



The bovine postpartum interval : blood plasma steroid levels and the effect of hormone treatment, mastectomy and induced parturition
by Verne Allan LaVoie

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Animal Science
Montana State University
© Copyright by Verne Allan LaVoie (1975)

Abstract:

Four studies were conducted to evaluate various aspects of bovine postpartum anestrus. In the first study, 18 beef cows were assigned to a NON-LACTATING, SUCKLED 2X DAILY or SUCKLED AD LIBITUM group to determine the effect of suckling during puerperium on blood plasma progesterone and testosterone concentrations and on the occurrence and intensity of estrus. Suckling increased ($P<.05$) the postpartum interval from 20 days in the NON-LACTATING group to 38 days in the SUCKLED AD LIBITUM group and increased intensity of estrus score ($P<.05$). In analyses excluding 3 cows with silent first estrus, progesterone averaged .8 ng/ml at parturition and ranged between .1 and .4 ng/ml through day 18 postpartum and was lowest in suckled cows ($P<.01$). Progesterone ranged between .1 and .4 ng/ml from 18 through 5 days, increased rapidly to 1.8 ng/ml at 3 days and returned to .3 ng/ml at 1 day prior to estrus. This pre-estrus progesterone peak was highest in SUCKLED AD LIBITUM cows ($P<.05$). Testosterone was low (mean=.2 ng/ml) over the postpartum period. The second study consisted of TRIALS A and B and progesterone and estrogen were given in single doses of 20 mg and 2 mg, respectively, between 30 to 90 days postpartum to determine if this treatment could be used in routine dairy management to improve reproductive performance.

The treatment decreased the interval from calving to estrus 25 days ($P<.07$, TRIAL A; $P<.01$ TRIAL B) but did not decrease the interval from calving to conception nor improve reproductive performance as measured by first service conception rate, conceptions per service and pregnancy rate. Treatment did not affect ovary diameter at 10 through 44 days or milk production from 3 through 50 days postpartum. The third study was designed to determine if mastectomized cows could be managed to reduce the average calving interval to less than 365 days. Nine mastectomized cows had a mean interval from calving to first estrus of 30 days but showed low fertility as measured by first service conception rate (56%) and conceptions per service (35%). The interval from calving to conception was 76 and 79 days for the mastectomized and control herds, respectively, indicating that mastectomy had little effect in reducing the calving interval to less than 365 days. In the fourth study, 31 cows were used to test the effect of flumethasone induced calving (5 mg injected 17 days prior to term) on postpartum reproduction. Treatment groups were: NT=nontreated, INDU=treated and induced and NI=treated but not induced. A breed difference was noted in response to treatment with more Angus responding than Herefords ($P<.01$) postpartum interval to first estrus was 45, 47 and 40 days in the NT, INDU and NI groups, respectively, but when postpartum interval was calculated from expected natural calving date, the intervals were 43, 32 and 37 days, respectively. Cows were in estrus 1.2, 1.1 and 1.3 times prior to 60 days postpartum, pregnancy rates were 58, 90 and 67% and days open were 76, 69 and 77 days in the NT, INDU and NI groups, respectively.

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 Vernon LaVoie

Date December 1, 1975

114

THE BOVINE POSTPARTUM INTERVAL: BLOOD PLASMA STEROID
LEVELS AND THE EFFECT OF HORMONE TREATMENT,
MASTECTOMY AND INDUCED PARTURITION

by

VERNE ALLAN LAVOIE

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

Approved:

Edward L. Moody
Chairman, Examining Committee

Oscar O. Thomas
acting Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1975

ACKNOWLEDGMENTS

I wish to express appreciation to my major professor, Dr. Edward Moody, for his encouragement and assistance during this project. Appreciation is also expressed to Drs. Peter Burfening, Larry Jackson and Robert Bellows who were the other members of my graduate committee.

I would also like to thank my fellow graduate students who gave assistance at various times during these studies, especially, Hardee Clark, James Winter, Greg Poncelet, David Han, David Griswold, Paul Lambert, Robert Friedrich, Gary Moss and John Stellflug. I would also like to thank Robert Brown for technical assistance and Mrs. Frankie Larson for typing the manuscript.

A special thanks is given to Dr. B. R. Moss and the personnel of the Montana State University Dairy Center for cooperation in providing dairy cows and facilities to conduct a portion of these studies.

The support and encouragement of my wife Sally, and my children, Buffy and Lyle, during this work is greatly appreciated as is the assistance and encouragement of my wife's family, Lyle (Bill) and Margaret O'Connor and children.

Appreciation is expressed to Dr. H. L. Self, Iowa State University, for his patience while I completed the final draft of this thesis.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENTS	iii
INDEX TO TABLES	vi
INDEX TO FIGURES	vii
INDEX TO APPENDIX TABLES	ix
INDEX TO APPENDIX FIGURES	xii
ABSTRACT	xiv
CHAPTER 1.	1
INTRODUCTION	1
CHAPTER 2.	4
LITERATURE REVIEW.	4
Postpartum Interval.	4
Postpartum Endocrinology	11
Lactation and the Postpartum Interval.	37
Techniques for Control of the Postpartum Interval.	41
Induced Parturition and the Postpartum Interval.	52
CHAPTER 3.	56
HORMONE ASSAYS	56
Progesterone Assay	56
Testosterone Assay	63
CHAPTER 4.	64
BLOOD PLASMA PROGESTERONE AND TESTOSTERONE LEVELS IN POSTPARTUM BEEF COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI.	64
Introduction	64
Materials and Methods.	65
Results and Discussion	70

	Page
CHAPTER 5.	106
USE OF PROGESTERONE AND ESTROGEN TO CONTROL POSTPARTUM REPRODUCTION IN DAIRY MANAGEMENT.	106
Introduction	106
Materials and Methods.	106
Results and Discussion	108
CHAPTER 6.	114
EFFECT OF MASTECTOMY ON POSTPARTUM REPRODUCTION IN BEEF COWS	114
Introduction	114
Materials and Methods.	114
Results and Discussion	114
CHAPTER 7.	119
EFFECT OF FLUMETHASONE INDUCED CALVING ON THE POSTPARTUM INTERVAL OF BEEF COWS	119
Introduction	119
Materials and Methods.	120
Results and Discussion	122
CHAPTER 8.	130
SUMMARY AND CONCLUSIONS.	130
CHAPTER 9.	135
APPENDIX	135
CHAPTER 10	175
LITERATURE CITED	175

INDEX TO TABLES

Table		Page
4-1	EXPERIMENTAL DESIGN	67
4-2	EFFECT OF THREE LEVELS OF SUCKLING STIMULI ON BEHAVIORAL ESTRUS AND PLASMA PROGESTERONE LEVELS IN POSTPARTUM BEEF COWS INCLUDING THREE COWS WITH SILENT FIRST OVULATION.	73
4-3	EFFECT OF THREE LEVELS OF SUCKLING STIMULI ON BEHAVIORAL ESTRUS AND PLASMA STEROID LEVELS IN POSTPARTUM BEEF COWS EXCLUDING THREE COWS WITH SILENT FIRST OVULATION.	74
4-4	RESIDUAL CORRELATIONS BETWEEN BEHAVIORAL ESTRUS, BODY WEIGHT AND BLOOD PLASMA PROGESTERONE IN POSTPARTUM COWS WITH EXPRESSED FIRST ESTRUS	89
5-1	REPRODUCTIVE PERFORMANCE OF STEROID TREATED COWS (TRIAL A).	112
5-2	REPRODUCTIVE PERFORMANCE OF STEROID TREATED COWS (TRIAL B).	113
6-1	REPRODUCTIVE PERFORMANCE OF MASTECTOMIZED COWS.	118
6-2	INDIVIDUAL PERFORMANCE OF MASTECTOMIZED COWS.	118
7-1	REPRODUCTIVE PERFORMANCE OF HEREFORD AND ANGUS COWS FOLLOWING FLUMETHASONE INDUCED PARTURITION	128
7-2	REPRODUCTIVE PERFORMANCE OF HEREFORD AND ANGUS COWS IN THE CONTROL GROUP (NT).	129

