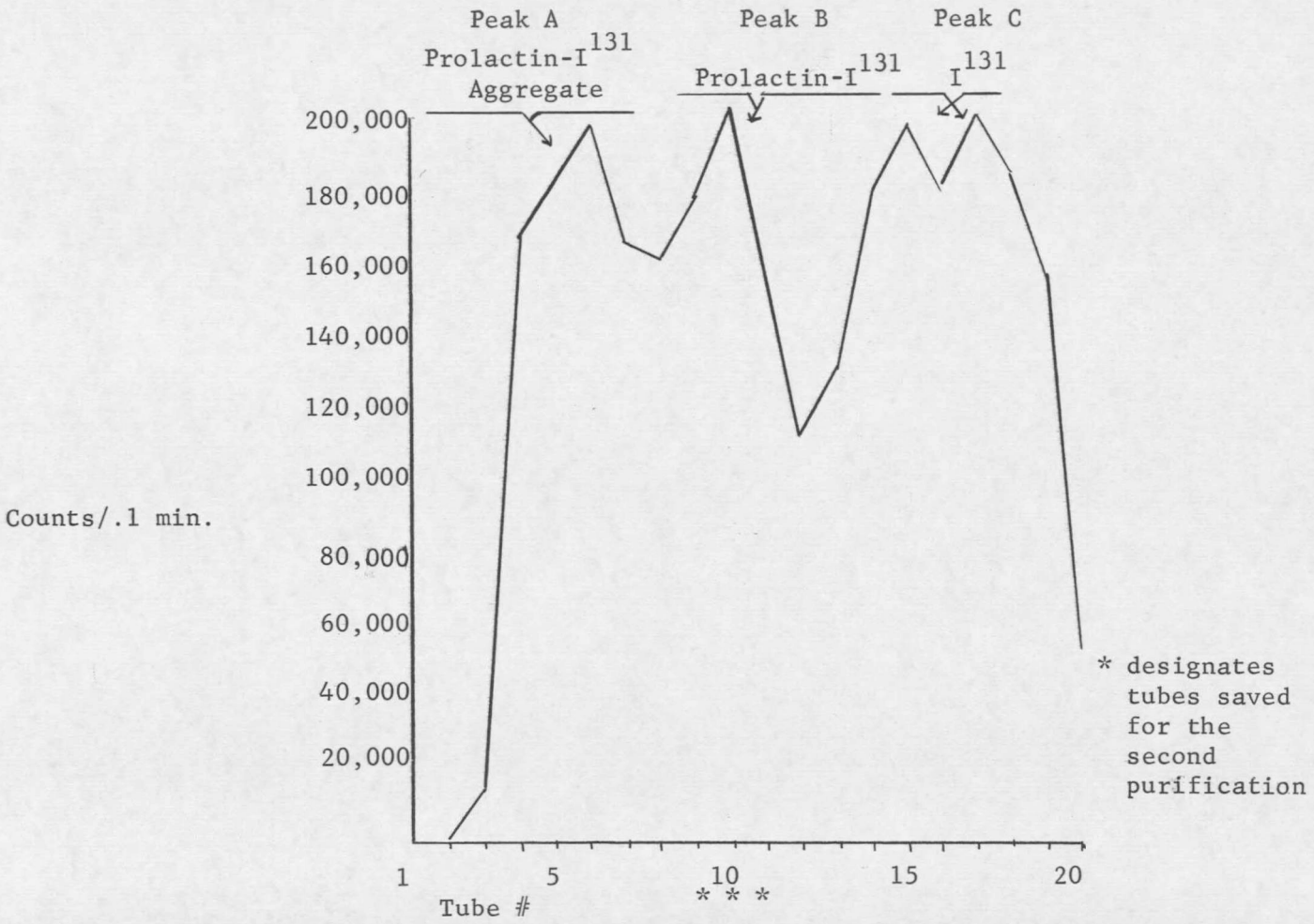


Figure 2. Elution pattern of radioiodinated prolactin from first purification (Sephadex G-75 column).

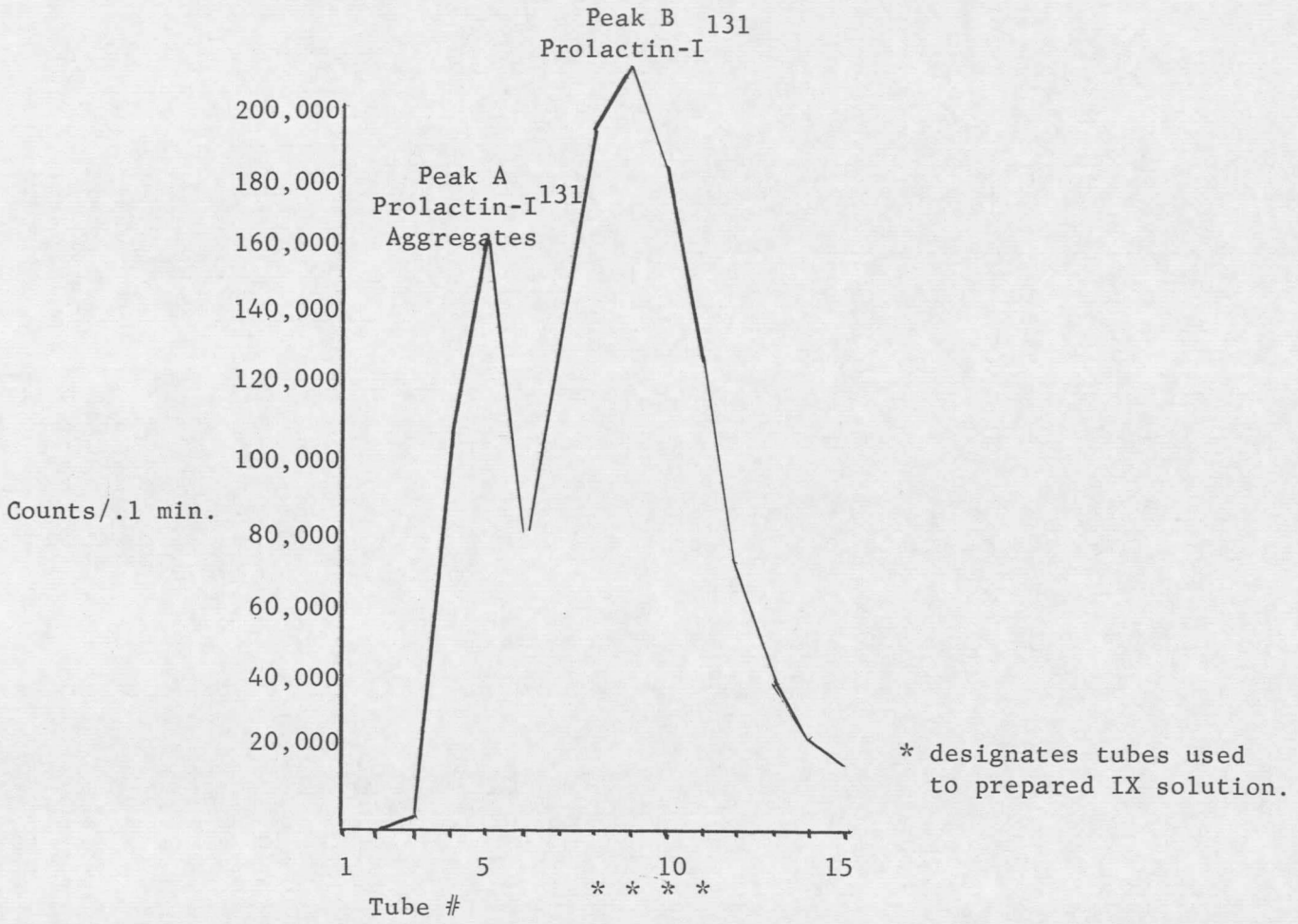


Figure 3. Elution pattern of radioiodinated prolactin from second purfication (Sephadex G-75 column).

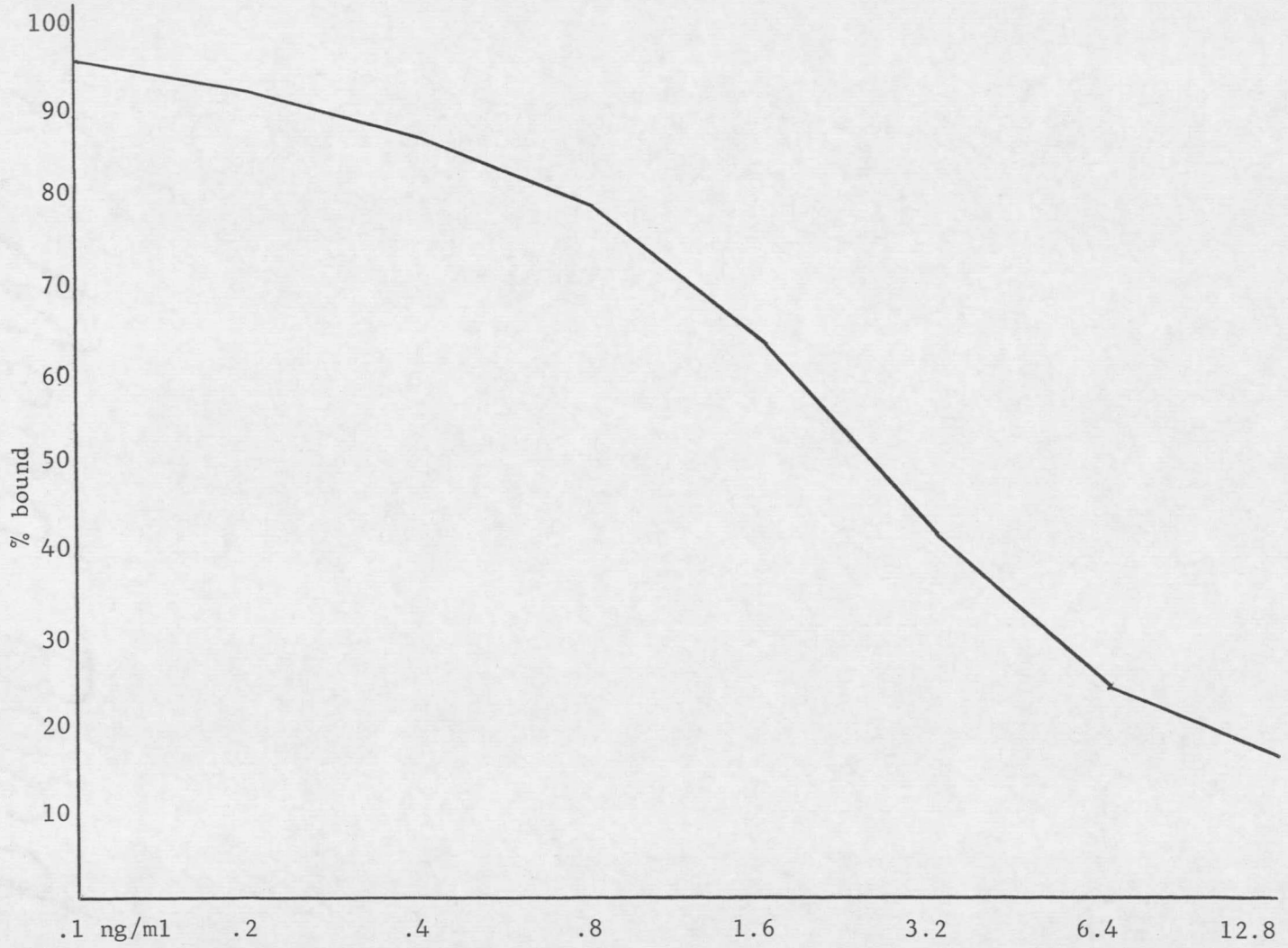


Figure 4. LH standard curve (NIH-LH-B7).

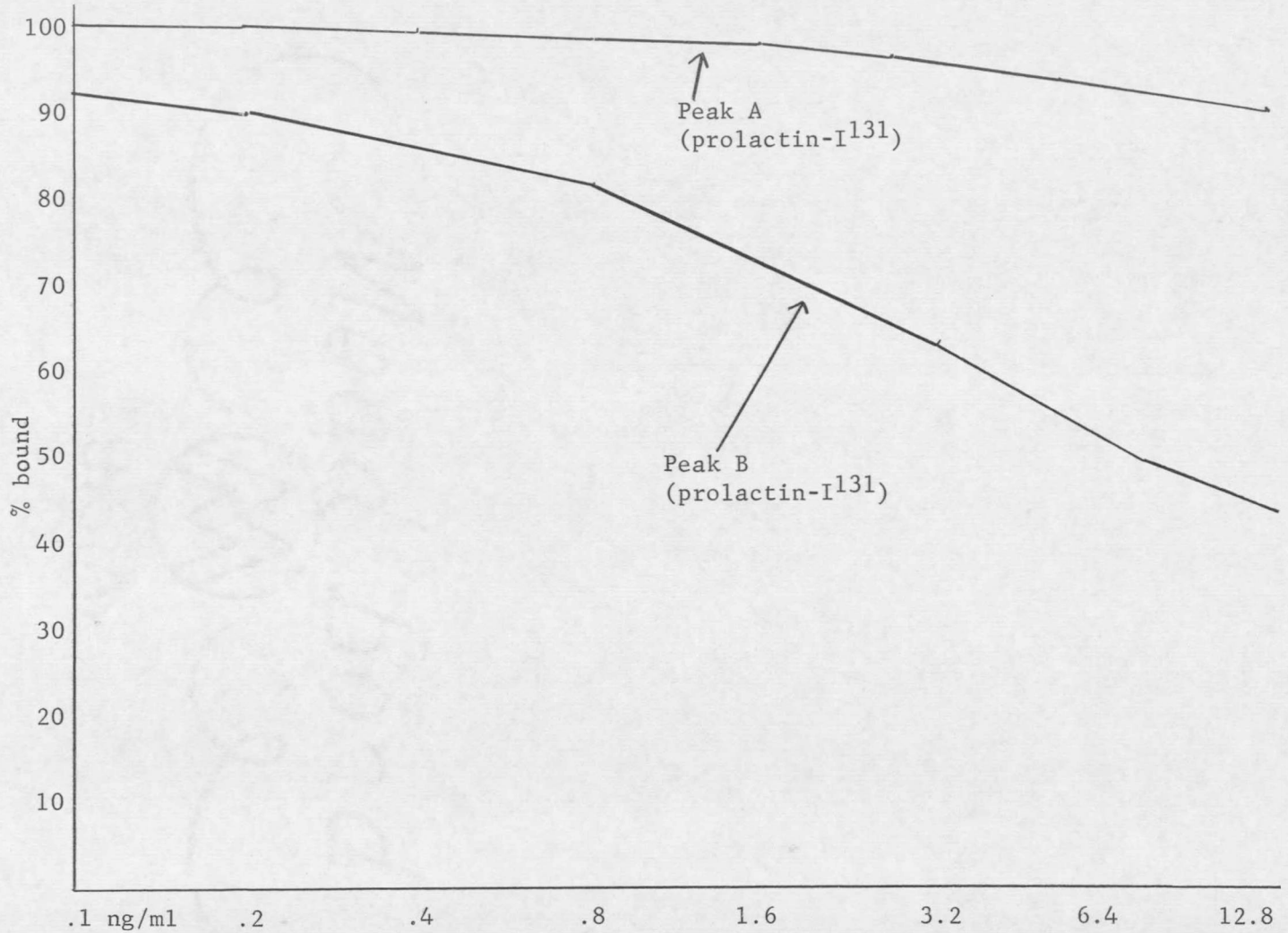


Figure 5. Comparison of prolactin standard curves from peaks A and B (Fig. 2) following single purification on Sephadex G-75 (NIH-P-B3).

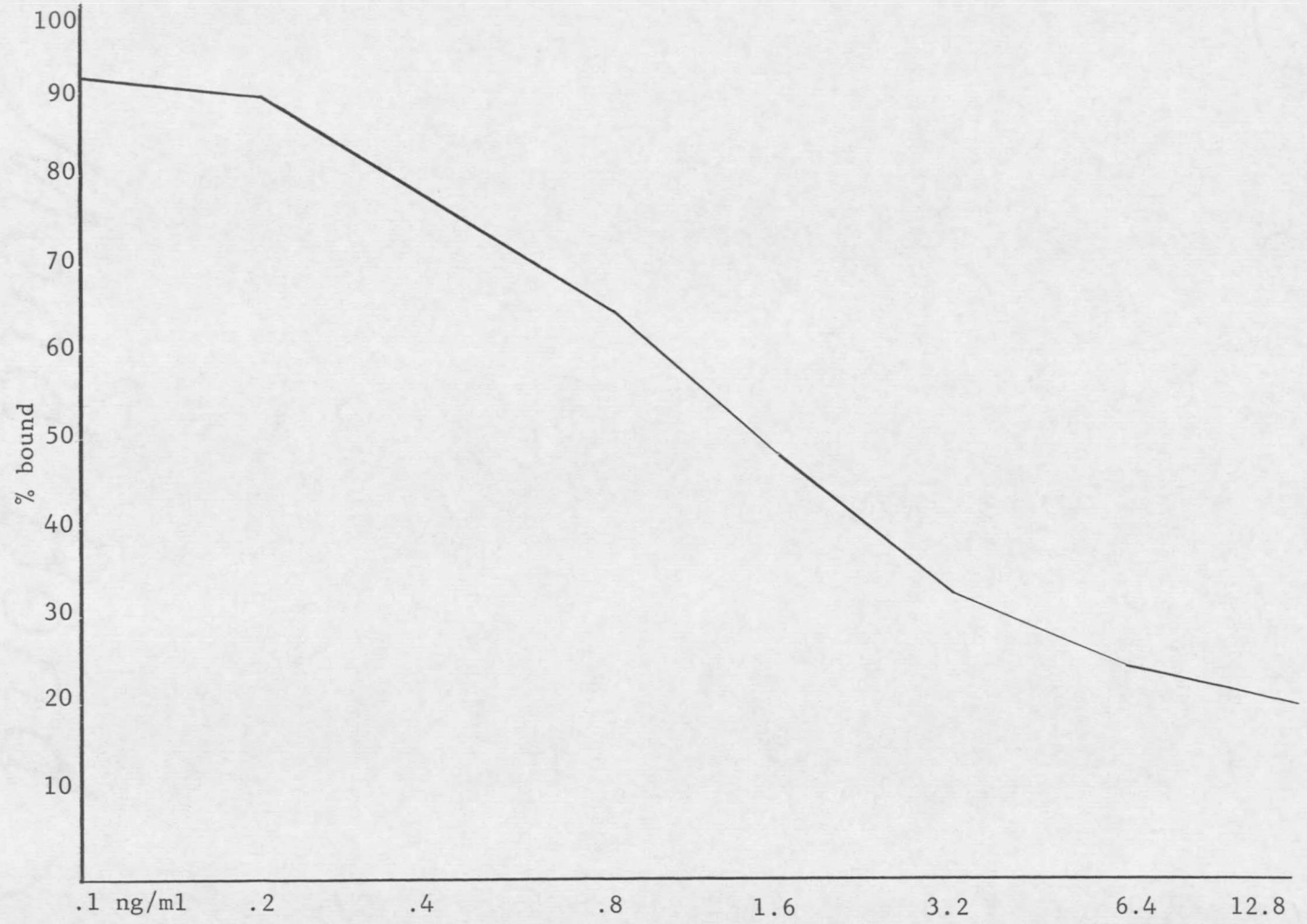


Figure 6. Prolactin standard curve following second purification (Fig. 3) on Sephadex G-75 column (NIH-P-B3).