INDEX TO FIGURES

CHAPTER. 2		Page
Figure		
2-1	Schematic representation of the hypothalamic control mechanisms for FSH and LH secretion. Adapted from Flerko (1970).	13
2-2	Schematic representation of the anatomic relationship between the hypothalamic area and the pituitary gland. Adapted from Gay (1972).	15
CHAPTER 4		
4-1	Body weights of cows following parturition with the average day of first observed postpartum estrus depicted by E ⁺ S.E.	72
4-2	Mean blood plasma progesterone levels and regression equations across all treatments (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle	77
4-3	Mean blood plasma progesterone levels and regression equations for the NON-LACTATING treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle	78
4-4	Mean blood plasma progesterone levels and regression equations for the SUCKLED 2X treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle	79
4-5	Mean blood plasma progesterone levels and regression equations for the SUCKLED <u>AD LIBITUM</u> treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle	80

Figure	Page
4-6 Mean blood plasma progesterone levels and regression equations across all treatments (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle.	81
4-7 Mean blood plasma progesterone levels and regression equations for the NON-LACTATING treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle	82
4-8 Mean blood plasma progesterone levels and regression equations for the SUCKLED 2X treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle	83
4-9 Mean blood plasma progesterone levels and regression equations for the SUCKLED <u>AD LIBITUM</u> treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle	84
4-10 Blood plasma testosterone levels in three cows, one from each treatment group, from parturition through the first postpartum estrus	88

INDEX TO APPENDIX TABLES

Table		Page
9-1	BASIC COMPUTER PROGRAM FOR COMPUTATION OF PROGESTERONE AND TESTOSTERONE CONCENTRATIONS IN BLOOD PLASMA	136
9-2	LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI (CHAP. 4).	140
9-3	LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN NON-LACTATING COWS (CHAP. 4).	140
9-4	LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUCKLED 2X DAILY (CHAP. 4)	141
9-5	LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUCKLED 2X DAILY (CHAP. 4)	141
9-6	LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)	142
9-7	LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI EXCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)	143
9-8	LEAST SQUARES ANALYSIS OF VARIANCE FOR INTENSITY OF SECOND OBSERVED ESTRUS AND LENGTH OF FIRST OBSERVED ESTROUS CYCLE IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4).	144
9-9	LEAST SQUARES ANALYSIS OF VARIANCE FOR INTENSITY OF SECOND ESTRUS AND LENGTH OF FIRST ESTROUS CYCLE IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI EXCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4).	144

Table		Page
9-10	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATIONS (CHAP. 4)	145
9-11	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN NON-LACTATING COWS INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	146
9-12	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED 2X DAILY INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	147
9-13	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED <u>AD LIBITUM</u> INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	148
9-14	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI EXCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)	149
9-15	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN NON-LACTATING COWS EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	150
9-16	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED 2X DAILY EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	151

Table		Page
9-17	LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED <u>AD LIBITUM</u> EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)	152
9-18	LEAST SQUARES ANALYSIS OF VARIANCE FOR BLOOD PLASMA TESTOSTERONE LEVELS DURING THE POSTPARTUM PERIOD IN THREE COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI (CHAP. 4)	153
9-19	LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS REPRODUCTIVE TRAITS OF STEROID TREATED COWS (CHAP. 5, TRIAL A).	154
9-20	LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS REPRODUCTIVE TRAITS OF STEROID TREATED COWS (CHAP. 5, TRIAL B).	154
9-21	LEAST SQUARES ANALYSIS OF VARIANCE FOR OVARY DIAMETER AT VARIOUS STAGES POSTPARTUM IN STEROID TREATED COWS (CHAP. 5, TRIAL B)	155
9-22	LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS AND CALVES SUBJECTED TO FLUMETHASONE INDUCED PARTURITION (CHAP. 7)	156

INDEX TO APPENDIX FIGURES

Figure		Page
9-1	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 142 (NON-LACTATING) during the postpartum period.	157
9-2	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 235 (NON-LACTATING) during the postpartum period.	158
9-3	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 715 (NON-LACTATING) during the postpartum period.	159
9-4	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 812 (NON-LACTATING) during the postpartum period.	160
9-5	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 609 (NON-LACTATING) during the postpartum period.	161
9-6	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 615 (NON-LACTATING) during the postpartum period.	162
9-7	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 314 (SUCKLED 2X) during the postpartum period.	163
9-8	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 601 (SUCKLED 2X) during the postpartum period.	164
9-9	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 613 (SUCKLED 2X) during the postpartum period.	165

Figure		Page
9-10	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 929 (SUCKLED 2X) during the postpartum period	166
9-11	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 722 (SUCKLED 2X) during the postpartum period	167
9-12	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 628 (SUCKLED 2X) during the postpartum period	168
9-13	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 315 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	169
9-14	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 371 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	170
9-15	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 804 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	171
9-16	Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 821 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	172
9-17	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 634 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	173
9-18	Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 756 (SUCKLED <u>AD LIBITUM</u>) during the postpartum period.	174

ABSTRACT

Four studies were conducted to evaluate various aspects of bovine postpartum anestrus. In the first study, 18 beef cows were assigned to a NON-LACTATING, SUCKLED 2X DAILY or SUCKLED AD LIBITUM group to determine the effect of suckling during puerperium on blood plasma progesterone and testosterone concentrations and on the occurrence and intensity of estrus. Suckling increased ($P<.05$) the postpartum interval from 20 days in the NON-LACTATING group to 38 days in the SUCKLED AD LIBITUM group and increased intensity of estrus score ($P<.05$). In analyses excluding 3 cows with silent first estrus, progesterone averaged .8 ng/ml at parturition and ranged between .1 and .4 ng/ml through day 18 postpartum and was lowest in suckled cows ($P<.01$). Progesterone ranged between .1 and .4 ng/ml from 18 through 5 days, increased rapidly to 1.8 ng/ml at 3 days and returned to .3 ng/ml at 1 day prior to estrus. This pre-estrus progesterone peak was highest in SUCKLED AD LIBITUM cows ($P<.05$). Testosterone was low (mean=.2 ng/ml) over the postpartum period. The second study consisted of TRIALS A and B and progesterone and estrogen were given in single doses of 20 mg and 2 mg, respectively, between 30 to 90 days postpartum to determine if this treatment could be used in routine dairy management to improve reproductive performance. The treatment decreased the interval from calving to estrus 25 days ($P<.07$, TRIAL A; $P<.01$ TRIAL B) but did not decrease the interval from calving to conception nor improve reproductive performance as measured by first service conception rate, conceptions per service and pregnancy rate. Treatment did not affect ovary diameter at 10 through 44 days or milk production from 3 through 50 days postpartum. The third study was designed to determine if mastectomized cows could be managed to reduce the average calving interval to less than 365 days. Nine mastectomized cows had a mean interval from calving to first estrus of 30 days but showed low fertility as measured by first service conception rate (56%) and conceptions per service (35%). The interval from calving to conception was 76 and 79 days for the mastectomized and control herds, respectively, indicating that mastectomy had little effect in reducing the calving interval to less than 365 days. In the fourth study, 31 cows were used to test the effect of flumethasone induced calving (5 mg injected 17 days prior to term) on postpartum reproduction. Treatment groups were: NT=nontreated, INDU=treated and induced and NI=treated but not induced. A breed difference was noted in response to treatment with more Angus responding than Herefords ($P<.01$) postpartum interval to first estrus was 45, 47 and 40 days in the NT, INDU and NI groups, respectively, but when postpartum interval was calculated from expected natural calving date, the intervals were 43, 32 and 37 days, respectively. Cows were in estrus 1.2, 1.1 and 1.3 times prior to 60 days postpartum, pregnancy rates were 58, 90 and 67% and days open were 76, 69 and 77 days in the NT, INDU and NI groups, respectively.