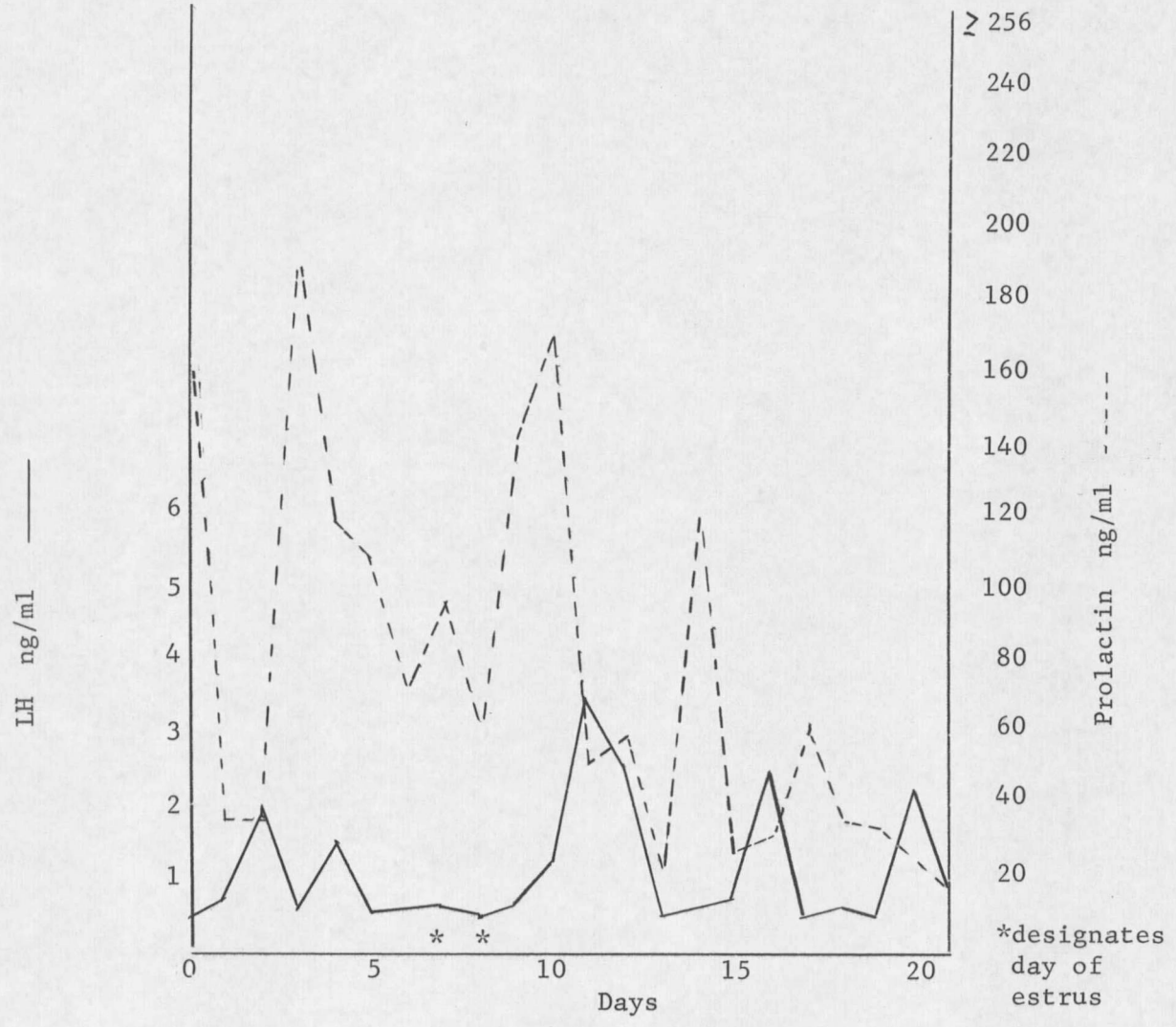


Figure 7. LH and prolactin levels for cow 603 from parturition until 21 days postpartum (non-suckled group).

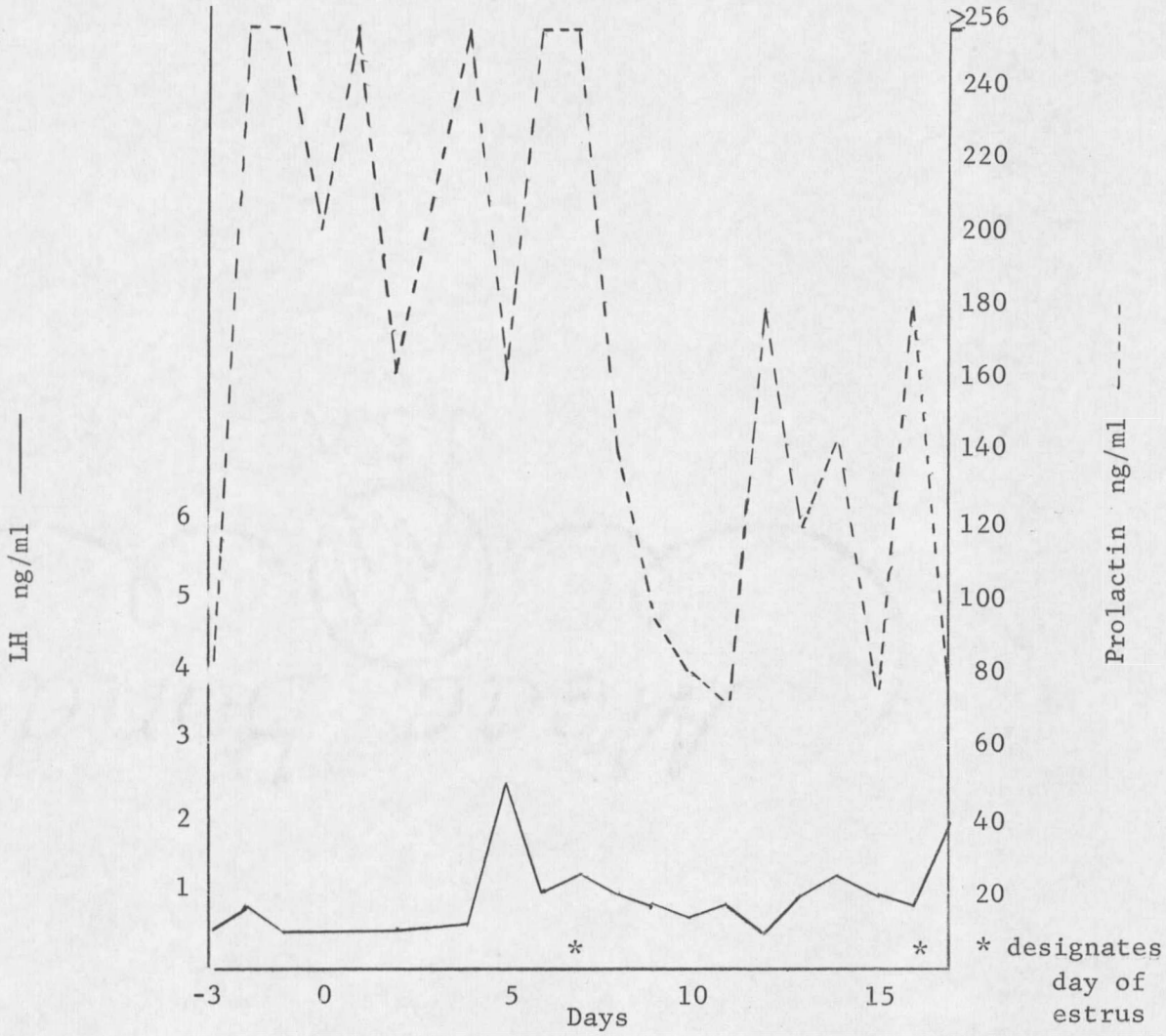


Figure 8. LH and prolactin levels for cow 621 from 3 days prepartum until 17 days postpartum (non-suckled group).

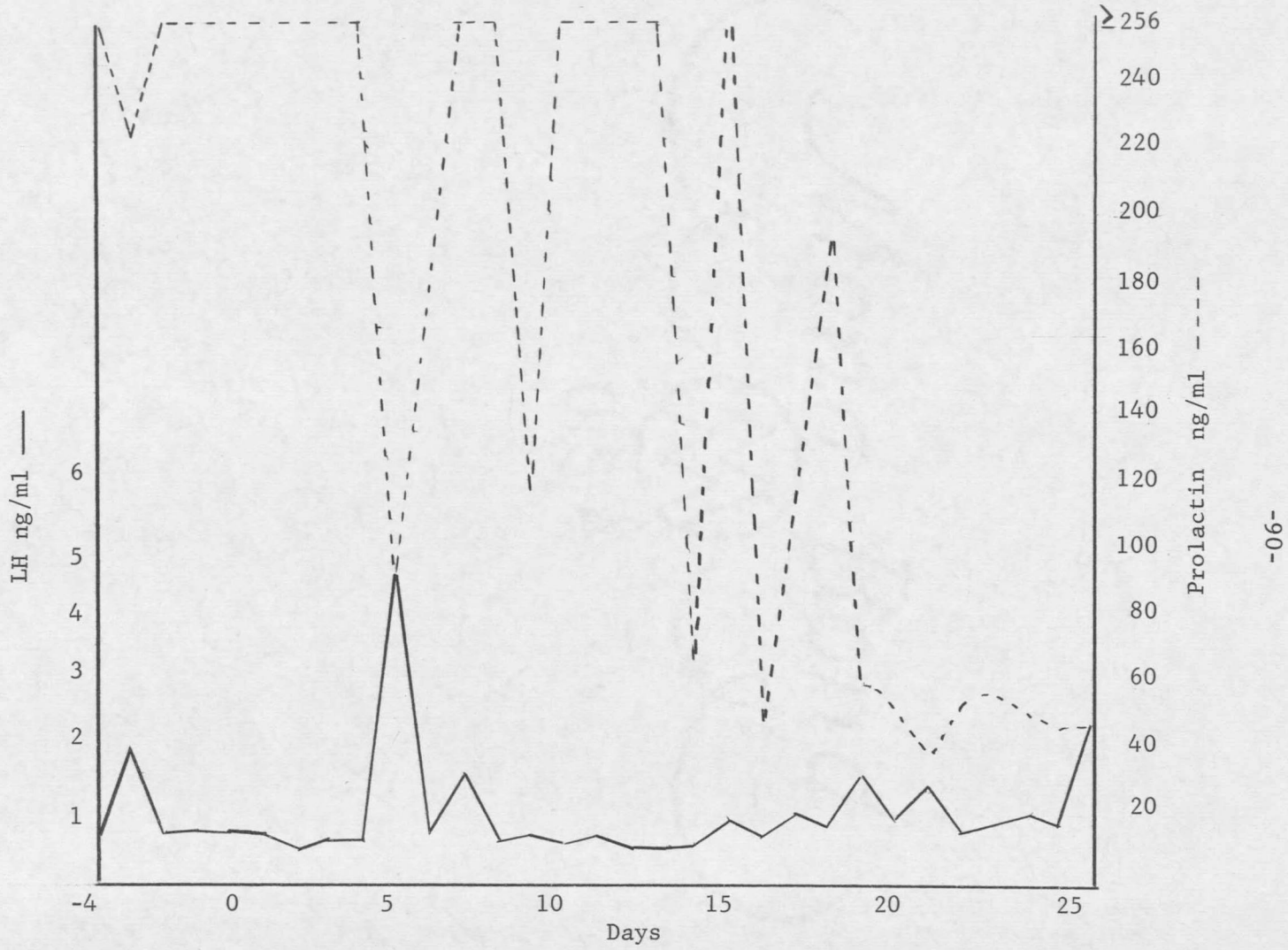


Figure 9. LH and prolactin levels for cow 613 from 4 days prepartum until 26 days postpartum (suckled group).

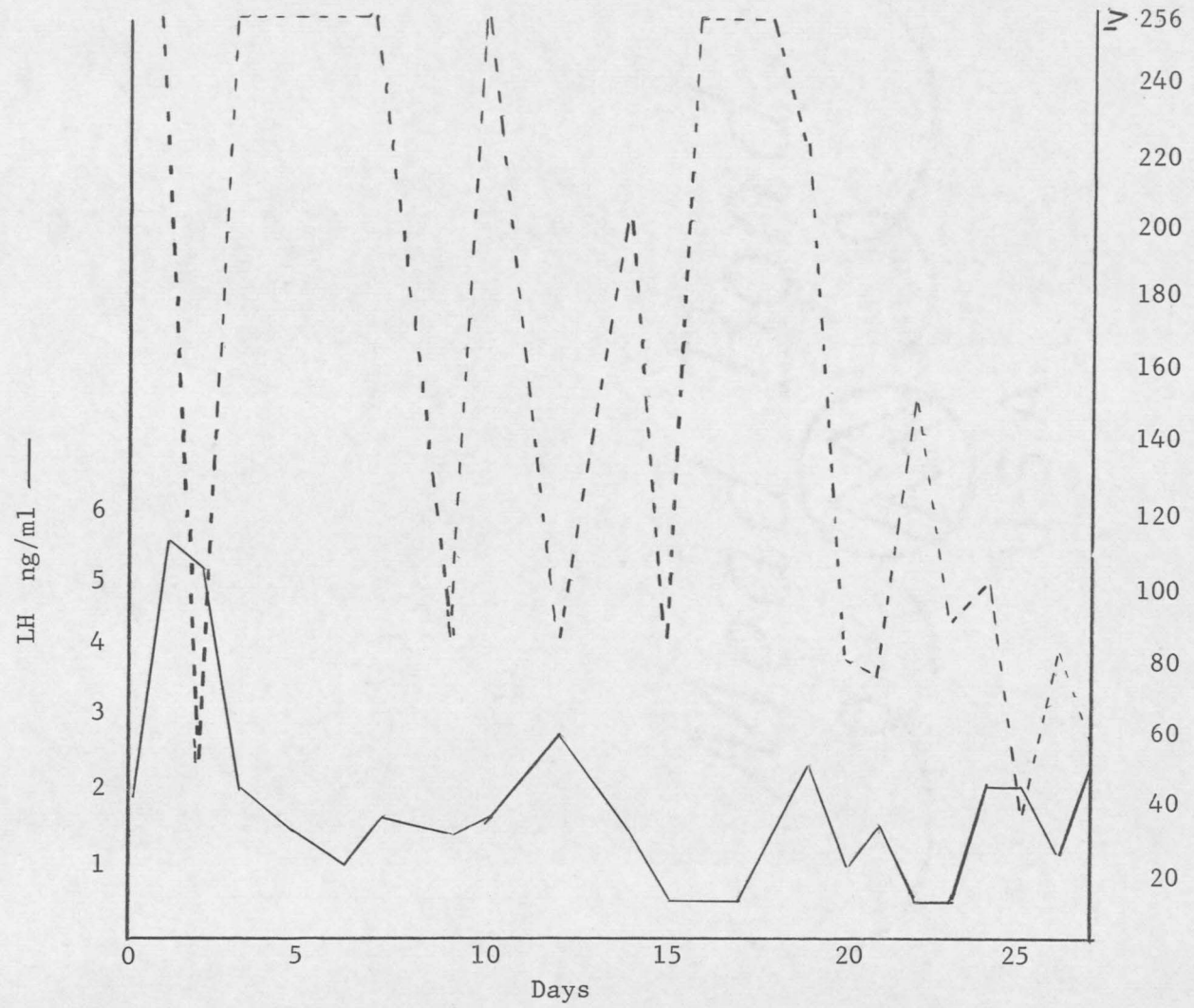


Figure 10. LH and prolactin levels for cow 673 from parturition until 27 days postpartum (suckled group).

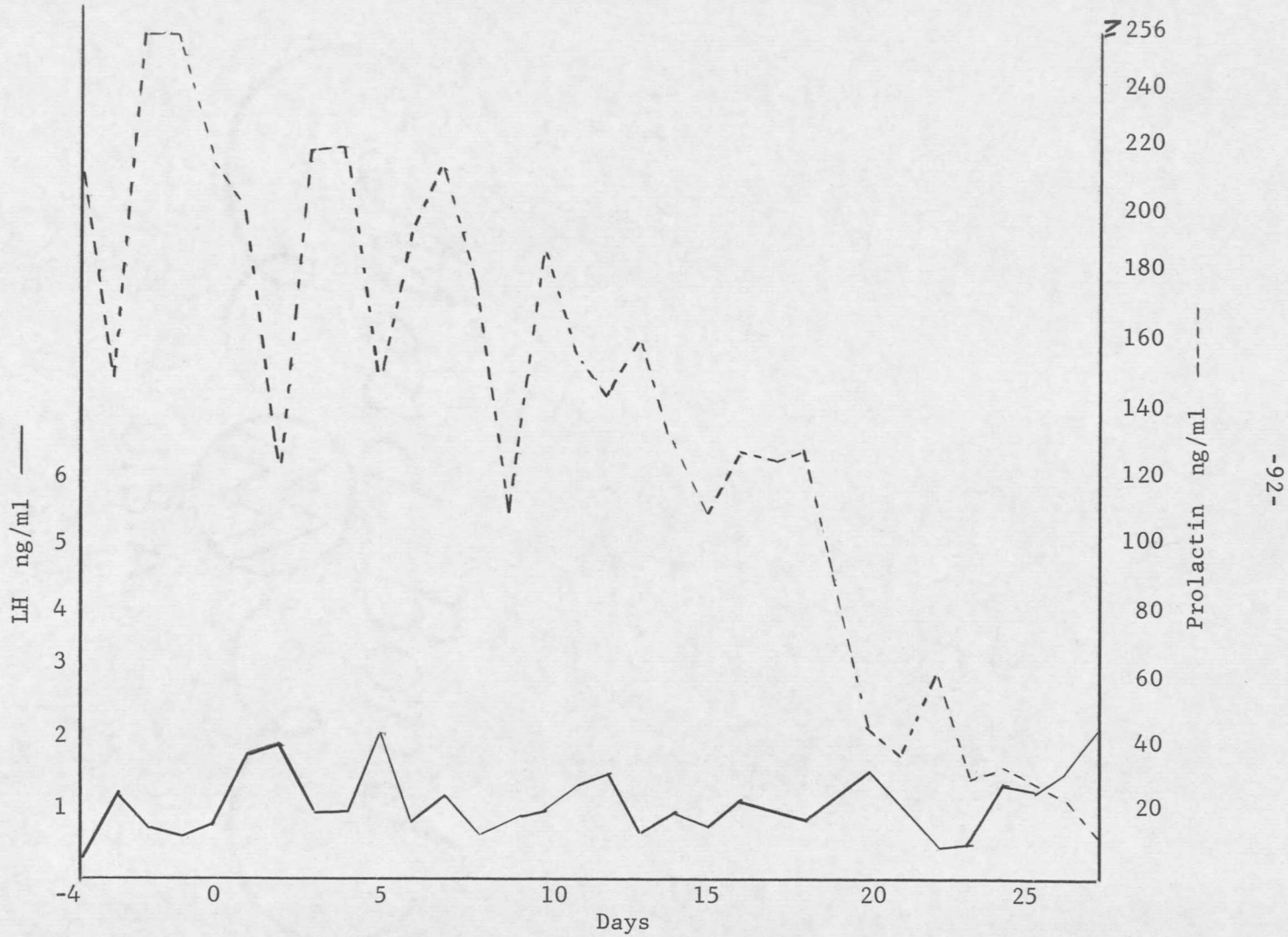


Figure 11. Least squares periparturient LH and prolactin means for combined suckled and non-suckled groups (Day 0 = parturition).

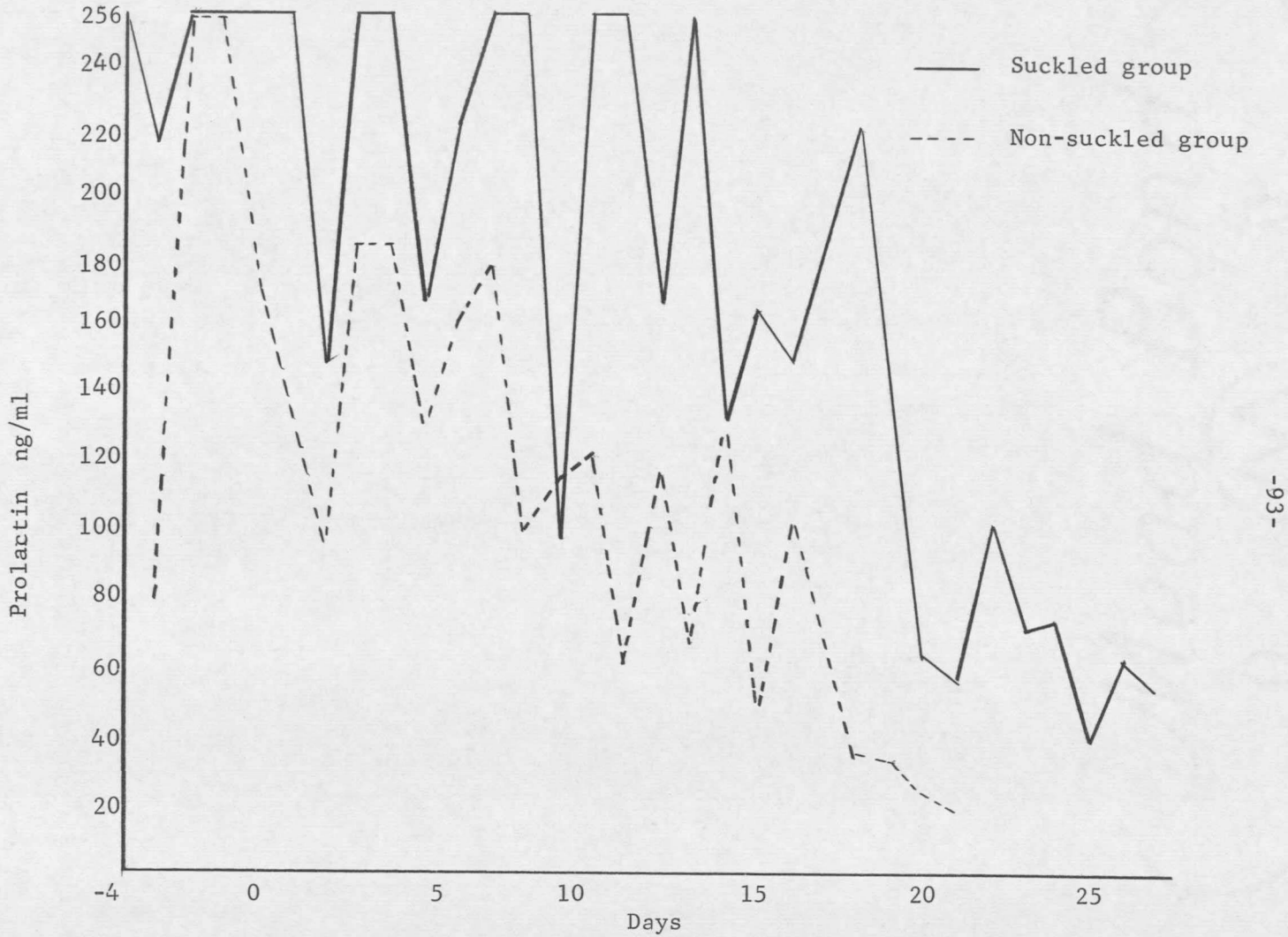


Figure 12. Least squares periparturient prolactin means for suckled and non-suckled groups (Day 0 = parturition).

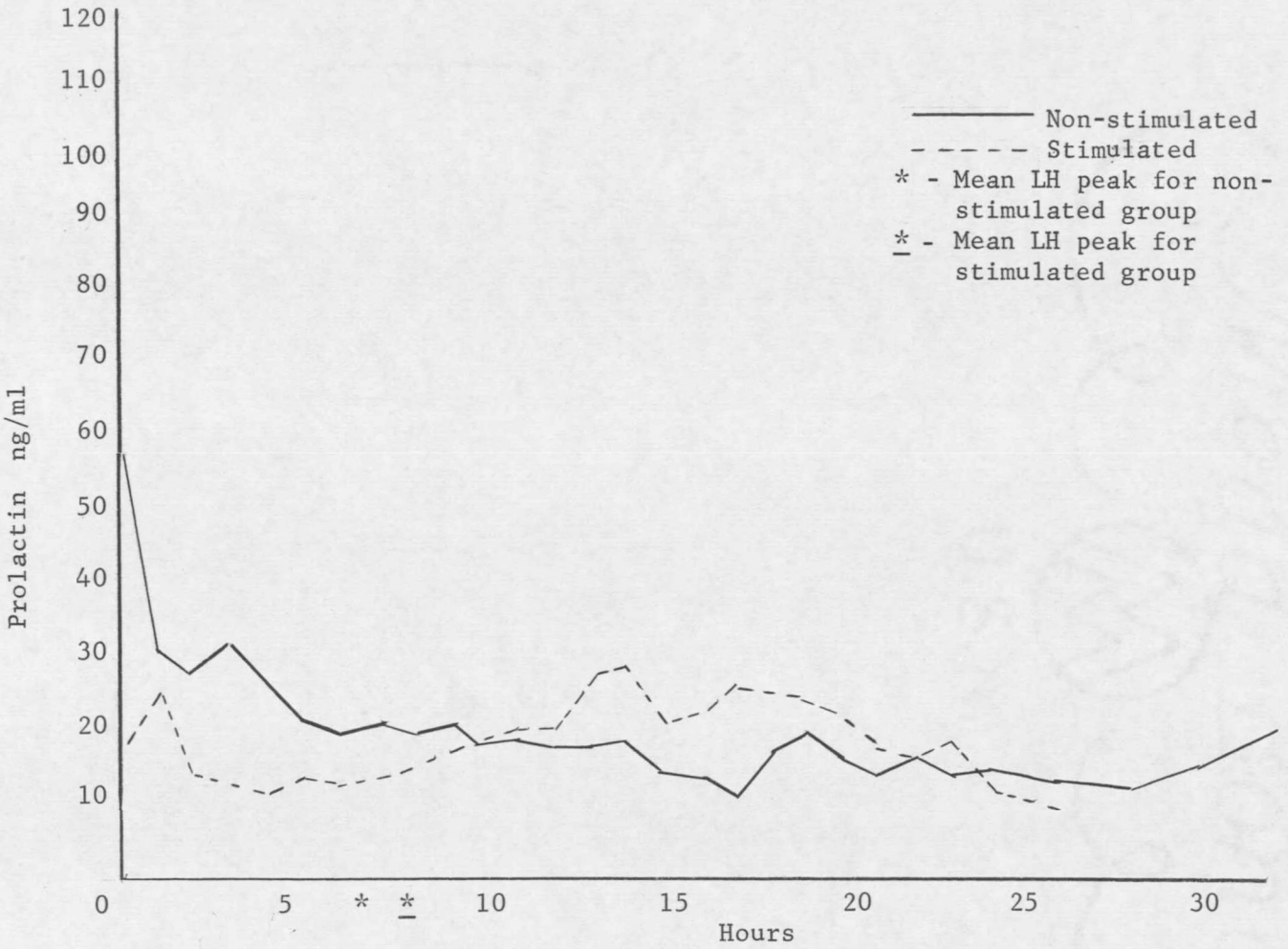


Figure 13. Least square prolactin means for non-stimulated and stimulated estrus synchronized cows from the onset of estrus to ovulation (Estrus = 0).

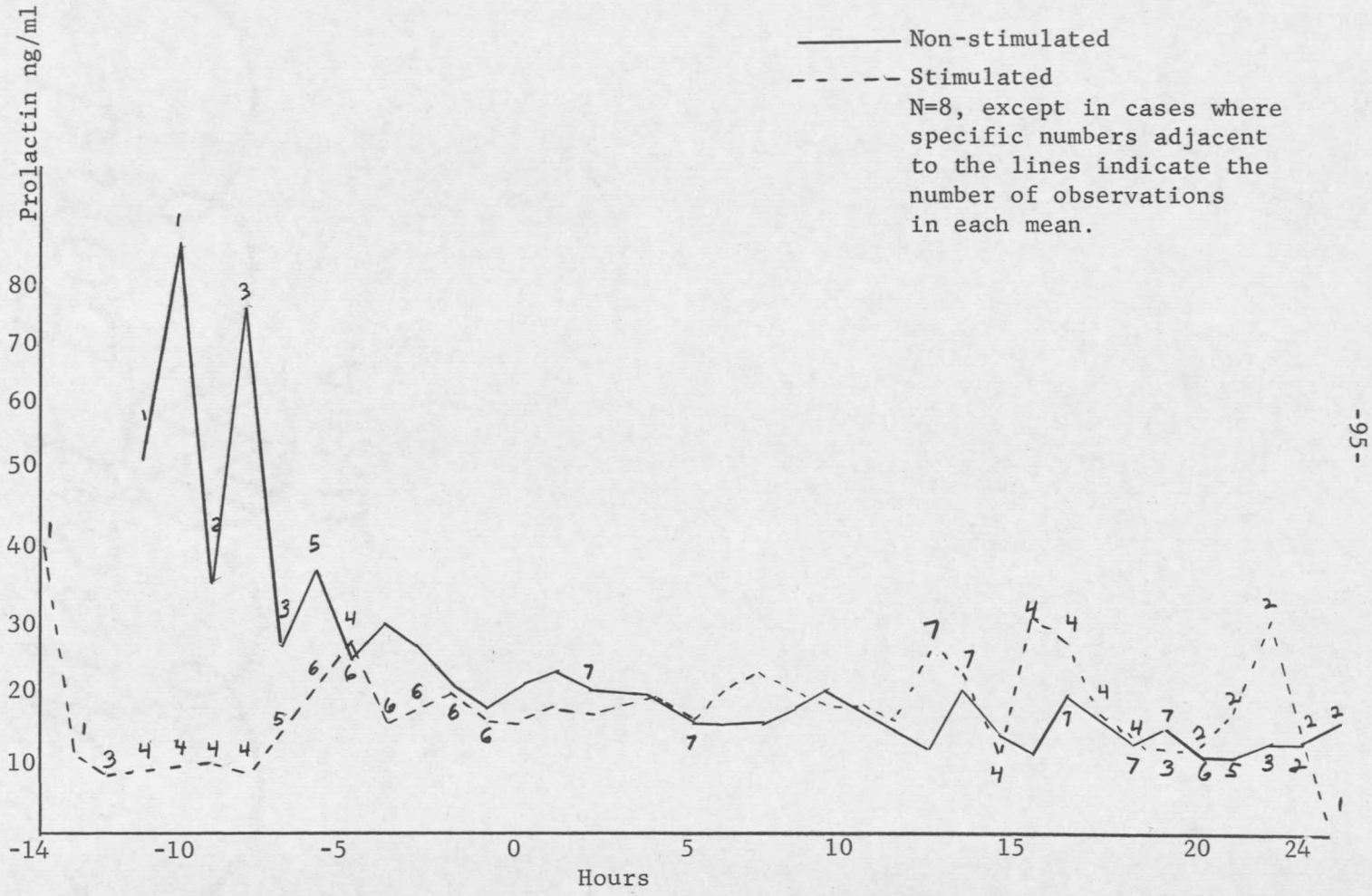


Figure 14. Least squares prolactin means for non-stimulated and stimulated groups before and after the LH peak (LH peak = 0).

SUMMARY

Specific bovine radioimmunoassays (RIA) were utilized for measuring blood levels of LH and prolactin in beef cattle. The lower level of sensitivity for both assays was in the range of .1 to .2 ng/ml. During the developmental stages of the prolactin assay, immunological problems were encountered with the prolactin-I^{131} preparation. These problems resulted from radioiodination damage to the prolactin-I^{131} molecules. The damaged prolactin-I^{131} molecules were characterized by the formation of molecular aggregates which exibited abnormal binding affinity with the prolactin antibody and were not displaced from the antibody in a direct relationship with increasing concentrations of unlabled prolactin. By using milder reaction conditions for preparation of the prolactin-I^{131} and two purification steps on Sephadex G-75 columns, the prolactin-I^{131} aggregates could be minimized. Following the second purification, the remaining prolactin-I^{131} was suitable for use in the assay system and produced very desirable standard curves. Prolactin-I^{131} was prepared by reacting 2.5 ug prolactin with 1 mCi I^{131}. The reaction was initiated with 20 ug CH-T and stopped after 20 sec. by the addition of 60 ug sodium metabisulfite.