CHAPTER 1

INTRODUCTION

Rising production costs, competition from other animal and synthetic protein sources and a rapidly increasing hungry world population emphasize the need for more efficient production in the cattle industry. One poorly understood but highly important aspect of beef and dairy cow reproduction that greatly affects efficiency of production is the postpartum interval.

The postpartum interval has been defined as the interval beginning with parturition and ending with a designated event. This event may be first ovulation, first estrus, completion of uterine involution, first breeding or conception (Casida, 1971). As implied by this definition, the postpartum interval is characterized by a series of events which culminate in the renewed ability to reproduce. Not only must the animal regain the ability to show estrus and ovulate but her uterus must return to a condition favorable to fertilization, implantation and normal gestation.

The relationship between the postpartum interval and fertility is negatively correlated. As the length of the interval increases, conception occurs later in the breeding season and if the interval is too long a return to a functional reproductive state does not occur until the breeding season is over. Therefore, considerable economic loss can result if the parturition to conception interval is unduly prolonged.

A cow must conceive within 82 days postpartum if she is to have a 12 month calving interval. As reviewed by Casida (1968) the literature reports mean intervals from parturition to estrus ranging from 46 to 104 days for beef herds and 30 to 72 days for dairy herds. Many dairy cows have intervals greater than 82 days (Casida, 1968). Wiltbank (1970) found in a group of beef cattle that the interval from calving to estrus was too long to permit a 12 month calving interval in 32% of three-year-old cows and 11% of cows age five years or older.

Application of these statistics show that Montana commercial beef producers lose an estimated \$11,000,000 annually because of prolonged postpartum anestrus and low postpartum fertility, and the annual loss of income to U. S. beef producers is conservatively estimated to be \$621,000,000. In dairy cattle not only is calf production affected, but average annual milk production is also decreased by long calving intervals (Schmidt and Van Vleck, 1974).

The purpose of this investigation was to study the postpartum interval to gain knowledge that could be used to improve reproductive efficiency in cattle. Four experiments were conducted using beef and dairy cows at the Montana Experiment Station, Bozeman. The first experiment was designed solely to obtain basic physiological information on hormonal mechanisms controlling postpartum anestrus while the other experiments consisted of hormone and surgical treatments that would hopefully lead to a better understanding of the postpartum

interval and to development of techniques to control the postpartum interval.

CHAPTER 2

LITERATURE REVIEW

Various aspects of bovine postpartum physiology have been reviewed by Casida (1971), Erb et al. (1971), Foote (1971) Morrow (1971) and Wagner and Oxenreider (1971) at the IX Biennial Symposium on Animal Reproduction at Purdue University in 1969. Other excellent reviews of the early literature on this subject include those by Casida et al. (1968), Morrow et al. (1969) and Moller (1970).

In the following review, the emphasis has been placed on the more recent publications that have direct bearing on the specific objectives of this investigation. The early literature is reported rather superficially as background information for the more extensive reviews of the postpartum interval in relation to blood hormone levels, exogenous hormone treatments, lactation, uterine environment and induced parturition.

Postpartum Interval

In order for a cow to resume a fully functional reproductive state following parturition not only must she regain the ability to show estrus and ovulate but her reproductive tract must return to a condition favorable to fertilization, implantation and gestation. The following discussion is a comprehensive review of postpartum ovarian activity and uterine involution, the factors that affect them and their relation to fertility.

Follicular activity

Follicles were not detected on the ovary at the time of parturition (Benesch and Wright, 1951; Labhsetwar et al., 1964) but follicles develop within 16 to 30 days postpartum (Casida and Venzke, 1936; Higaki et al., 1959; Labhsetwar et al., 1964). Mean days to ovulation and corpus luteum development range from 20 to 71 days in different studies (Casida et al., 1968) and ovulation has been reported to occur prior to 20 days in some instances (Labhsetwar et al., 1964; Morrow et al., 1966; Wagner and Hansel, 1969).

First estrus

There is wide variation in reports on the interval from parturition to first estrus with means of 30.0 to 76.3 days reported in 13 studies of dairy cattle and 52.2 to 80.2 days in five studies of beef cattle (Morrow et al., 1969), Casida et al. (1968) in a survey of the literature indicated that different groups of dairy cows had averages ranging from 30 to 72 days and of beef cows from 46 to 104 days.

Numerous studies indicate that a high percent of early postpartum ovulations are not accompanied by observed behavioral estrus, and that the incidence of these silent estruses decreases as days from parturition increase (Casida and Wisnicky, 1950; Kidder et al., 1952; Trimberger and Fincher, 1956; Menge et al., 1962; Labhsetwar et al., 1963). Saiduddin et al. (1968) reported that the incidence of non-standing behavioral estrus also decreases as time from parturition

increases.

In cows with short intervals to first estrus there is a tendency for them to have a shortened first estrous cycle of about 13 days (Olds et al., 1949; Menge et al., 1962).

Uterine involution

The time after parturition required for complete involution of the uterus as determined by rectal palpation varies considerably but ranges between 25 and 50 days. As reviewed by Morrow et al., (1969) postpartum interval to uterine involution ranges from 26.2 to 52.0 in six studies with dairy cows and 37.7 to 56.0 days in three studies with beef cows. Two studies based on histological criteria suggest a parturition to involution interval of 25 to 30 days for most animals (Gier and Marion, 1968; Wagner and Hansel, 1969).

Several workers have shown that a delay in uterine involution may result in a delay of first postpartum estrus (Albrechtsen, 1917; Buch et al., 1955; Higaki, 1959) while others show no significant relationship between the two events (Foote et al., 1960; Menge et al., 1962).

Cervical involution

Information on involution of the cervix is scanty but available studies indicate that it occurs very rapidly and the cervix is completely closed by 36 to 96 hours postpartum (Benesch and Wright, 1951; Jubb and Kennedy, 1963; Boyd, 1925).

Factors affecting postpartum interval

Parturition and postparturient disease. The postpartum interval to first estrus (Chapman and Casida, 1937; Buch et al., 1955; Morrow, 1971) and to uterine involution is increased by abnormal parturitions, dystocia and disease including retained placenta, brucellosis, pyometria and others. A high proportion of cows have uteri contaminated with microorganisms shortly after parturition (Rasbech, 1950; Higaki et al., 1959; Elliott et al., 1968; Gier et al., 1962).

Suckling. Suckling delayed the onset of first estrus as compared to milking (Clapp, 1937; Wiltbank and Cook, 1968, Wagner and Hansel, 1969) and drying cows off soon after calving (Saiduddin et al., 1967a). The effect of suckling on uterine involution is not clear as one research group reports that suckling hastens involution (Lauderdale et al., 1968; Riesen et al., 1968), another reports no effect (Wagner and Hansel, 1969), while yet another reports that suckling prolongs involution (Wiltbank and Cook, 1958).