Plasma LH and prolactin were measured in four postpartum Hereford cows. Blood samples were collected daily through indwelling jugular cannulas. All cows were randomly divided into a non-suckled and a suckled group. Calves from the non-suckled group were removed from their dams on the day of parturition. Data were analyzed by the

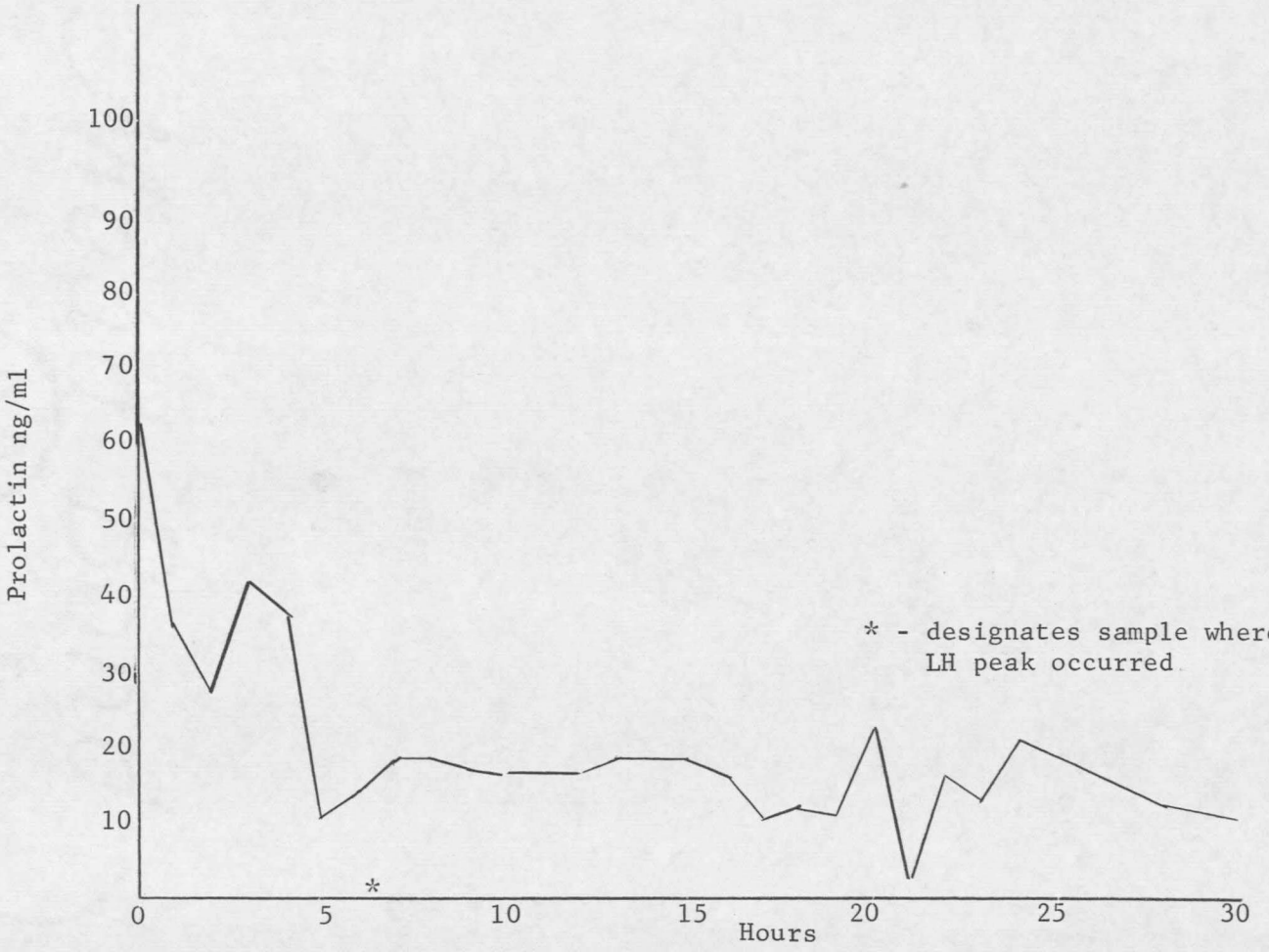
method of least-squares. The least squares prolactin means were significantly different ($P<.01$) between non-suckled (94.9 ± 13.6 ng/ml) and suckled (176.3 ± 10.7 ng/ml) groups. A signficiant negative linear regression ($b = -6.795 \pm 0.985$) of prolactin on the day postpartum was observed in a combined group analysis. No significant differences in LH were detected between groups. The within subclass correlation coefficient ($r = -.29$) between LH and prolactin was significant ($P<.06$) for the combined group analysis.

In a second study, serum prolactin was measured in 16 mature cycling beef cows. Blood samples were collected through indwelling jugular cannulas at hourly intervals for 24 hrs from the onset of estrus and at 2 hr intervals thereafter until ovulation. Cannulas were inserted at the onset of estrus. Estrus was synchronized by feeding 180 mg MAP/head/day for 11 days and injecting 5 mg estradiol benzoate on day 2 of MAP feeding.

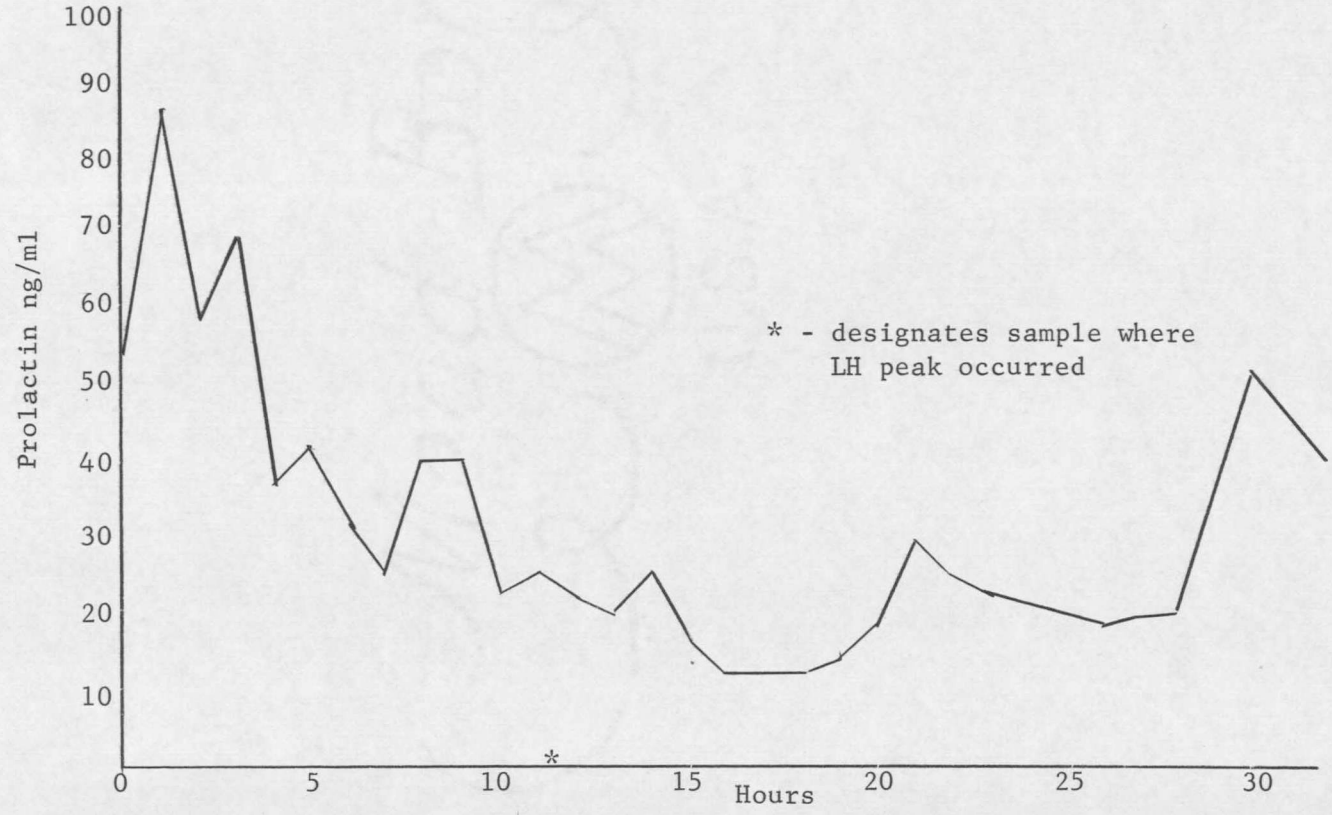
All cows were divided into two treatment groups and subjected to different mating stimuli. The control group (NS) received no clitoral stimulation while the treated group (S) received either manual clitoral stimulation or natural mating to surgically sterilized bulls. Data were analyzed by the method of least-squares. In the first analysis all cows were grouped with the onset of estrus as a distinct physiological event. This analysis revealed a significant ($P<.01$) group by sample time interaction. In the second analysis, all cows were

aligned with their LH peaks representing a distinct physiological event. This revealed a significant difference ($P<.01$) in group, time interval (before or after LH peak) and group by time interval interaction. The least squares prolactin means for the NS and S groups before the LH peak were 30.0 ± 2.0 ng/ml and 16.4 ± 1.9 ng/ml, respectively, while the least square prolactin means for the NS and S groups after the LH peak were 16.8 ± 1.2 ng/ml and 19.8 ± 1.4 ng/ml, respectively. The third analysis was designed to look specifically at all samples before the LH peak. This analysis revealed a significant difference ($P<.01$) in group and group by sample time interaction. The least squares prolactin means for the NS and S groups before the LH peak were 39.7 ± 3.6 ng/ml and 16.7 ± 2.6 ng/ml, respectively.

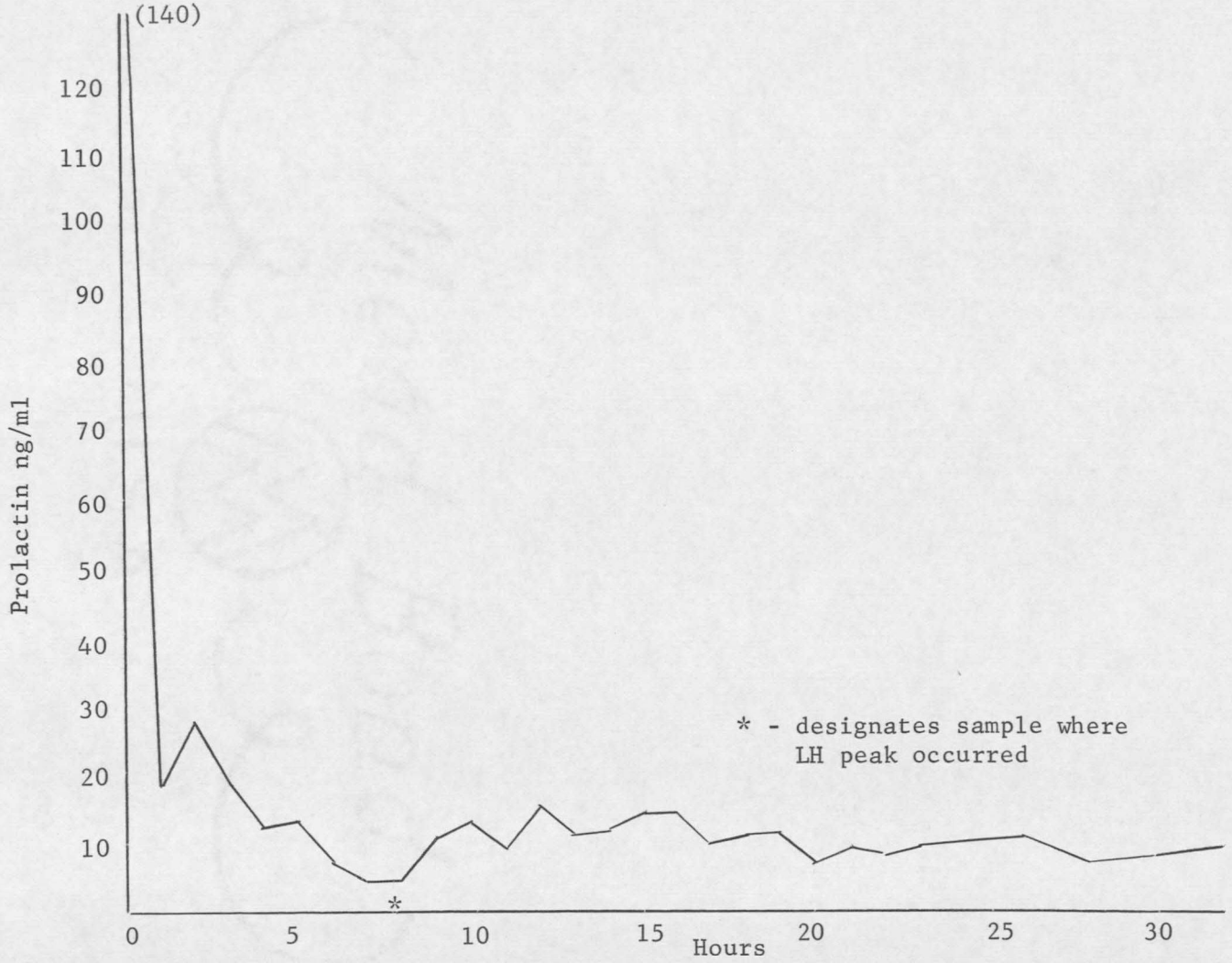
APPENDIX



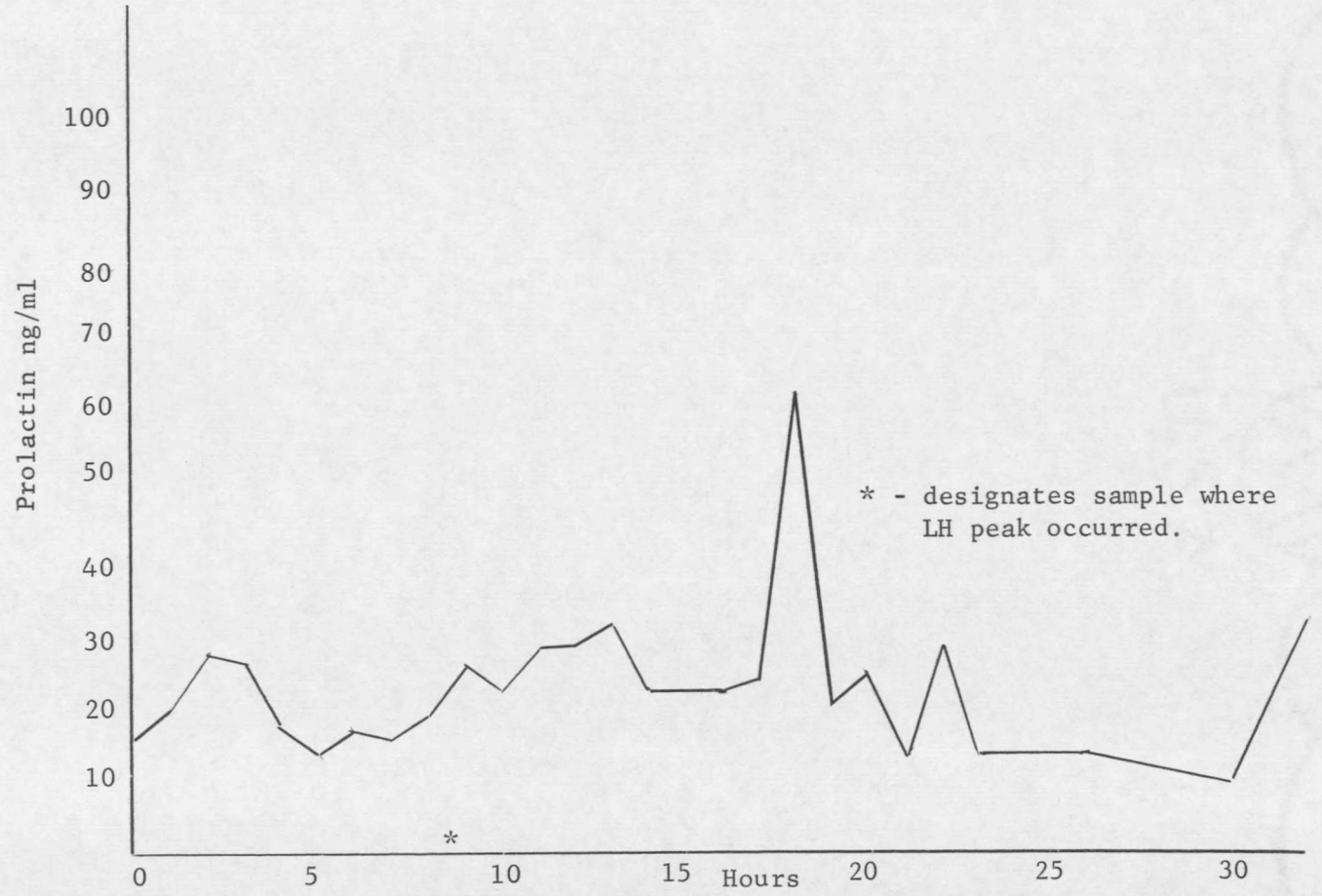
Appendix Figure I. Prolactin levels for cow 7939 from estrus until ovulation (Group I).



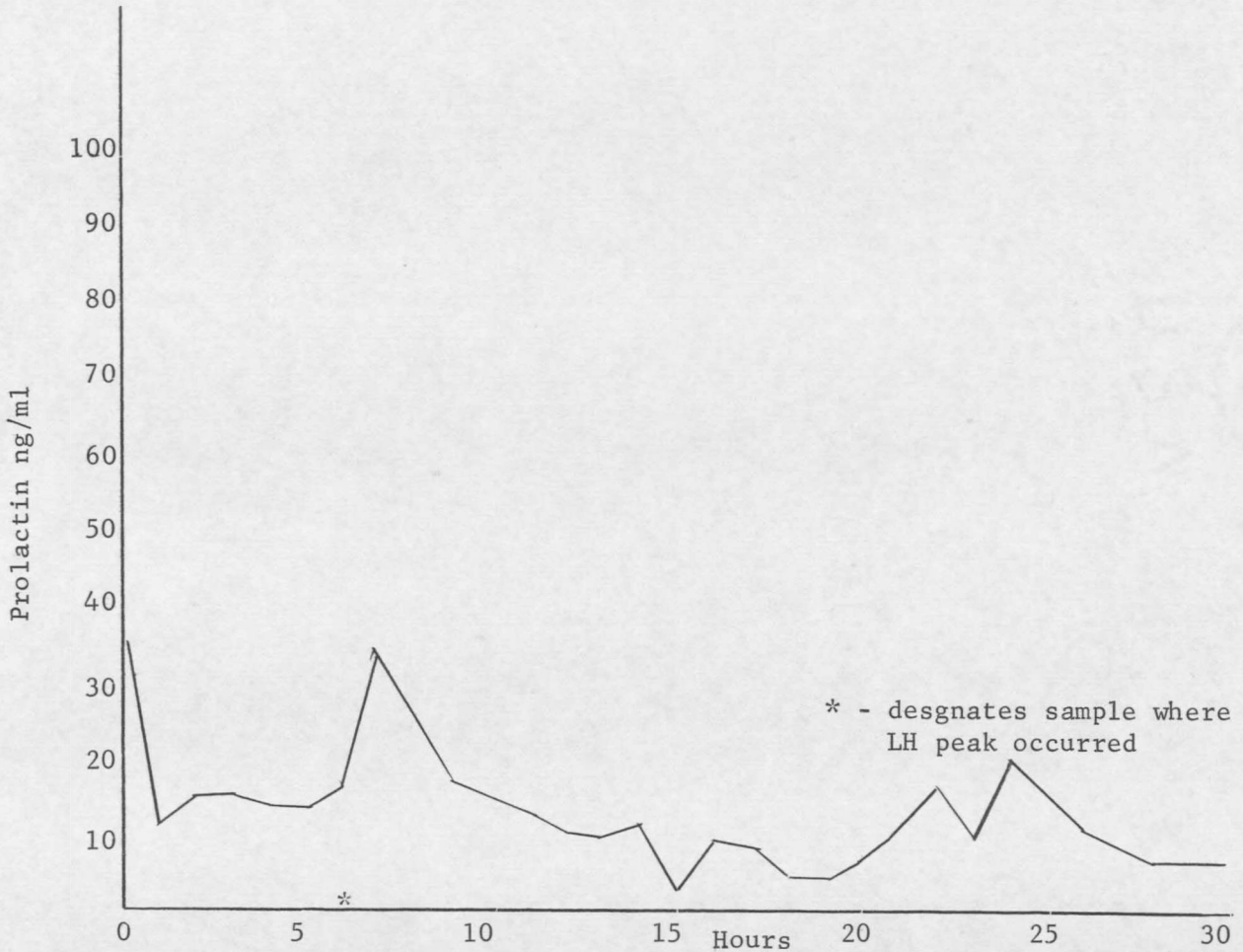
Appendix Figure II. Prolactin levels for cow 9704 from estrus until ovulation (Group I).



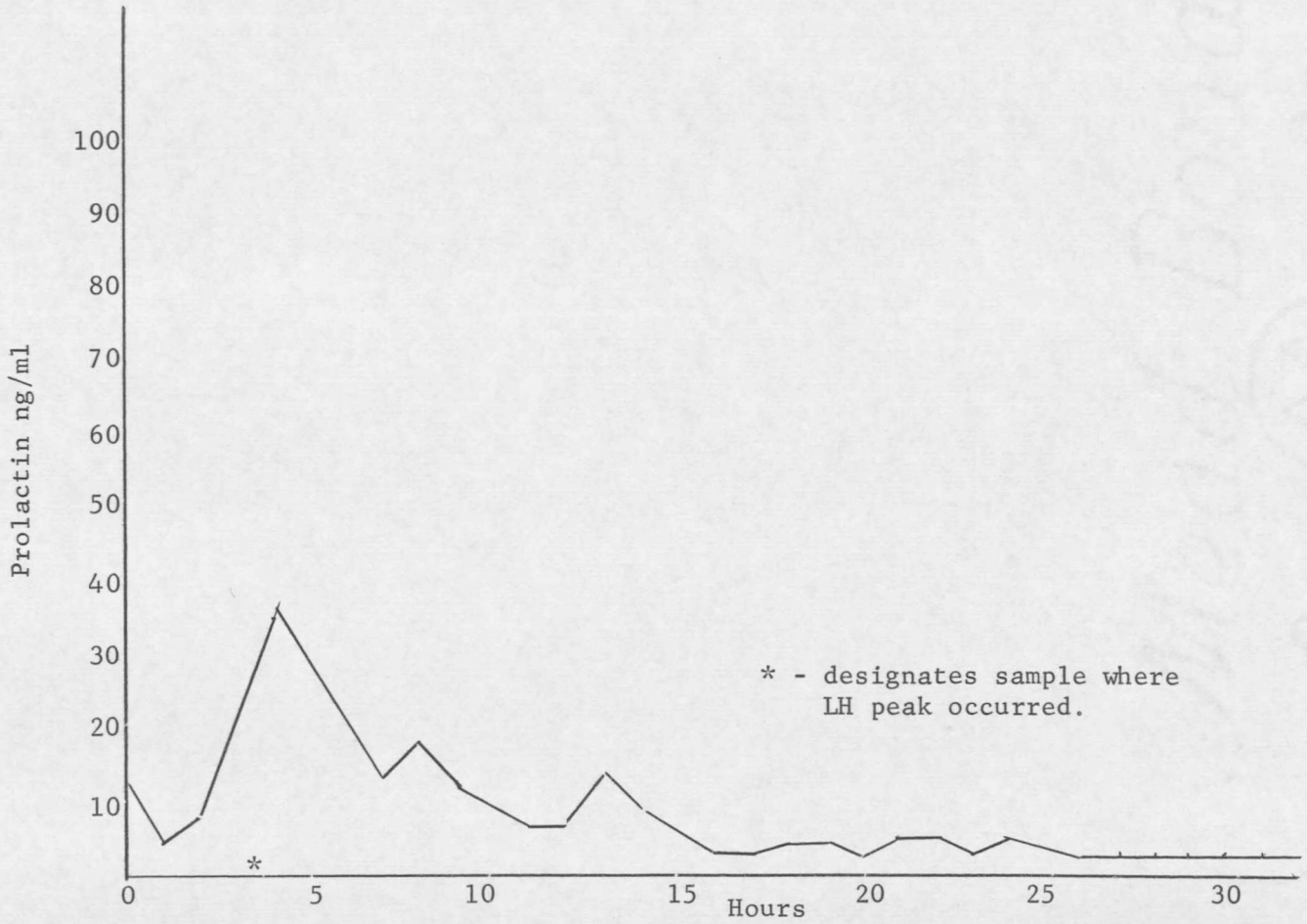
Appendix Figure III. Prolactin levels for cow 9107 from estrus until ovulation (Group I).



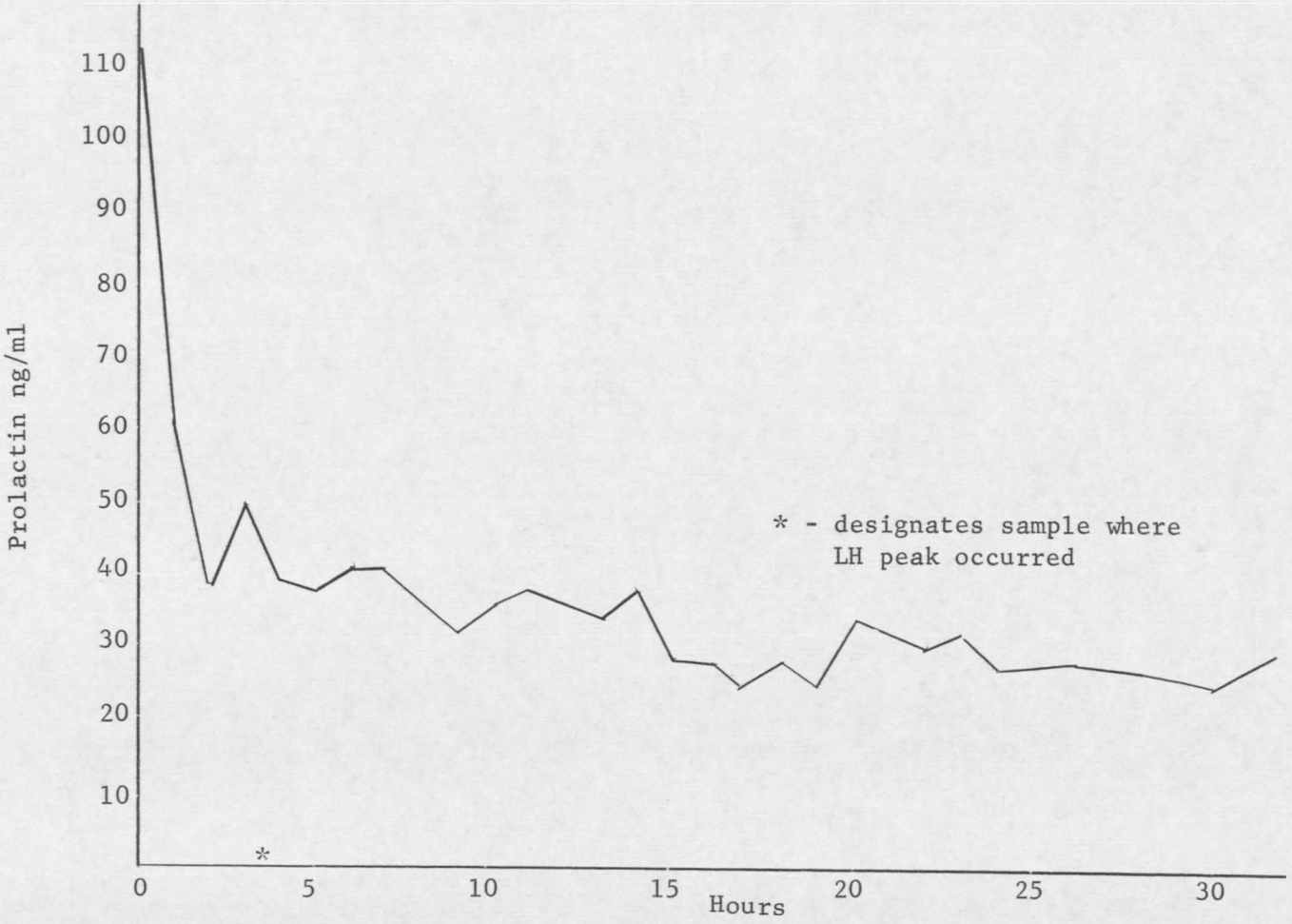
Appendix Figure IV. Prolactin levels for cow 9041 from estrus until ovulation (Group I).



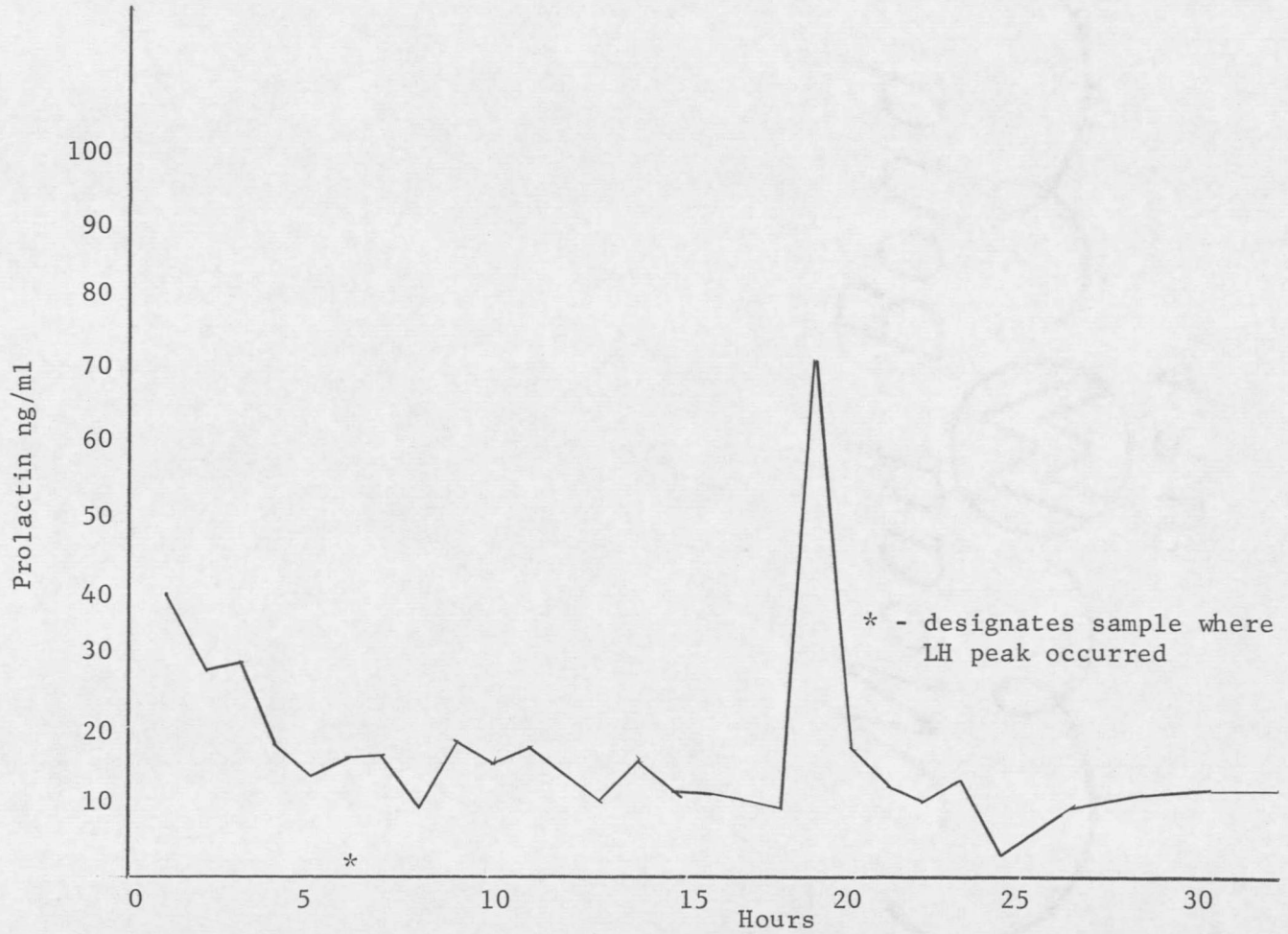
Appendix Figure V. Prolactin levels for cow 9771 from estrus until ovulation (Group II).



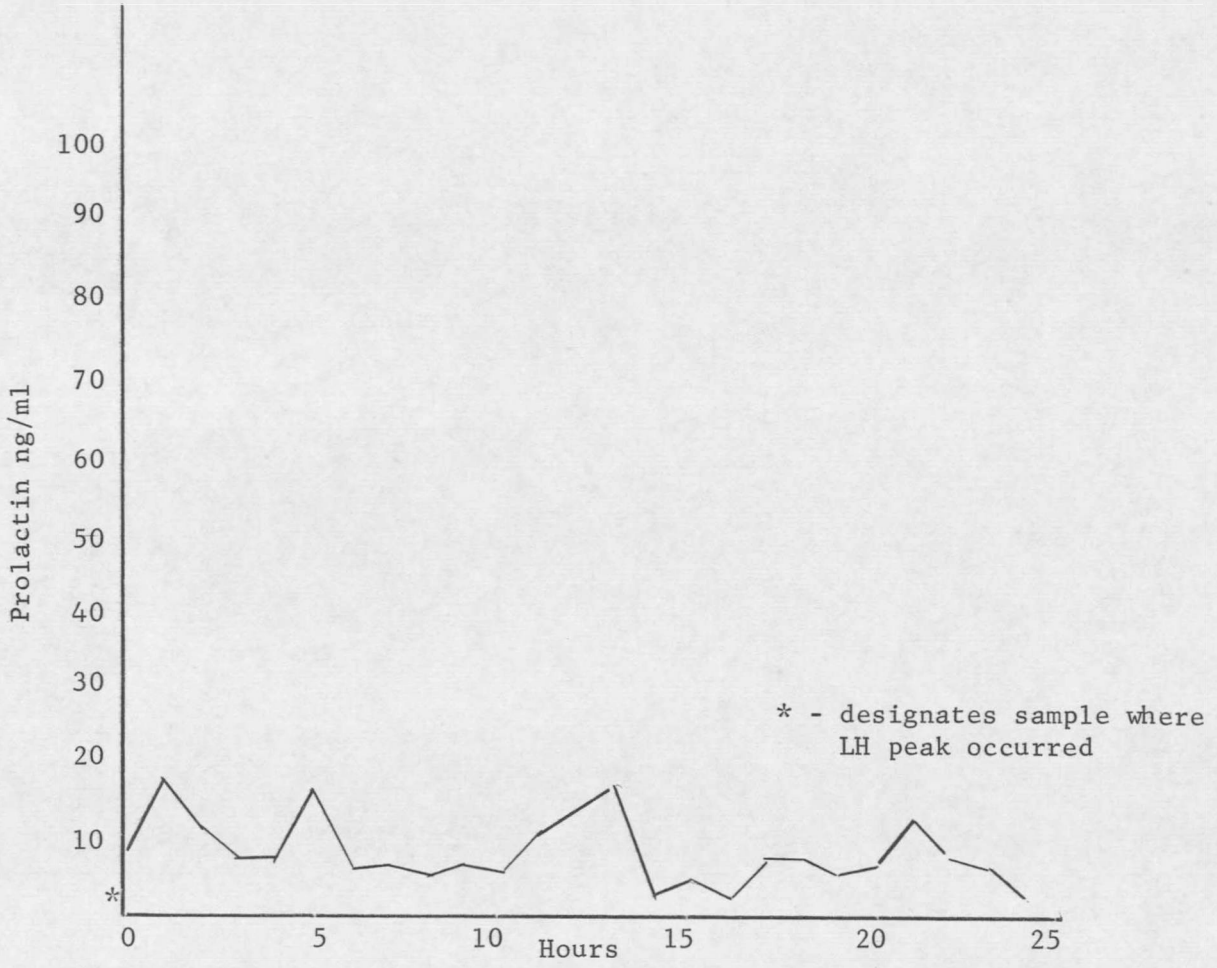
Appendix Figure VI. Prolactin levels for cow 9379 from estrus until ovulation (Group II).