Level of production. There is conflicting data on the effect of level of milk production on the interval to first estrus with some researchers reporting that the interval is prolonged by increased production (Olds and Seath, 1953; Carman, 1955; Saiduddin et al., 1969) while others report no effect (Clapp, 1937; Herman and Edmondson, 1950). In one study there was no effect of level of milk production on interval to uterine involution (Morrow et al., 1966), but Menge et al. (1962)

reported a significant positive correlation of production level on this interval.

Nutrition. Low pre- and postpartum energy levels have been reported to decrease the interval from parturition to first estrus (Wiltbank et al., 1962; Wiltbank et al., 1964; Dunn et al., 1969) and to increase the incidence of silent estrus (Saiduddin et al., 1967b). Hypoglycemia has been associated with a decrease in follicular development and could be an important factor affecting postpartum interval (Oxenreider and Wagner, 1971).

Season. Postpartum interval to first estrus appears to be shortest in the fall and longest in the spring (Chapman and Casida, 1937; Buch et al., 1955, Carman, 1955) while interval to uterine involution is shortest in the summer and spring (Marion et al., 1968). However, several reports find no effect of season on these intervals (Herman and Edmondson, 1950; Warnick, 1955; Wiltbank and Cook, 1958; Morrow et al., 1966).

Age and parity. Most authors conclude that postpartum interval to first estrus tends to decrease with increased parity up to 7 years of age and then increases again (Hammond and Sanders, 1923; Herman and Edmondson, 1950; Wiltbank and Cook, 1958). Other reports show this interval to increase with parity (Casida and Wisnicky, 1950) or to not be affected by parity (Clapp, 1937; Buch et al., 1955; Foote et al., 1960). Postpartum interval to uterine involution is longer in

primiparous cows (Rasbech, 1950; Morrow et al., 1966). However, Tennant et al. (1967) maintained that age had no effect on the rate of involution.

Other factors. Other factors reported to delay the appearance of postpartum estrus are the occurrence of cystic follicles (Morrow et al., 1966), anemia, feeding of thyroprotein (Wagner and Hansel, 1969), and the occurrence of cystic and retained corpora lutea although the latter two are probably not causes of postpartum anestrus (Asdell et al., 1949; Morrow et al., 1966; Donaldson and Hansel, 1968).

Postpartum interval and fertility

Reproductive efficiency of beef cattle has been observed to improve as the time from parturition to first service increase (Edwards, 1950; Shannon et al., 1952; Warnick, 1955; Perkins and Kidder, 1963; Graves et al., 1968; Saiduddin et al., 1968).

VanDemark and Salisbury (1950) found in a 28 year study involving 1,674 pregnancies in 593 dairy cows that fertility increased with the length of the postpartum interval in a curvilinear relationship. In the cows bred less than 20 days after calving, only 35% conceived while nearly 58% of the cows conceived when first bred 100 to 120 days after calving. Conception rate of cows bred 201 days or more following calving was 46.3%. There was a significant linear regression ($r = -.116$) between the postpartum interval to first service and the number of services required for conception within 80 days after parturition.

Casida et al. (1968) reviewed literature on fertility at first service following parturition and found conception at first service (unweighted means of 5 studies) to be 39.3, 53.2, 61.6, 62.2, 64.7 and 64.3% for consecutive months one through six postpartum in dairy cows. In beef cows, conception at first service (unweighted mean of 3 studies) was 33.4, 58.1, 68.6 and 74.4% for consecutive months one through four postpartum.

Ovarian activity usually occurs before uterine involution is completed (Buch et al., 1955) and the question arises as to whether cows can conceive at ovulation prior to complete involution. Most reports indicate that the degree of uterine involution does not seriously affect fertility (Foote et al., 1960; Perkins and Kidder, 1963; Tennant and Peddicord, 1967) and the interval to first estrus is a more important effect. It is difficult to see how the effect of uterine involution and the interval to first estrus can truly be separated.

Foote et al. (1960) estimated the relative importance of the effect of interval to first estrus and interval to uterine involution on fertility. Of the two the interval to first breeding was the most important with standard partial regression on fertility on interval to involution of the uterus being .02 and on interval to first service being .40. However, these studies did not consider the degree of involution at the time of breeding.

Postpartum Endocrinology

The hormonal mechanisms controlling postpartum anestrus are not well understood. It is evident from the literature to be presented later that this anestrus is not caused by a lack of gonadotrophins nor is there a lowered sensitivity of the ovaries to gonadotrophins (Casida et al., 1943). Follicles large enough to ovulate develop on the ovary shortly after parturition, yet ovulation does not normally occur until sometime later (Wagner and Oxenreider, 1971). This inability to ovulate early postpartum is undoubtedly related to the lack of an ovulatory surge of LH and this concept will be further developed.

In the following discussion, literature will be reported relative to mechanisms of LH release, pituitary and blood levels of hormones during the postpartum interval and other endocrine factors of postpartum anestrus.

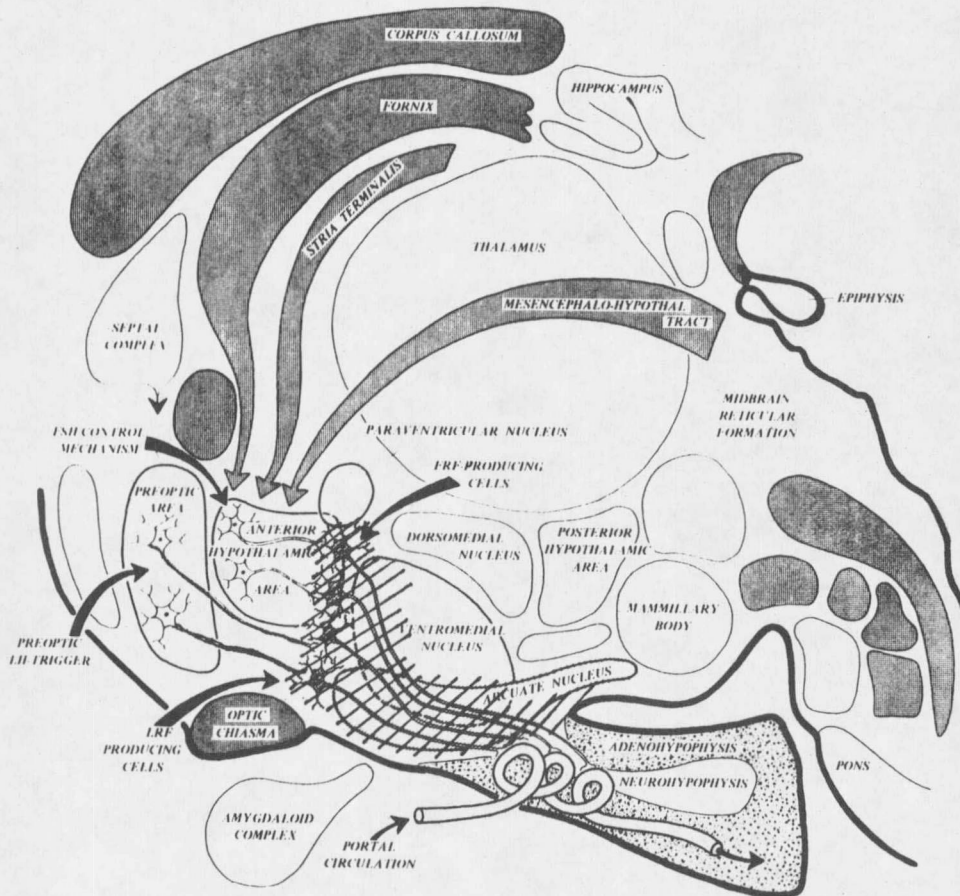
Postpartum anestrus and prepubertal anestrus are similar in some respects and may be related. Therefore, a study of one may give insight into the other and for this reason data on the prepubertal heifer has been included when appropriate. In the section on mechanisms of gonadotrophin release data from other species including rats are included.