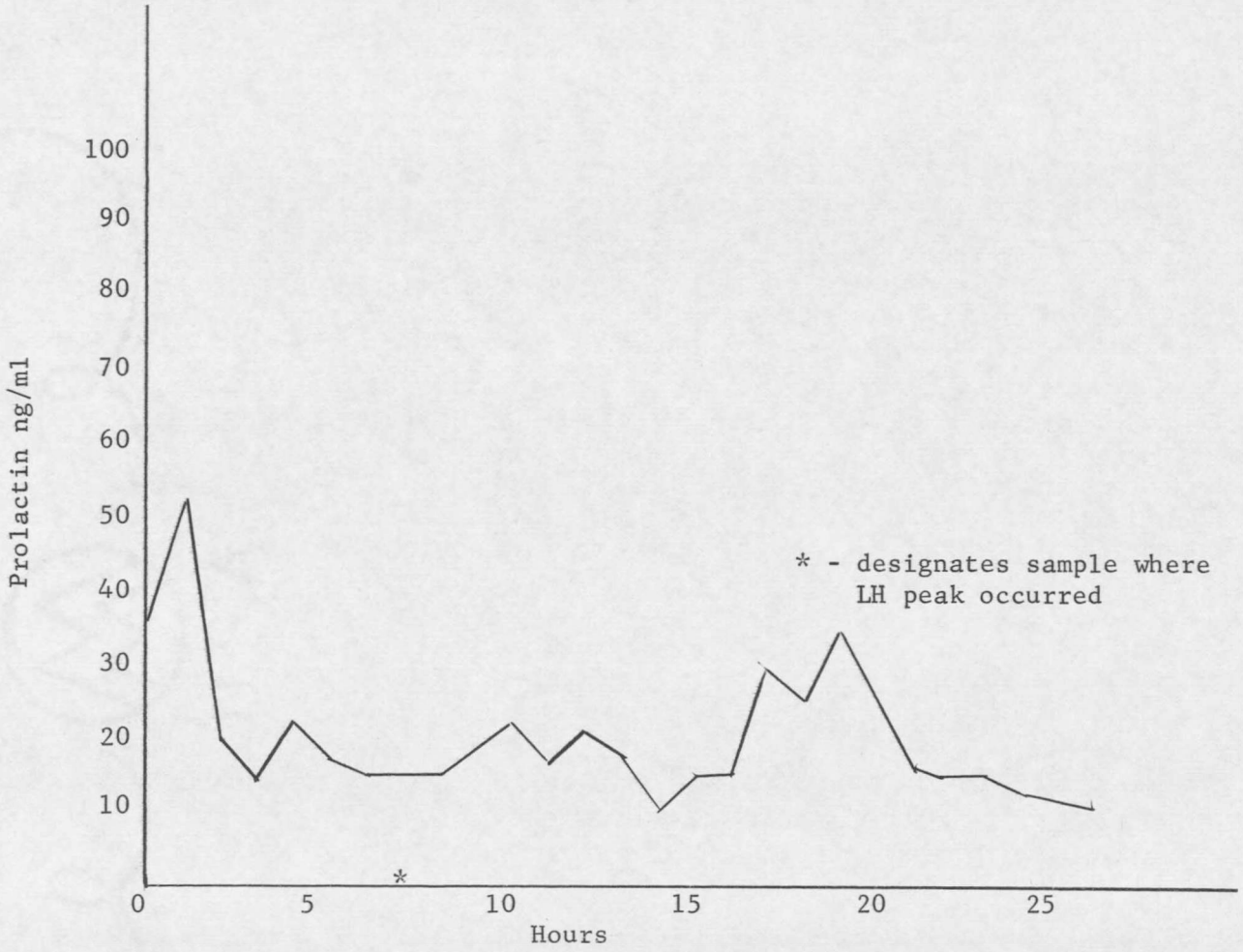
Appendix Figure VII. Prolactin levels for cow 8343 from estrus until ovulation (Group II).



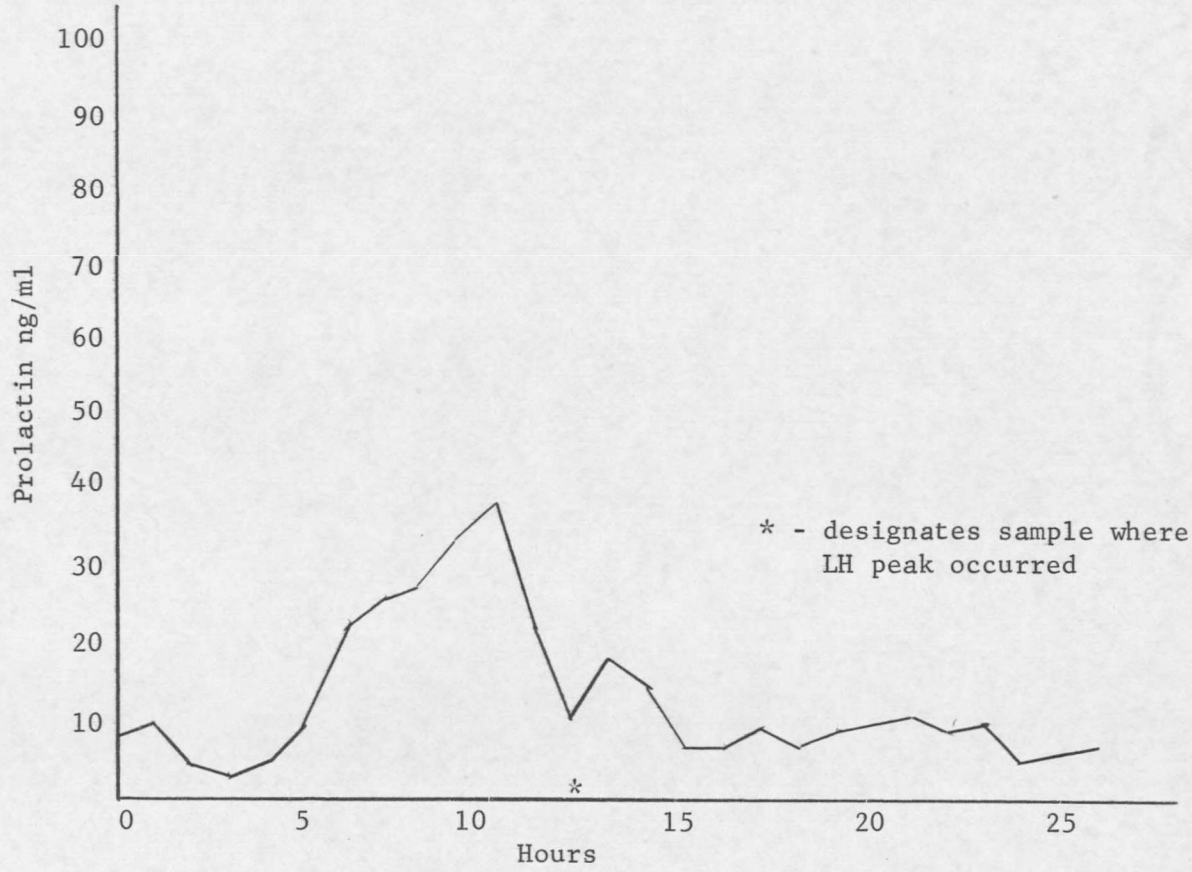
Appendix Figure VIII. Prolactin levels for cow 8056 from estrus until ovulation (Group II).



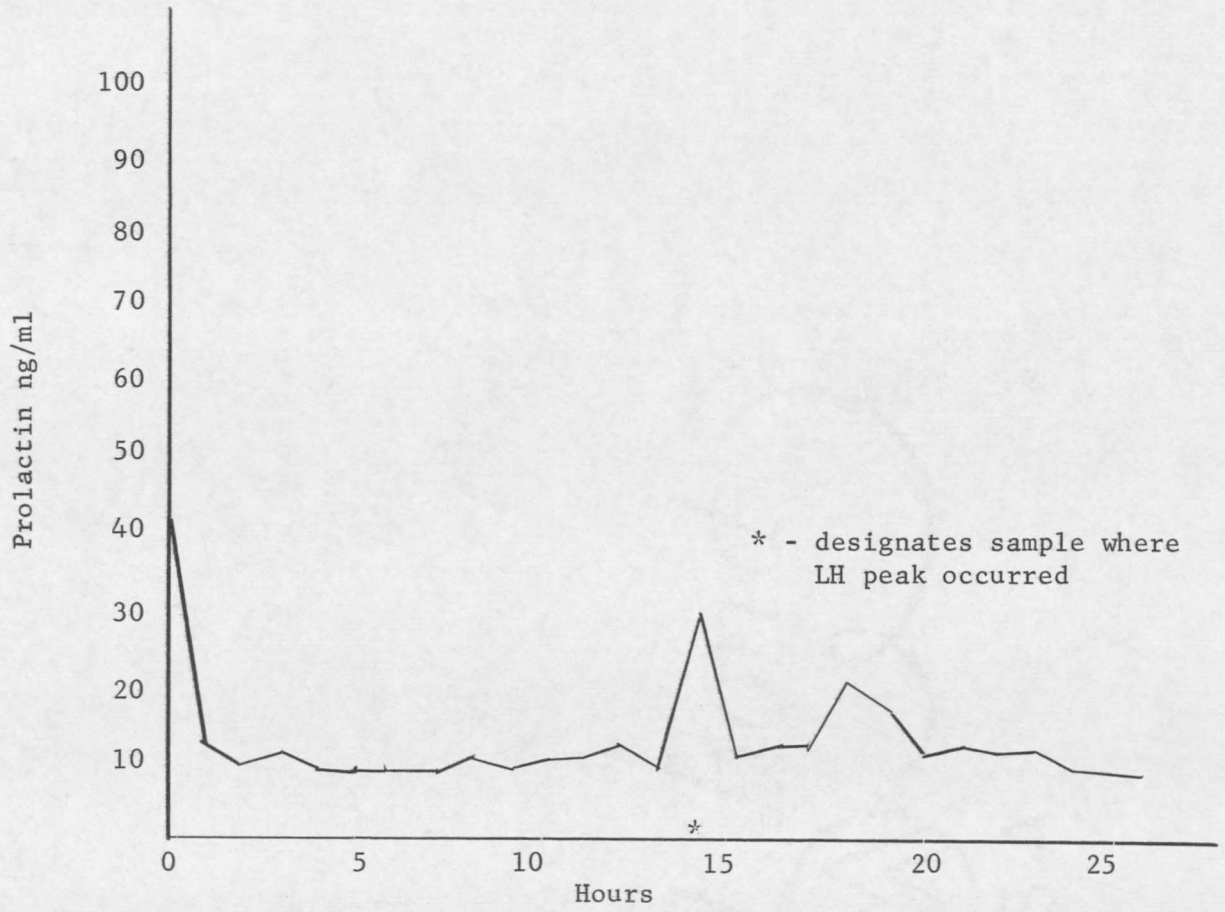
Appendix Figure IX. Prolactin levels for cow 9117 from estrus until ovulation (Group III).



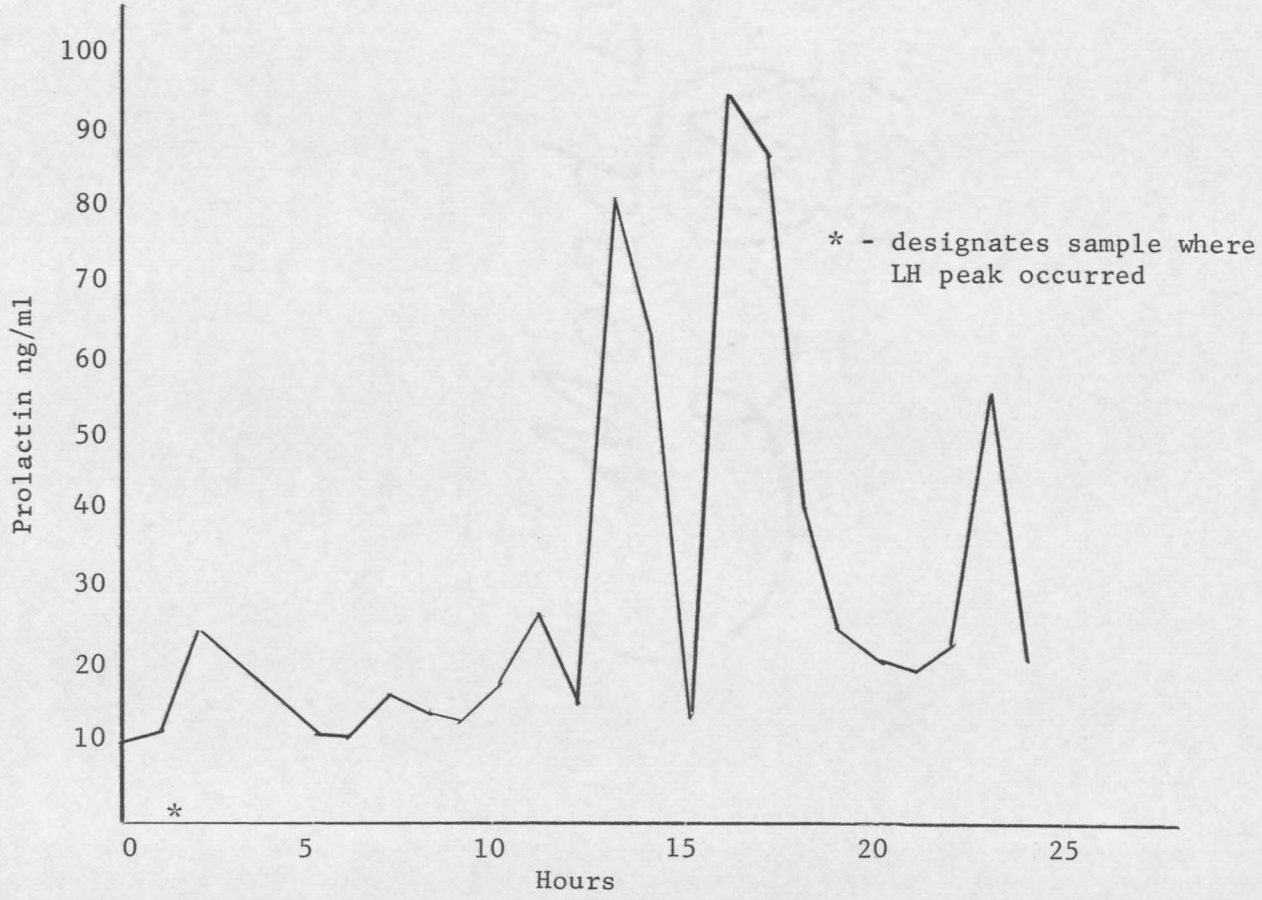
Appendix Figure X. Prolactin levels for cow 9834 from estrus until ovulation (Group III).



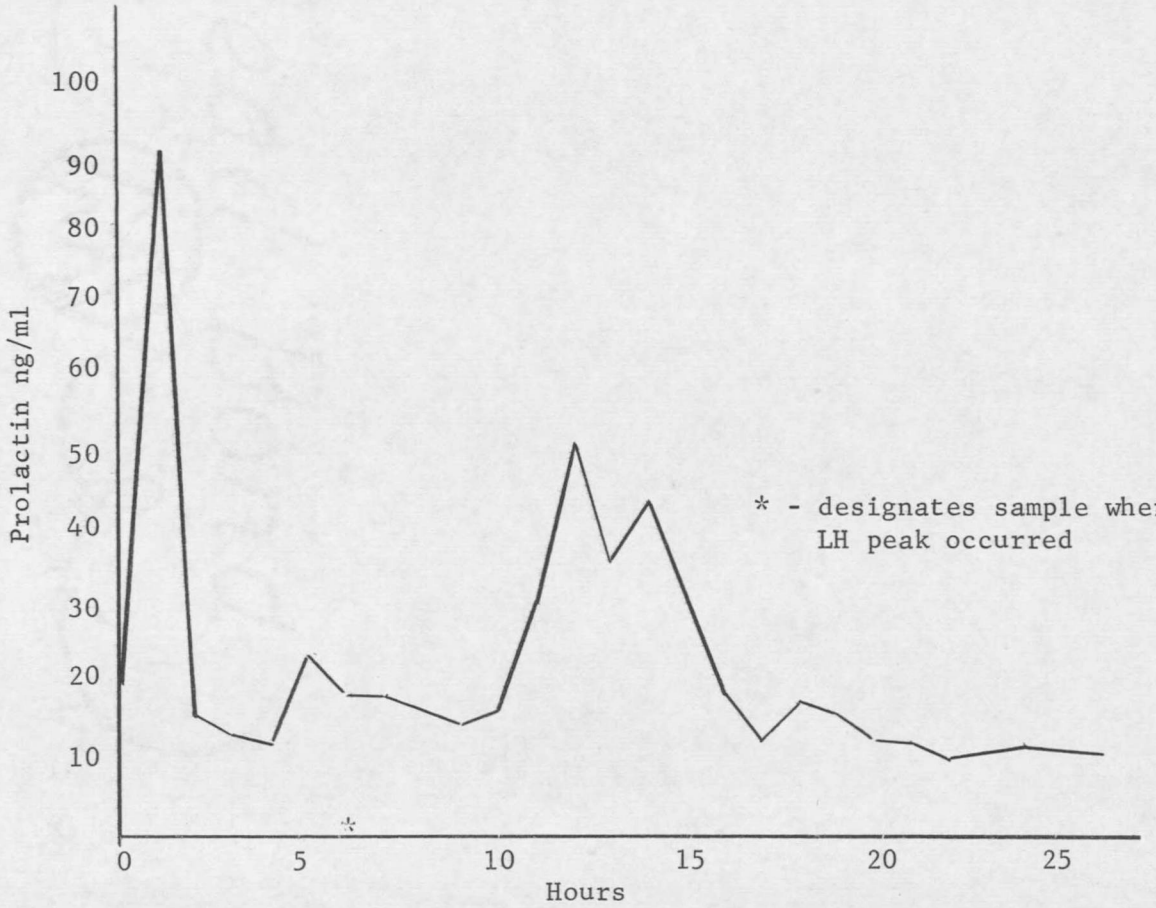
Appendix Figure XI. Prolactin levels for cow 9656 from estrus until ovulation (Group III).