Role of the hypothalamo-hypophyseal axis

Mechanisms of ovulation. Ovulation in the bovine is the result of a surge of LH that may increase as much as 200 times above basal luteal phase levels within a 12 hr period (Geschwind, 1972). Prolactin also increases coincidentally with the LH peak (Davis et al., 1971; Swanson and Hafs, 1972). In rats it has been demonstrated that increased levels of prolactin are not necessary for ovulation to occur (Wuttke, Cassell and Meites, 1971).

Rondell (1970) has postulated a mechanism by which LH may cause ovulation. In this model, LH either acts directly on the follicular wall or indirectly via an induced steroid secretion (progesterone?) causing fibrocytes and/or other cells common to both thecal layers to produce a collagenase enzyme. This enzyme has been identified in follicular tissue and its action affects the bonding between collagen fibrils to increase the distensibility and plasticity of the follicular wall. The wall becomes thinner and thinner until rupture occurs. This proposed mechanism is supported by the fact that intrafollicular pressure does not increase and in fact may decrease slightly just prior to ovulation.

Hypothalamic control of gonadotrophins. The secretions of the anterior pituitary are controlled by small peptide molecules arising from the hypophysiotrophic area of the hypothalamus (figure 1). Releasing hormones exist for GH (GRH), TSH (TRH), ACTH (CRH), LH (LRH)



GRAY AREAS ■■■ ARE NERVE TRACTS

HATCHED AREA ▨▨▨▨ REPRESENTS HYPOPHYSIOTROPHIC AREA

Figure 2-1. Schematic representation of the hypothalamic control mechanisms for FSH and LH secretion. Adapted from Flerko (1970).

and FSH (FRH). Control of prolactin is mediated by an inhibitory hormone (PIH) but there is also evidence for a prolactin releasing factor (PRF) (Gay, 1972). Evidence exists that LRH and FRH are the same compound which has been named gonadotrophin releasing hormone (GnRH) (Schally et al., 1971).

These hypothalamic hormones exert their control over the cells of the pituitary via the hypothalamic-hypophyseal portal system which is schematically represented in figure 2. Long and short portal vessels, arising from the superior and inferior hypothalamic arteries, respectively, serve as the method of communication between hypothalamic neurons and hormone secreting cells of the adenohypophysis. Releasing factors may be secreted on or near the capillary network of the portal vessels to be rapidly transported to their target cells (Gay, 1972).

In figure 2, three possible types of neuronal stimuli are indicated: Neuron 1 may stimulate neuron 3 to cause the release of a releasing factor. Neuron 2, although located more distant from the capillary network also may secrete releasing factors near the capillary network. The pathway of neuron 4 suggests the transport of releasing factor to the short portal vessels which supply the periphery of the adenohypophysis. However, the involvement of the short portal vessels is not well supported by experimental data (Gay, 1972).

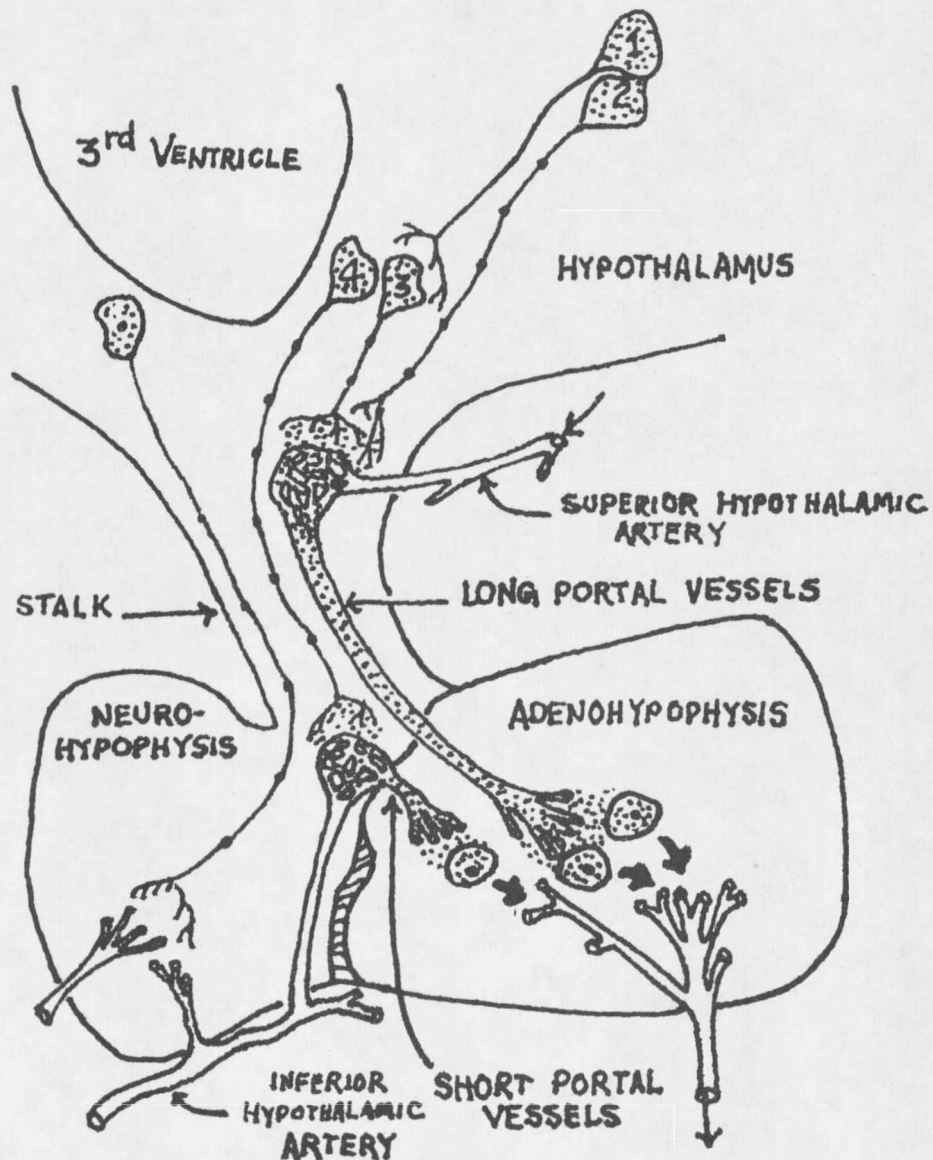


Figure 2-2. Schematic representation of the anatomic relationship between the hypothalamic area and the pituitary gland. Adapted from Gay (1972).

Data from rats indicates that two levels exist in the neural control of gonadotrophin secretion. One level is represented by the tonic mechanism situated in the hypophysiotrophic area. This tonic mechanism acts directly on the anterior pituitary cells by means of the hypophysiotrophic factors (releasing hormones). This level of neural control cannot secure by itself the cyclic output of gonadotrophin hormone, but it is able to maintain a tonic basal level of FSH and LH in an amount sufficient to maintain ovarian follicular growth and estrogen secretion (Flerko, 1970).

The other level of neural control of gonadotrophin secretion is the cyclic mechanism that triggers the burst of LH secretion responsible for ovulation. Using techniques developed by Halász and Pupp (1965) to interrupt neural connections of the hypophysiotrophic area without altering pituitary connections, Halász (1969) demonstrated that the neurogenic stimulus that causes the release of LRF in amounts necessary for ovulation comes mostly from the preoptic area.

The concept is that a message is sent via the nervous system from the LH trigger mechanism (LH surge center) and from the FSH control mechanism to the neurons in the hypophysiotrophic area causing a release of LRH and FRH (GnRH?). This is depicted in figure 1 where the FRF and LRF producing neurons of the tonic mechanism are indicated by the neural units with dotted bodies. They are situated in the hypophysiotrophic area and their nerve endings terminate on the

capillary loops of the portal system. Neurons belonging to the hypothalamic cyclic mechanism (preoptic LH-trigger and FSH-control mechanism) are represented by clear bodies located in the preoptic and anterior hypothalamic areas, respectively, (Flerko, 1970).

This concept is further illustrated in figure 2. Because surgical transection of the anterior hypothalamus (axons 1 and 2) appears to abolish the cyclic release of LH in the female rat but not the basal secretions of LH in either sex (Halász, 1969) it has been proposed that the preovulatory surge of LH is controlled by one group of neurons (neurons 1 and 2) while the basal secretion in both sexes is regulated by other neurons (neurons 3 and 4) (Gay, 1972).

It is now generally accepted that estrogen evokes the surge of LH during the cycle (Geschwind, 1972) and increasing levels of estrogen are associated with the LH peak in cycling rats (Motta et al., 1970), cycling cows (Christensen et al., 1971) and in cows experiencing the postpartum ovulatory surge of LH (Arije et al., 1974).

This action of estrogen may be via the preoptic LH surge center. It has been shown that estrogen exerts a stimulatory effect on LH and FSH release, via the anterior hypothalamic-preopticsuprachiasmatic region in rats, but the site of action is unknown in monkeys (Arimura and Findlay, 1974). In rats, estradiol is accumulated in the anterior hypothalamus (Kato and Villet, 1967) and this increases electrical activity in the preoptic area (Cross, 1973).

Small amounts of progesterone are needed in order for the estrogen evoked LH surge to occur in the rat (Motta et al., 1970). It is interesting to note that in the prepubertal and postpartum cow, no functional corpus luteum exists (Wagner et al., 1969) and that progesterone levels are low (Arjie et al., 1974; Gonzales-Padilla et al., 1975).

LaVoie and Moody (1971) gave indirect evidence for the existence of steroid binding sites in the head of the cow. They compared levels of progesterone in the carotid artery and jugular vein and noted that progesterone levels were highest in the carotid artery compared to the jugular vein on all days except on day -1, 0 and 1 in relation to estrus when they were highest in the jugular vein. It was postulated that progesterone was bound or used in the head as evidenced by the fact that more went to the head than came from the head. On the days around estrus this was reversed and it was suggested that high levels of estrogen were displacing progesterone from binding sites in the head.

Some factors affecting hypothalamic control. Estrogen, progesterone and testosterone also exhibit an inhibitory affect on gonadotrophin secretion but this action is on the tonic mechanisms and any inhibitory effects of steroids on the cyclic mechanism are not well understood (Armimura and Findlay, 1974). The various progestin compounds used to synchronize estrus (Lammond, 1964) and androgens

(Johnson et al., 1956; Berry et al., 1958) may block ovulation by inhibition of the cyclic release mechanism. Binding sites exist for testosterone in the preoptic area of the rat brain (Naess and Attramadal, 1974).

Currently there is much interest in the effects of prolactin secretion on gonadotrophin production and it is thought that the high levels of prolactin associated with early lactation may be related to postpartum anestrus (Han, 1973).

In the lactating rat follicular quiescence results from the suppression of LH and FSH and levels of these vary with the intensity of the suckling stimuli. Conversely, prolactin increases with increased suckling stimuli and evidence exists for an antagonism between gonadotrophin and prolactin secretion. This evidence includes: (1) observations that when endogenous prolactin is suppressed by auto feed back mechanisms (median eminence prolactin implants) the pituitary increases gonadotrophin secretion; (2) decreased ability of the gonadotrophin secreting pituitary to respond to a prolactin stimulator (perphenazine) until a gonadotrophin suppressor (methallibure) is given; (3) histological changes including a reduction in size and number of secretory granules in pituitary secreting cells and an increased number and size of gonadotrophins in response to prolactin producing pituitary tumors (MtT-W10 pituitary tumors) and (4) the fact that various neurotransmitters placed in the third

ventricle produce inverse effects on the secretions of gonadotrophins and prolactin (as reviewed by Han, 1973).

Extrapolating this concept to the cow one would suppose that postpartum anestrus in cattle may occur from insufficient gonadotrophin secretions but LH levels are not low in the postpartum cow as measured by radioimmunoassay (Han 1973; Arjie *et al.*, 1974). Also, FSH levels appear to be adequate to cause the follicular growth that occurs at this time (Wagner, *et al.*, 1969).

The ergot alkaloid, ergocorine, effectively reduces serum and pituitary levels of prolactin and LH in the rat, but does not interfere with ovulation nor induce alterations in the estrous cycle of rats (Wuttke *et al.*, 1971). Niswender (1974) using cycling ewes demonstrated that 2-Br- α -ergocryptine effectively reduced serum levels of prolactin to less than .05 ng/ml while controls showed levels ranging from 10 to 100 ng/ml. Serum levels of LH, FSH and progesterone were not different between ergot treated ewes and controls. Assuming that an antagonism exists between prolactin and the cyclic release of LH such a treatment should prove effective in reducing the postpartum interval of cattle. (Hubenov *et al.*, 1964 as quoted by Wagner, 1968) reported that ergotamine treatment in cows shortened the interval to uterine involution by 3.5 days and decreased days open by 32.3 days.

Pituitary levels of gonadotrophins

The data on pituitary hormone levels in the postpartum cow may be summarized by stating that LH levels generally rise after parturition while FSH is usually at a peak level at parturition and declines following parturition.

Saiduddin and Foote (1964) using pituitaries recovered from Hereford cows at 5, 17 and 30 days after calving reported increased depletion of ascorbic acid with advancing postpartum stage and suggested, based on limited numbers, that pituitary LH activity increases as postpartum anestrus progresses toward resumption of estrous cycles.

Utilizing 20 pluriparous Holstein cows Labhsetwar et al. (1964) found that pituitary activity of FSH was significantly higher on the day of calving compared with activity found 21 days postpartum and that the inverse was true for pituitary LH activity. It was found that follicular development was greatest at 21 days postpartum when pituitary FSH was lowest and it was suggested that the higher FSH activity on the day of calving was due to the rate of FSH synthesis having been higher for a period of time than its rate of release from the gland.

In the same study it was found that administration of 100 mg progesterone per day for 20 days postpartum did not affect pituitary FSH or LH activity nor did it depress follicular development. None of

the progesterone treated cows ovulated within the 21 day period but of the non-treated cows two ovulated without expressed estrus, one showed estrus and ovulated and the remaining two had no corpora lutea at slaughter 21 days postpartum.

Saiduddin et al. (1968) found FSH content was high on the day after calving and decreased by day 20 postpartum while LH increased in Holstein cows that were suckled or non-lactating. Although differences in pituitary gonadotrophin levels were not detected, ovulations occurred in the non-suckled cows by day 20 but not until day 30 in the suckled cows. In a similar study by the same group Riesen et al., 1968) it was found that suckling decreased pituitary prolactin activity.

Wagner et al. (1969) found a nonsignificant rise in pituitary LH concentration from day 7 postpartum to day 30 postpartum in grade Holstein cows. Neither nursing of calves nor anemia caused a significant change in pituitary LH content, but both control cyclic and thyroprotein fed cyclic cows had significantly higher levels of pituitary LH than did the postpartum cows.