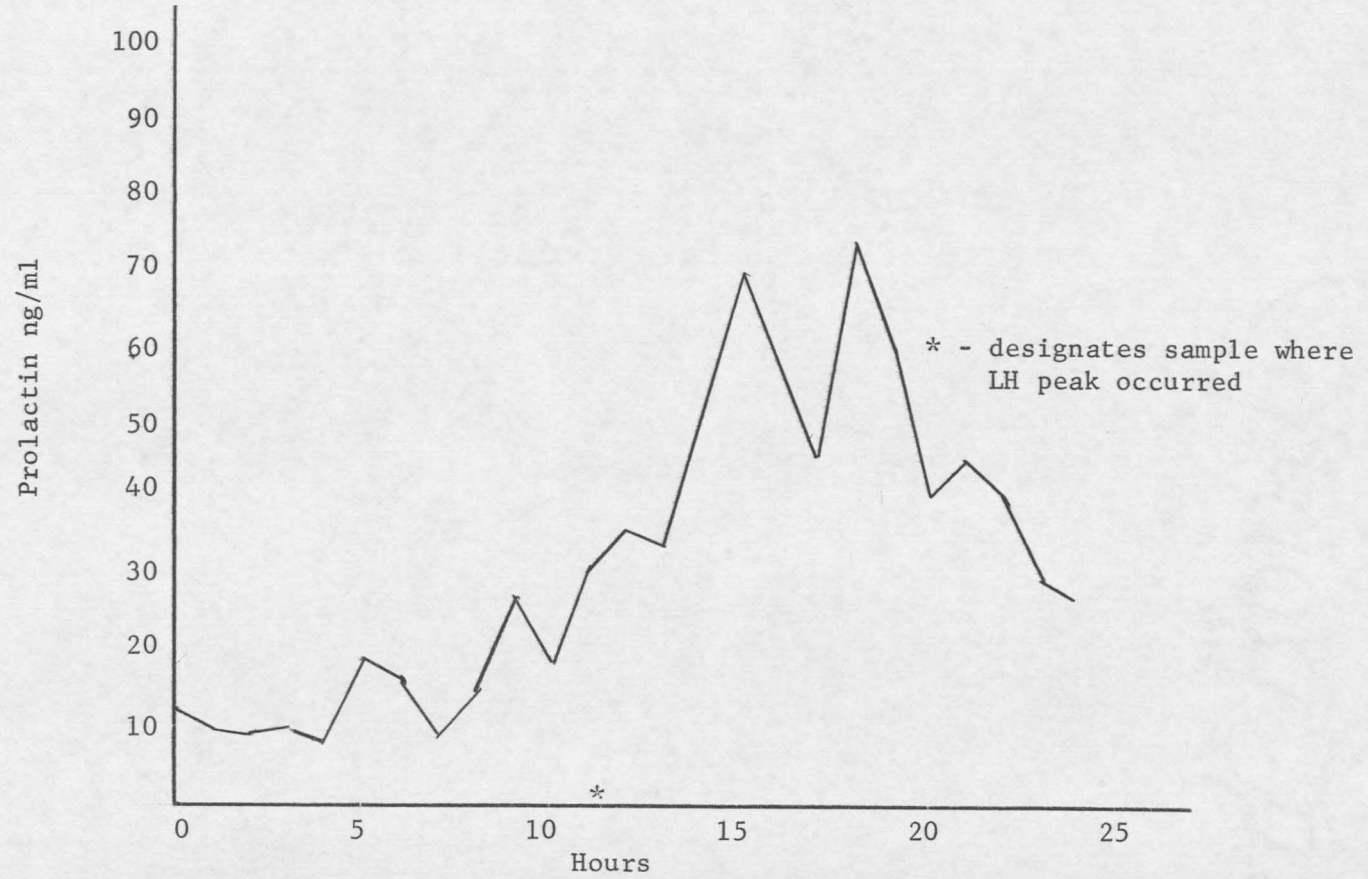
Appendix Figure XII. Prolactin levels for cow 9871 from estrus until ovulation (Group IV).



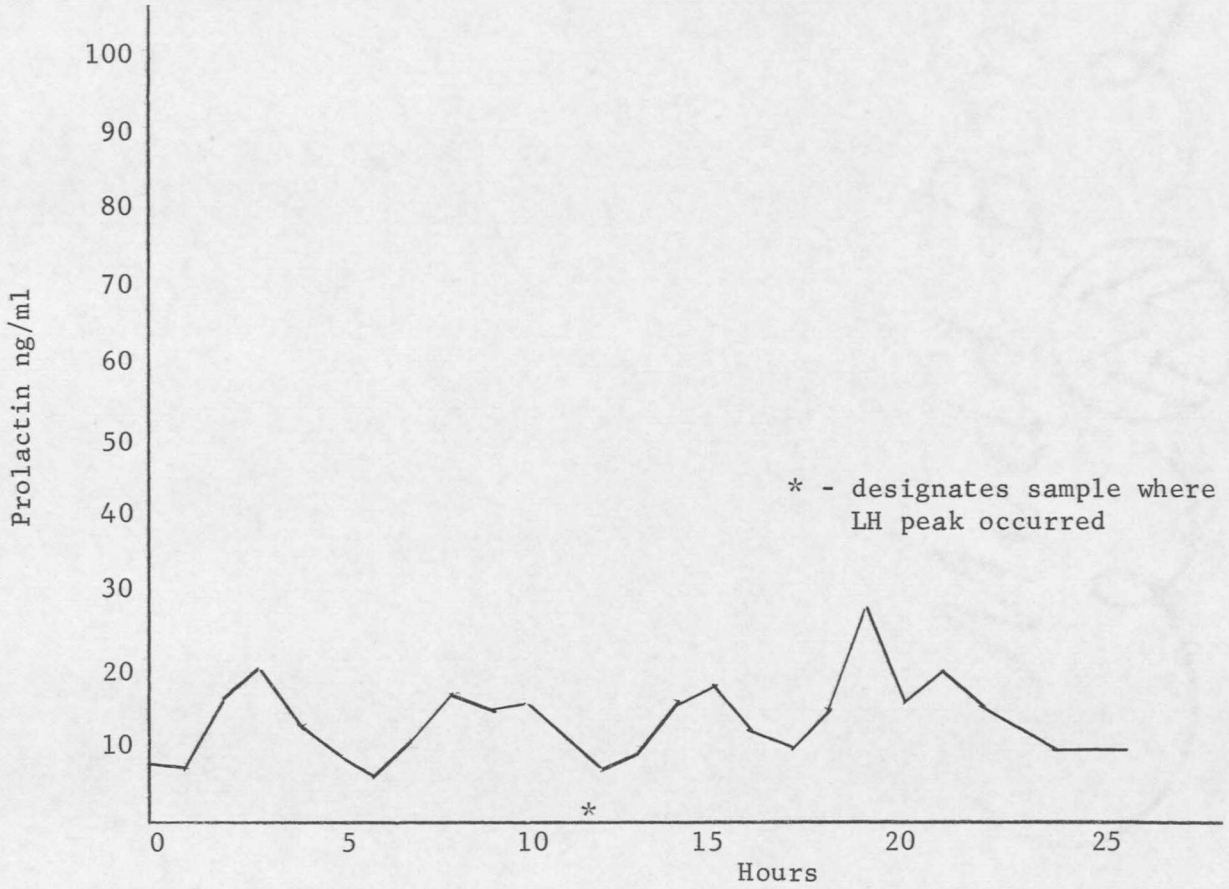
Appendix Figure XIII. Prolactin levels for cow 7720 from estrus until ovulation (Group IV).



Appendix Figure XIV. Prolactin levels for cow 9809 from estrus until ovulation (Group V).



Appendix Figure XV. Prolactin levels for cow 7566 from estrus until ovulation (Group V).



Appendix Figure XVI. Prolactin levels for cow 5386 from estrus until ovulation (Group V).

LITERATURE CITED

Ajika, K., L. Krulich, C. P. Fawcett and S. M. McCann. 1972. Effects of estrogen on plasma and pituitary gonadotropins and prolactin, and on hypothalmic releasing and inhibiting factors. Neuroendocrinology 9:304.

Amenomori, Y., C. L. Chen and J. Meites. 1970. Serum prolactin levels in rats during different reproductive states. Endocrinology 86: 506.

Apostolakis, M. and J. A. Loraine. 1967. Pituitary Gonadotropins. In. Hormones in Blood. Gray and Bacharach, Academic Press, N.Y. 1:275.

Arije, G. F., J. N. Wiltbank and M. L. Hopwood. 1971. Hormone levels in pre- and post-parturient beef cows. J. Anim. Sci. 33:247 (Abstr.).

Armstrong, D. T. and R. O. Creep. 1962. Effect of gonadotrophic hormones on glucose metabolism by luteinized rat ovaries. Endocrinology 70:701.

Armstrong, D. T. and D. L. Black. 1966. Influence of luteinizing hormone on corpus luteum metabolism and progesterone biosynthesis throughout the bovine estrous cycle. Endocrinology 78:937.

Astwood, E. B. 1941. Regulation of corpus luteum function by hypophysial luteotrophin. Endocrinology 28:309.

Barraclough, C. A. 1961. Production of anovulatory, sterile rats by single injections of testosterone propionate. Endocrinology 68:62.

Barraclough, C. A. and R. A. Gorski. 1961. Evidence that the hypothalmus is responsible for androgen-induced sterility in the female rat. Endocrinology 68:68.

Barraclough, C. A. 1967. Modification in reproductive function after exposure to hormones during the prenatal period. In. Neuroendocrinology. Martini and Ganong. Academic Press, N.Y. 2:61.

Bartosik, D., E. B. Romanoff, D. J. Watson and E. Scricco. 1967. Luteotrophic effects of prolactin in the bovine ovary. Endocrinology 81:186.

Bates, R. W., M. M. Garrison and J. Cornfield. 1963. An improved Bio-assay for prolactin using adult pigeons. Endocrinology 73:217.

Ben-David, M., A. Danon, R. Benveniste, C. P. Weller and F. G. Sulman. 1971. Results of radioimmunoassays of rat pituitary and serum prolactin after adrenalectomy and perphenazine treatments in rats. J. Endocr. 50:599.

Ben-David, M., A. Danon and F. G. Sulman. 1971. Evidence of antagonism between prolactin and gonadotrophin secretion: Effect of methallibure and perphenazine-induced prolactin secretion in ovareictomized rats. J. Endocr. 51:719.

Berson, S. A., R. S. Yalow, A. Bauman, M. A. Rothschild and K. Newerly. 1956. Insulin-I^{131} metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. 35:170.

Berson, S. A. and R. S. Yalow. 1964. Immunoassay of protein hormones. In. The Hormones. Pincus, Thimann and Astwood, Academic Press, N.Y. 4:557.

Bradley, T. R. and P. M. Clarke. 1956. The response of rabbit mammary glands to locally administered prolactin. J. Endocr. 14:28.

Brown, J. G., D. W. Peterson and W. D. Foote. 1972. Reproductive response of beef cows to exogenous progestogen, estrogen and gonadotropins at various stages postpartum. J. Anim. Sci. 35:362.

Bryant, G. D., R. M. Connan and F. C. Greenwood. 1968. Changes in plasma prolactin induced by acepromazine in sheep. J. Endocr. 41:613.

Bryant, G. D., F. C. Greenwood and J. L. Linzell. 1968. Plasma prolactin levels in the goat: Physiological and experimental modification. J. Endocrin. 40:IV.

Bryant, G. D., F. C. Greenwood, G. Kann, J. Martinet and R. Denamur. 1971. Plasma prolactin in the oestrous cycle of the ewe: Effect of pituitary stalk secretion. J. Endocr. 51:405.

Buch, L. N., H. L. Woehling and L. E. Casida. 1955. Postpartum estrus and involution of the uterus in an experimental herd of Holstein-Friesian cows. J. Dairy Sic. 38:73.

Butler, W. R., L. B. Willett and P. V. Malven. 1971. Patterns of release of ovine prolactin. J. Anim. Sci. 33:250 (Abstr.).

Caligaris, L., J. J. Astrada and S. Teleisnik. 1967 Pituitary FSH concentration in the rat during the estrous cycle. Endocrinology 81:1261.

Casida, L. E. 1968. The Postpartum Cow - A Resume. In. Studies on the Postpartum Cow. Wis. Agr. Exp. Sta. Bull. 270:48.

Chadwick, A. 1963. Detection and assay of prolactin by the lactogenic response in the rabbit. J. Endocr. 27:253.

Chen, C. L., H. Minaguchi and J. Meites. 1967 Effects of transplanted pituitary tumors on host pituitary prolactin secretion. Proc. Soc. Exp. Biol. Med. 126:317.

Chen, C., J. L. Voogt and J. Meites. 1968. Effect of median eminence implants of FSH, LH or prolactin on luteal function in rats. Endocrinology 83:1273.

Chen, C. L. and J. Meites. 1970. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Endocrinology 86:502.

Clemens, J. A. and J. Meites. 1968. Inhibition by hypothalmic prolactin implants of prolactin secretion, mammary growth and luteal function. Endocrinology 82:878.

Clemens, J. A., M. Sar and J. Meites. 1968. Inhibition of lactation by median eminence implant of prolactin and ACTH. Fed. Proc. 27:269 (Abstr.).

Clemens, J. A., M. Sar and J. Meites. 1969. Inhibition of lactation and luteal function in postpartum rats by hypothalmic implantation of prolactin. Endocrinology 84:868.

Clemens, J., J. Keber, C. Shaar and W. Tandy. 1970. Effect of electrochemical stimulation of the preoptic area on plasma LH and FSH levels in rats. Fed. Proc. 29:312 (Abstr.).

Clemens, J. A., C. J. Shaar, J. W. Kleber and W. A. Tandy. 1971a. Reciprocal control by the preoptic area of LH and prolactin. Exp. Brain Res. 12:250.

Clemens, J. A., C. J. Shaar, J. W. Kleber and W. A. Tandy. 1971b. Areas of the brain stimulatory to LH and FSH secretion. Endocrinology 88:180.

Coble, Y. D., Jr., P.O. Kohler, C. M. Cargille and G. T. Ross. 1969. Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. J. Clin. Invest. 48:359.

Convey, E. M., H. A. Tucker, V. G. Smith and J. Zolman. 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. Endocrinology 92:471.

Corbin, A. and A. I. Cohen. 1966. Effect of median eminence implants of LH on pituitary LH in female rats. Endocrinology 78:41.

Corbin, A and J. C. Story. 1967. "Internal" feedback mechanism: Response of pituitary FSH and of stalk-median eminence follicle stimulating hormone-releasing factor to median eminence implants of FSH. Endocrinology 80:1006.

Cummins, L. J., M. A. de B. Blockey, L. R. Fell and J. R. Goding. 1973. Plasma prolactin release about oestrus and after suckling in the beef cow. J. Reprod. Fert. 32:319 (Abstr.).

Cutuly, E. 1941. Implantation following mating in hypophysectomized rats injected with lactogenic hormone. Proc. Soc. Exp. Biol. Med. 48:315.

Daane, T. A. and A. F. Parlow. 1971. Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle: Effects of pentobarbital. Endocrinology 88:653.

Davis, S. L., L. E. Reichert, Jr and G. D. Niswender. 1971. Serum levels of prolactin in sheep as measured by radioimmunoassay. Biology of Reprod. 4:145.

Davis, S. L. and M. L. Borger. 1973. Metabolic clearance rates and secretion rates of prolactin in sheep. Endocrinology 92:1414.

Denamur, R., J. Martinet and R. V. Short. 1966. Secretion de la progesterone par les corps jaunes de la brebis apres hypophysectomie section de la tage pituitaire et hysterectomie. Acta endocrinologica 52:72.

Desclin, L. and J. Flament-Durand. 1969. Effects of reserpine on the morphology of pituitaries grafted into the hypothalmus or under the kidney capsule in the rat. J. Endocr. 43:LIX.

Donaldson, L. E. and W. Hansel. 1965. Prolongation of life span of the bovine corpus luteum by single injections of bovine luteinizing hormone. J. Dairy Sci. 48:903.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman and J. N. Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci. 29:719.

Ensminger, M. E. 1970. The Stockmans Handbook, (4th Ed.) The Interstate Printers and Pub., Inc.; Danville, Ill.

Evans, H. M., M. E. Simpson, W. R. Lyons and K. Turpeinen. 1941. Anterior pituitary hormones which favor the production of traumatic uterine placentomata. Endocrinology 28:933.

Everett, J. 1966. The control of the secretion of prolactin. In. The Pituitary Gland. Harris and Donovan, Univ. Calif. Press. Berkeley, Calif. 2:166.

Everett, J. W. and D. L. Quinn. 1966. Differential hypothalmic mechanism inciting ovulation and pseudopregnancy in the rat. Endocrinology 78:141.

Fell, L. R., C. Beck, M. A. de B. Blockey, J. M. Brown, K. J. Catt, I. A. Cumming and J. R. Goding. 1971. Prolactin in the dairy cow during suckling and machine milking. J. Reprod. Fert. 24:144 (Abstr.).

Fell, L. R., G. S. Perry, N. J. Chandler and J. R. Goding. 1973. The effect of interval between milkings on prolactin release and milk production in the cow. J. Reprod. Fert. 32:317 (Abstr.).

Ferin, M., A. Tempone, P. Zimmering and R. Vande Wiele. 1969. Effects of antibodies to 17β-estradiol and progesterone on the estrus cycle of the rat. Endocrinology 85:1070.

Flerko, B. 1966. Control of gonadotropin secretion in the female. In. Neuroendocrinology. Ganong, Academic Press, N. Y. 1:613.

Florsheim, W. H., S. M. Velcoff and R. E. Bodfish. 1959. Gonadotrophin assay based on augmentation of radiophosphate uptake by the chick testis. Acta. Endocrinologica. 30:175.

Foote, W. D. and J. E. Hunter. 1964. Postpartum intervals of beef cows treated with progesterone and estrogen. J. Anim. Sci. 23:517.

Foote, W. D. 1971. Endocrine changes in the bovine during the post-partum period. J. Anim. Sci. 32:73 (Suppl. I).

Forsyth, I. A. 1967. Prolactin and placental lactogens. In. Hormones in Blood. Gray, Academic Press, N.Y. 1:233.