Desjardins and Hafs (1968) noted that in rats and pigs (Ramirey and McCann, 1963; Parlow et al., 1964) pituitary LH and FSH was higher in prepuberal than in postpuberal animals, so they collected comparable data in the bovine. Pituitary FSH and LH were determined in 65 Holstein heifers slaughtered in groups of five at monthly intervals

from birth through 12 months of age. FSH concentrations were 1.7, 2.7 and 1.1 mcg equivalents NIH-FSH-52 per mg at 0, 1 and 2 months, respectively, but did not change significantly thereafter. Pituitary LH concentrations increased fourfold from 2.4 at birth to 9.1 mcg equivalents NIH-LH-B2 per mg at 3 months of age, varied considerably from 3 to 7 months and declined from 10.4 at 7 months to 4.8 mcg equivalents at 12 months. Pituitary LH in these heifers was twice that found in a group of 42 non-pregnant adult cows while FSH levels were equal. Puberty was associated with decreased levels of pituitary LH.

Blood levels of hormones

Only within the last 10 years have methods been developed to measure hormones in blood rapidly and reliably, and only within the last five or six years have these methods been turned toward the postpartum cow. Generally, the postpartum interval is marked by low levels of gonadal hormones and an absence of cyclic LH release. It appears that the resumption of cyclic LH release may be mediated by progesterone arising from an as yet unknown source.

Pope et al. (1969) measured plasma progesterone at two day intervals in eight Friesian cows from before parturition through several ovarian cycles but failed to comment on the patterns of progesterone secretion during the anestrus period. There appeared to be a slight elevation in progesterone prior to the first ovulation.

All of the cows but two had one or more silent ovulations and four had shortened first ovarian cycles (12, 14, 14 and 17 days). The mean peak progesterone level during the 10 days preceding ovulation with estrus was 9 ng/ml compared to 3.7 ng/ml preceding quiet ovulations ($P < .001$).

Donaldson et al. (1970) measured plasma progesterone in 13 beef cows bled twice weekly through the anestrus period and found that progesterone levels indicated that six animals exhibited estrus without a previous cycle, five animals had one silent estrus prior to the first observed estrus and two animals had two silent estrous periods. A small but highly significant increase ($P < .001$) in progesterone concentration was seen 3 to 4 days prior to the start of the first postpartum cycle in ten of the cows and this rise was associated with follicular activity. It must be pointed out that the specificity of the competitive protein binding assay was limited and it was not certain that the material measured was entirely progesterone.

Ingalls et al. (1971) collected jugular blood samples daily from 32 dairy heifers around parturition and twice weekly thereafter until first estrus. They found that LH did not change measurably, and that production fell from >200 ng/ml in the two days prior to parturition to 60 ng/ml 60 hr postpartum and ranged from 50 to 100 ng/ml until estrus. Growth hormone was elevated at parturition but no changes postpartum were noted.

Callahan et al. (1971) and Erb et al. (1971) reported on ovarian activity and urine and plasma hormone levels in 65 dairy cows which were classified into postpartum groups consisting of clinically normal cows, cows with metritis, cows with cystic ovaries and cows with both metritis and cystic ovaries. Blood and urine were collected at 0.5, 3, 8 and 13 days after calving and weekly thereafter. If estrus or ovulation intervened samples were collected on the day of expressed estrus or 0 to 2 days after silent estrus and weekly thereafter until the end of the ovarian cycle.

Urinary estrogens were high at calving (average total estrogens all groups = 1756 ng/mg creatinine) and decreased markedly by day 3 postpartum (226 ng/ml creatinine) and remained steady throughout the rest of the experimental period. There were no significant differences in urinary estrogens between genital condition groups. These authors indicated that estrogen synthesis may be high at this time as compared to normal estrous cycles occurring after fertility has been re-established.

No differences associated with time after calving were found for plasma progesterone, corticosterone and cortisol. Plasma progesterone was 8, 7 and 8 ng/ml at 0.5, 3 and 8 days postpartum which is much higher than that reported by Echternkamp and Hansel (1973), Corah et al. (1974) and Arije et al. (1974) who found progesterone levels to be low during postpartum anestrus. A follicular source was suggested for this

progesterone.

Plasma LH was significantly ($P < .005$) lower at 0.5 and 3 days than 8 and 13 to 60 days after calving. Plasma LH was consistently lower ($P < .05$) and plasma corticosterone was consistently higher ($P < .005$) in the metritis group than for other groups.

Sixty-five percent of the cows in these studies (Callahan et al., 1971; Erb et al., 1971) had their first postpartum ovulation unaccompanied by estrous behavior, but no obvious differences in hormones were found to explain the phenomena. A slightly different estrogen excretion pattern was noted 3 to 1 days prior to ovulation with cows having anestrus ovulations exhibiting lower levels in relation to levels before and after this 3 day period. Subnormal development of luteal function and decreased luteal life span was indicated following anestrus ovulations in the clinically normal cows.

Henricks et al. (1972) measured progesterone and total estrogens concurrently in daily plasma samples from 10 dairy cows and found that in three cows which were detected in estrus prior to 35 days postpartum progesterone levels were low but present in a cyclic pattern within 20 days after parturition. There was a peak of estrogen which coincided with estrus followed by a marked increase in progesterone levels. In 3 cows that were detected in estrus between 35 and 49 days, postpartum progesterone levels were lower and did not undergo the early cyclic pattern observed in cows that exhibited estrus by day thirty-five.

There was a peak in estrogen 20 to 25 days prior to estrus followed by a progressive rise in progesterone, then a decline indicating that a silent estrus occurred.

In three of the four cows that did not exhibit estrus until later than 60 days postpartum, estrogen remained below 10 pg/ml during the 60 day period following calving. In the other cow, estrogen peaked at 34 days postpartum and was followed by a 10 day increase in progesterone indicating corpus luteum development. In two of the three cows with low estrogen levels progesterone remained low, but in the other a cyclic pattern was observed with peak levels occurring on day 26 and 48 indicating the development of functional corpora lutea during each period.

These data demonstrated luteal function prior to the first detected estrus and it was noted that estrus coincided with the peak in estrogen in only those cows in which cyclic patterns in progesterone levels had been present prior to estrus. This indicates that the difference in postpartum interval appears to be related to the incidence of plasma progesterone.

Schams et al. (1972) collected blood at 6 hr intervals from eight cows over two complete pregnancies including the postpartum periods and measured LH in each sample and progesterone every 4 to 5 days. Unfortunately these authors did not report on the levels of these hormones in relation to the first postpartum estrus. Distinct LH

peaks were observed between 7 and 20 days postpartum but progesterone levels remained low and it was concluded that neither ovulation nor luteinization occurred.

Echternkamp and Hansel (1973) measured estradiol, progesterone, total corticoids and LH in plasma from 35 postpartum lactating Holstein cows at 4 day intervals. Estradiol levels fluctuated considerably between parturition and first estrus in some cows but in others remained low until 2 to 3 days prior to estrus. They related these erratic changes in estradiol to pituitary FSH release (Nalbandov and Casida, 1940) and variable follicular activity seen at this time (Morrow et al., 1966). Progesterone levels were low or undetectable from parturition until the development of the corpus luteum following first ovulation. Elevated progesterone levels found in a few individuals during postpartum anestrus were attributed to either follicular luteinization or adrenal progesterone secretion. Plasma LH concentration increased gradually from 1 to 2 ng/ml for the first 12 days postpartum which paralleled the increase in bovine pituitary LH content seen during the first 30 days postpartum by Saiduddin et al. (1966) and Wagner et al. (1969). Corticoid levels appeared unrelated to postpartum interval.