Gay, V. L., A. R. Midgley, Jr. and G. D. Niswender. 1970. Patterns of gonadotrophin secretion associated with ovulation. Fed. Proc. 29:1880.

Geschwind, I. 1972. Dynamics of pituitary gonadotropin secretion. J. Anim. Sci. 34:19 (Suppl. I).

Gorski, R. A. 1966. Localization and sexual differentiation of the nervous structures which regulate ovulation. J. Reprod. Fert. Suppl. I:67.

Grandison, L. and J. Meites. 1972. Luteolytic action of prolactin during estrous cycle of the mouse. Proc. Soc. Exp. Biol. Med. 140:323.

Graves, W. E., J. W. Lauderdale, E. R. Hauser and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows. In. Studies on the Postpartum Cow. Wis. Agr. Exp. Sta. Bull. 270:23.

Greenwood, F. C., W. M. Hunter and J. S. Glover. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. 89:114.

Grosvenor, C. E. 1965a. Effect of nursing and stress upon prolactin-inhibiting activity of the rat hypothalmus. Endocrinology 77:1037.

Grosvenor, C. E. 1965b. Evidence that exterioceptive stimuli can release prolactin from the pituitary gland of the lactating rat. Endocrinology 76:340.

Grosvenor, C. E., S. M. McCann and R. Nallar. 1965. Inhibition of nursing-induced and stress-induced fall in pituitary prolactin concentration in lactating rat by injection of acid extracts of bovine hypothalmus. Endocrinology 76:883.

Grosvenor, C. E., F. Mena, A.P.S. Dhariwal and S. M. McCann. 1967. Reduction of milk secretion by prolactin-inhibiting factor: Further evidence that exteroceptive stimuli can release pituitary prolactin in rats. Endocrinology 81:1021.

Grosvenor, C. E., F. Mena and D. A. Schaefgen. 1967. Effect of non-suckling interval and duration of suckling on the suckling-induced fall in pituitary prolactin concentration in the rat. Endocrinology 81:449.

Hafs, D. and D. A. Morrow. 1970. Blood prolactin and LH during puberty in heifers. J. Anim. Sci. 31:232 (Abstr.).

Hammons, Jr., M. Velasco and I Rothchild. 1973. Effect of the sudden withdrawal or increase of suckling on serum LH levels in ovariectomized postparturient rats. Endocrinology 92:206.

Harris, G. W. and H. J. Campbell. 1966. The regulation of the secretion of luteinizing hormone and ovulation. In: The Pituitary Gland. Harris and Donovan. Univ. Calif. Press, Berkeley, Calif. 2:99.

Harvey, W. R. 1968. Least-squares analysis of data with unequal subclass number. AR 20:8.

Hays, R. L. and N. L. VanDemark. 1953. Effect of stimulation of the reproductive organs of the cow on the release of an oxytocin-like substance. Endocrinology 52:634.

Henricks, D. M., J. F. Dickey and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346.

Hunter, W. M. 1969. Assessment of radioiodinated hormone preparations. In. Immunoassay of Gonadotropins. Karolinska Symposia on Research Methods in Reproductive Endocrinology 1:134.

Ingalls, W., H. D. Hafs and W. D. Oxender. 1971. Growth hormone, prolactin and lutenizing hormone in heifers before and after parturition. J. Dairy Sci. 54:768.

Johke, T. 1970. Factors affecting the plasma prolactin level in the cow and goat as determined by radioimmunoassay. Endocrinol. Japan 17:393.

Jones, E. E. and A. V. Nalbandov. 1972. Effects of intrafollicular injection of gonadotrophins on ovulation or luteinization of ovarian follicle. Biol. of Reprod. 7:87.

Kaprowski, J. A., E. M. Convey and H. A. Tucker. 1971. Response of serum prolactin to stimulation of the udder or brisket. J. Dairy Sci. 54:769 (Abstr.).

Kaprowski, J. A., H. A. Tucker and E. M. Convey. 1972. Prolactin and growth hormone circadian periodicity in lactating cows. Proc. Soc. Exp. Biol. Med. 140:1012.

Kaprowski, J. A. and H. A. Tucker. 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology 92:1480.

Kato, J. and C. A. Villee. 1967. Preferential uptake of estradiol by the anterior hypothalmus of the rat. Endocrinology 80:567.

Koch, Y., Y. F. Chow and J. Meites. 1971. Metabolic clearance and secretion rates of prolactin in the rat. Endocrinology 89:1303.

Kohler, P.O., G. T. Ross and W. D. Odell. 1968. Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. J. Clin. Invest. 47:38.

Kovačić, N. 1962. Prolongation of dioestrus in the mouse as a quantitative assay of luteotrophic activity of prolactin. J. Endocr. 24:227.

Kovačić, N. 1963. The Deciduoma Assay: A method for measuring prolactin. J. Endocr. 28:45.

Kovačić, N. 1965. Prolactin assay by the decidual reaction in the mouse. J. Endocr. 33:295.

Kwa, H. G. and F. Verhofstad. 1967. Prolactin levels in the plasma of female rats. J. Endocr. 39:455.

Labhsetwar, A. P., W. E. Collins, N. J. Tyler and L. E. Casida. 1964. Some pituitary-ovarian relationships in the periparturient cow J. Reprod. Fert. 8:85

Labhsetwar, A. P. 1970. Synergism between LH and FSH in the induction of ovulation. J. Reprod. Fert. 23:517.

Labhsetwar, A. P. 1972. Further evidence for synergism between LH and FSH in the induction of ovulation in rats: Lack of effects of prolactin. J. Reprod. Fert. 29:435.

Lincoln, D. W. and B. A. Cross. 1967. Effect of oestrogen on the responsiveness of neurons in the hypothalmus, septum and preoptic area of rats with light induced persistent oestrus. J. Endocr 37:191.

Lyons, W. R. and E. Page 1935. Detection of mammotropin in the urine of lactating women. Proc. Soc. Exp. Biol. Med. 32:1049.

Malven, P. V. and C. H. Sawyer. 1966. A luteolytic action of prolactin in hypophysectomized rats. Endocrinology 79:268.

Malven, P. V. 1970. Development of a double antibody radioimmunoassay (RIA) for ovine prolactin. In. Annual Report of Regional Research Project NE-72. Purdue Univ. Expt. Sta. 1:4.

Malven, P. V. and W. R. Hoge. 1971. Effect of ergocornine on prolactin secretion by hypophysial homografts. Endocrinology 88:445.

Mason, N. R., J. M. Marsh and K. Savard. 1962. An action of gonadotopins in vitro. J. Biol. Chem. 237:1801.

McNeilly, J. R. and G. E. Lamming. 1971. Effect of perphenozine on the level of prolactin in sheep blood. J. Endocr. 50:359.

Meites, J., P. K. Talwalker and C. S. Nicoll. 1960. Initiation of lactation in rats with hypothalmic or cerebral tissue. Proc. Soc. Exp. Biol. Med. 103:298.

Meites, J., C. S. Nicoll and P. K. Tallwalker. 1963. The central nervous system and the secretion and release of prolactin. In. Advances in Neuroendocrinology. Nalbandov. Univ. Ill. Press, Urbana. 1:238.

Meites, J. 1970. Modification of synthesis and release of hypothalmic releasing factors induced by exogenous stimuli. In. Neurochemical Aspects of Hypothalmic Function. Martini and Meites, Academic Press, N.Y. 1:1.

Menge, A. C., S. E. Mares, W. J. Tyler and L. E. Casida. 1962. Variation and association among postpartum reproduction and production characteristics in Holstein-Friesian cattle. J. Dairy Sci. 45:233.

Mishkinsky, J., K. Khozen and F. G. Sulman. 1968. Prolactin-releasing activity of the hypothalmus in postpartum rats. Endocrinology 82:611.

Morrow, D. A. 1971. Effects of peripartuient disease on postpartum reproduction in dairy cattle. J. Anim. Sci. 32:17 (Suppl. I).

Nakayama, I. and P. A. Nickerson. 1973. Suppression of anterior pituitary in rats bearing a transplantable growth hormone and prolactin-secreting tumor (Mt-T-W 10). Endocrinology 92:516.

Neill, J. D. 1970. Effects of "stress" on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. Endocrinology 87:1192.

Neill, J. D., M. E. Freeman and S. A. Tillson. 1971. Control of proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. Endocrinology 89:1448.

Neill, J. D. 1972. Sexual differences in the hypothalmic regulation of prolactin secretion. Endocrinology 90:1154.

Nicoll, C. S. and J. Meites. 1964. Prolactin secretion in vitro: Effects of gonadal and adrenal cortical steroids. Proc. Soc. Exp. Biol. Med. 117:579.

Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Niswender, G. D. 1972. The effect of ergocornine on reproduction in sheep. Biol. Reprod. 7:138 (Abstr.).

Oxenreider, S. L. 1968. Effects of suckling and ovarian function on postpartum reproductive activity in beef cows. Amer. J. Vet. Res. 29:2099.

Pfaff, D. W. 1968. Uptake of ^{3}H-estradiol by the female rat brain. An Autoradiographic Study. Endocrinology 82:1149.

Prop, F. J. A. 1962. On the development of a sensitive quantitative reaction for mammotrophic activity. Acta Endocrinologica Suppl. 67:67.

Ramirez, V. D., R. M. Abrams and S. M. McCann. 1964. Effect of estradiol implants in the hypothalamo-hypophysial region of the rat on the secretion of luteinizing hormone. Endocrinology 75:243.

Ramirez, V. D. and S. M. McCann. 1964. Induction of prolactin secretion by implants of estrogen into the hypothalamo-hypophisial region of female rats. Endocrinology 75:206.

Randel, R. D., R. E. Short, D. S. Christensen and R. A. Bellows. 1973. Effect of various mating stimuli on the LH surge and ovulation time following synchronization of estrus in the bovine. J. Anim. Sci. 37:128.

Ratner, A. and J. Meites. 1964. Depletion of prolactin-inhibiting activity of rat hypothalmus by estradiol or suckling stimulus. Endocrinology 75:377.

Raud, H. R., C. A. Kiddy and W. D. Odell. 1971. The effect of stress upon the determination of serum prolactin by radioimmunoassay. Proc. Soc. Exp. Biol. Med. 136:689.

Reece, R. P. and C. W. Turner. 1937. Effects of stimulus of suckling upon galactin content of the rat pituitary. Proc. Soc. Exp. Biol. Med. 35:621.

Riesen, J. W., S. Saiduddin, W. J. Tyler and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity and uterine involution in dairy cows. Wisc. Agr. Exp. Sta. Res. Bull. 270:27.

Robertson, H. A. and A. S. M. Hutchinson. 1962. The levels of FSH and LH in the pituitary of the ewe in relation to follicular growth and ovulation. J. Endocr. 24:143.

Rothchild, I. 1960. The corpus luteum-pituitary relationship: The association between the cause of luteotrophin secretion and the cause of follicular quiescence during lactions; the basis for a tentative theory of the corpus luteum-pituitary relationship in the rat. Endocrinology 67:9.

Saiduddin, S. and W. D. Foote. 1964. Pituitary luteinizing hormone activity of postpartum bovine. J. Anim. Sci. 23:592 (Abstr.).

Saiduddin, S., J. W. Riesen, W. E. Graves, J. W. Tyler and L. E. Casida. 1966. Pituitary luteinizing hormone activity in the postpartum cow. J. Anim. Sci. 25:930 (Abstr.).

Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in dairy cows. Wisc. Agr. Exp. Sta. Res. Bull. 270:15.

Santolucito, J. A., M. T. Clegg and H. H. Cole. 1960. Pituitary gonadotrophins in the ewe at different stages of the estrous cycle. Endocrinology 66:273.

Sawhney, D. S. 1966. Pituitary and ovarian activities and inter-relationship in beef cows before and after parturition. M. S. Thesis. University Nevada, according to W. D. Foote, 1971.

Schally, A. V., A. Arimura, Y. Baba, R. M. G. Nair, H. Matsuo, T. W. Redding and L. Debeljuk. 1971a. Isolation and properties of the FSH and LH-releasing hormone. Biochem. Biophys. Res. Commun. 43:393.

Schally, A. V., A. Arimura, A. J. Kastin, H. Matsuo, Y. Baba, T. W. Redding, R. M. G. Nair, L. Debeljuk and W. F. White. 1971b. Gonadotropin-releasing hormone: One polypeptide regulates secretion of luteinizing and follicle-stimulating hormone. Science 173:1036.

Schally, A. V., T. W. Redding, H. Matsuo and A. Arimura. 1972. Stimulation of FSH and LH release in vitro by natural and synthetic LH and FSH releasing hormone. Endocrinology 90:1561.

Schams, D. and H. Karg. 1969. Radioimmunologische LH-bestimmung im blutserum vam rind unter besonderer berücksichtigung des brunstzyklus. Acta. Endocrinologica 61:96.

Schams, V. D. and S. Bohm. 1972. Influence of milking stimulus, manipulation on the udder, genital stimulation and exogenous oxytocin supply on the prolactin blood level in cattle. Milchwissenschaft 27(5):300.

Schwartz, N. B., C. E. McCormak. 1972. Reproduction: Gonadal function and its regulation. Ann. Rev. Phsiol. 34:425.

Shaar, C. J. and J. A. Clemens. 1971. Estrogen requirement for neural stimulation of prolactin secretion. Fedn. Proc. 30:254 (Abstr.).

Shelesnyak, M. C. 1958. Maintenance of gestation in ergotoxine-treated pregnant rats by exogenous prolactin. Acta Endocrinologica 27:99.

Shino, M., E. G. Rennels and M. G. Williams. 1971. Ultrastructural observations of pituitary release of prolactin in the rat by suckling stimulation. Anat. Rec. 169:427 (Abstr.).

Short, R. E., R. A. Bellows, E. L. Moody and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34:70.

Sinha, Y. N and H. Tucker. 1968. Pituitary prolactin content and mammary development after chronic administration of prolactin. Proc. Soc. Exp. Biol. Med. 128:84.

Sinha, Y. N. and A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507.

Snook, R. B. M. A. Brunner, R. R. Saatman and W. Hansel. 1969. The effect of antisera to bovine LH in hysterectomized and intact heifers. Biol. Reprod. 1:49.

Snook, R. B., R. R. Saatman and W. Hansel. 1971. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinology 88:678.

Sprague, E. A., M. L. Hopwood, G. D. Niswender and J. N. Wiltbank. 1971. Progesterone and luteinizing hormone levels in peripheral blood of cycling beef cows. J. Anim. Sci. 33:99.

Steelman, S. L. and F. M. Pohley. 1953. Assay of follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. Endocrinology 53:604.

Sulman, F. G. 1970. Hypothalmic control of lactation. Spring-Verlag, New York.

Taleisnik, S. and S. M. McCann. 1961. Effect of hypothalmic lesions on the secretion and storage of hypophysial luteinizing hormone. Endocrinology 68:263.

Török, B. 1964. Structure of the vascular connections of the hypothalmo-hypophysial region. Acta. Anat. 59:84.

Tucker, H. A. 1971. Hormonal response to milking. J. Anim. Sci. 32:137 (Suppl.I).

Valverde, R. C., V. Chieffo and S. Reichlin. 1972. Prolactin-releasing factor in porcine and rat hypothalmic tissue. Endocrinology 91:982.

Van Demark, N. L. and R. L. Hays. 1952. Uterine motility responses to mating. Amer. J. Physiol. 170:518.

Van Dyke, D. C., M. E. Simpson, S. Lepkovsky, A. A. Koneff and J. R. Brobeck. 1957. Hypothalmic control of pituitary function and corpus luteum formation in the rat. Proc. Soc. Exp. Biol. Med. 95:1.

Veomett, M. J. and J. C. Daniel, Jr. 1971. Termination of pregnancy after accelerated lactation in the rat. J. Reprod. Fert. 26:415.

Voogt, J. L., J. A. Clemens and J. Meites. 1969. Stimulation of pituitary FSH release in immature female rats by prolactin implant in median eminence. Neuroendocrinology 4:157.

Wagner, W. C., R. Saatman and W. Hansel. 1969. Reproductive physiology of the postpartum cow. II. Pituitary, adrenal and thyroid function. J. Reprod. Fert. 18:501.

Wagner, W. C. and G. L. Oxenreider. 1971. Endocrine physiology following parturition. J. Anim. Sci. 32:1 (Suppl. I).

Watson, J. T. and I. E. Danhoff. 1971. Distribution volume, secretion rate and turnover time for FSH, LH and prolactin in intact male rats. Fed. Proc. 30:254 (Abstr.).

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J. Anim. Sci. 21:219.

Wiltbank. J. N., W. W. Rowden, J. E. Ingalls and D. R. Zimmerman. 1964. Influence of postpartum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23:1049.

Wolthuis, O. L. 1963. An assay of prolactin based on a direct effect of this hormone on cells of the corpus luteum. Acta. Endocrinologica 42:364.

Wright, A. D. and K. W. Taylor. 1967. Immuno-assay of hormones. In. Hormones in the Blood. Gray and Bacharch. Academic Pres, N.Y. 1:23.

Wuttke, W. and J. Meites. 1970. Effects of ether and pentobarbital on serum prolactin and LH levels in proestrus rats. Proc. Soc. Exp. Biol. Med. 135:648.

Wuttke, W., E. Cassell and J. Meites. 1971. Effects of ergocorine on serum prolactin and LH and on hypothalmic content of PIF and LRF. Endocrinology 88:737.

Wuttke, W. and J. Meites. 1971. Luteolytic role of prolactin during the estrous cycle of the rat. Proc. Soc. Exp. Biol. Med. 137:988.

MONTANA STATE UNIVERSITY LIBRARIES
3 1762 10014186 8

N378
H19
cop.2
Han, David K
LH and prolactin levels in postpartum beef cows

NAME AND ADDRESS
JAN 24 1980 Anne Perkins
Box 431
994-2323
FEB 6 '80
'80

N378
H19
cop.2