Corah et al. (1974) measured progesterone and estradiol in plasma collected at 3 day intervals from 12 first calf 2-year-old Hereford heifers fed two prepartum levels of energy. While energy levels

increased to the 4 to 7 pg/ml range and stayed at this level until first estrus. Progesterone declined from 2.5 ng/ml just prior to parturition to 0.9 ng/ml at parturition and 0.16 ng/ml 13 days postpartum. Levels remained low from 13 to 21 days postpartum then gradually increased until peaking at 0.93 ng/ml 1 day prior to estrus.

Of special interest in this study was the fact that of three heifers that conceived on the first postpartum estrus all had elevated progesterone levels (1.7 ng/ml) 1 to 3 days prior to estrus while none of the heifers that did not conceive showed elevated progesterone levels at this time (approximately 0.4 ng/ml). In three heifers exhibiting a short first estrous cycle (6 to 8 days) there was no increase in progesterone which was interpreted to mean that no functional corpora lutea had developed.

Arije et al. (1974) measured hormones in daily blood serum samples collected via indwelling jugular cannulae from three lactating (suckled), pluriparous beef cows during their postpartum anestrus which lasted an average of 98 ± 7 days. LH levels were low from parturition to estrus and varied from 0.5 to 3 ng/ml. An LH peak of 42.4 ng/ml occurred an average of 5 hr after the start of estrus. Progestins fluctuated between 0.2 to 2.0 ng/ml from the day after parturition through most of the postpartum interval. Serum progestins increased steadily from 0.3 ng/ml at 7 days before estrus to 2.0 ng/ml at 3 days before estrus and then fell to 0.2 ng/ml at estrus. Through most of

the postpartum period estrogens fluctuated around 200 pg/ml with occasional peaks of 300 to 400 pg/ml and increased during the 5 days preceding estrus from 149 pg/ml to as much as 540 pg/ml, indicating a rise in estrogens preceding the LH peak.

Mean prolactin levels fluctuated widely between 50 and 500 pg/ml the first 40 days of the postpartum interval but levels in one cow were five to 10 times higher than for the other two cows and for values observed routinely in the bovine. Prolactin fluctuated around 150 ng/ml from 30 days postpartum until 2 days before estrus when levels of 200 ng/ml were achieved. These elevated levels were maintained until 2 days after estrus. Corticosteroid levels varied between 11 and 62 ng/ml during the postpartum interval and reached relatively high values of 64, 48 and 52 ng/ml 2 days before, 1 day before and on the day of estrus.

Five suckled and five non-suckled primiparous Hereford heifers were used to study postpartum ovarian activity by Connor et al. (1974) and blood hormone analyses were conducted on three of the non-suckled and two of the suckled heifers by Tribble et al. (1974). The heifers were observed for estrus every 6 hr at the time of each blood collection via jugular cannulae, and rectal palpation was conducted every other day.

Tribble et al (1974) found that the hormonal changes preceding the first postpartum estrus were similar for the suckled and

non-suckled heifers with the only real difference being in the length of the postpartum interval (76 vs 47 days for the suckled and non-suckled, respectively). Serum prolactin levels were erratic but were higher at times of suckling than during non-suckling periods. LH levels fluctuated considerably throughout the postpartum interval in all five heifers with a major LH peak occurring at standing estrus. Six to 14 days preceding standing estrus a significant elevation of progesterone and 20 B-hydroxyprogesterone occurred, persisted for several days and declined rapidly one day prior to standing estrus.

One suckled and one non-suckled heifer showed elevated progestin levels a duration of 16 and 14 days, respectively, while the other suckled and two non-suckled heifers had a much shorter progesterone release period, with lower levels recorded. Also, there was elevated levels of LH approximately one cycle length prior to standing estrus. Of special interest was a peak of 17-hydroxyprogesterone that occurred at or near the time of estrus in four of the five heifers.

Connor et al. (1974) have postulated that the source of this progesterone must be from a luteinized follicle and that luteinization might be necessary to elicit the first postpartum standing estrus with ovulation. This postulation was based on the following observations: (1) No estrus, ovulation or corpus luteum was detected prior to the increase in progestins measured by Tribble et al. (1974) on the

same heifers, (2) both 17-hydroxyprogesterone, found only in follicular tissue (Cupps, 1968), and 20-B-hydroxyprogesterone, found only in luteal tissue (Saward and Telegdy, 1965), were found prior to estrus with ovulation, (3) it had previously been reported that luteinization begins before ovulation (Short, 1962; Lobel and Levy, 1968), (4) Short (1962) quantified both 17-hydroxyprogesterone and 20-B-hydroxyprogesterone from luteinized follicles, (5) an increase in ovarian size prior to first estrus was accompanied by an increase in plasma progestin levels and (6) since the major difference between the suckled and non-suckled groups was the length of the postpartum interval and not a difference in hormone profile it seemed likely that luteinization must be necessary to elicit the first postpartum estrus and ovulation.

Han and Moody (1974) measured LH, prolactin and progesterone in daily plasma samples collected from parturition through 17 days postpartum in two non-suckled and two suckled beef cows. Prolactin levels in the non-suckled but not in the suckled group exhibited a significant negative linear regression ($P < .05$) with increasing days postpartum and mean prolactin levels were lower ($P < .01$) in the non-suckled group compared to the suckled group. LH and progesterone levels were not significantly different between groups nor did they exhibit any significant regressions. In a combined analysis a significant ($P < .06$) correlation ($r = -.29$) between LH and prolactin was detected.

Three possibilities were discussed as factors involved in the regression of prolactin on days postpartum and in the group differences in mean prolactin levels: (1) Physiological differences between suckled and non-suckled animals may exist and the differences may indicate that reduced prolactin secretion is necessary for the initiation of estrus. The authors pointed out that estrus occurred on day 7 and greater than 26 days after parturition in the non-suckled and suckled groups, respectively, but failed to acknowledge that the negative regression of prolactin in the non-suckled group could be a result of the events leading to estrus and not the cause. The other factors discussed were (2) the variable response of prolactin to the stress of blood collection, and (3) between assay variation both of which may have contributed to the differences seen in the prolactin profile between groups.

It was stated that the significant negative correlation between LH and prolactin plasma levels support the concept of an antagonism between gonadotrophins and prolactin as demonstrated in the rat. No comment was made relative to the low postestrous levels of progesterone in the non-suckled group.

Gonzales-Padilla et al. (1973) measured blood serum concentrations of LH, FSH, GnRH, prolactin, progesterone and estradiol 17-B in samples collected at 6 hr intervals or less from six Angus heifers before and at puberty. There were no marked changes in FSH, prolactin, GnRH or

estrogens as puberty approached and there were no elevations of either estradiol 17-B or GnRH associated with LH peaks. LH levels fluctuated markedly but two distinct LH peaks were observed in every heifer: One occurred on day 0 ("pubertal LH peak") and the other occurred 8 to 10 days earlier ("priming LH peak"). Between days -20 and 0, every heifer showed two progesterone elevations. The first occurred between days -18 to -11 and always preceded the "priming" LH peak, and the second occurred between the "priming" and "pubertal" LH peaks. It was suggested that the source of the second progesterone elevation was probably from a luteinized follicle that occurred in response to the "priming" LH peak and that the first elevation was at least partially produced by the adrenal cortex.

Prolactin was negatively correlated with GnRH and estradiol-17-B during the prepubertal period but this correlation disappeared after puberty. Progesterone showed a small negative correlation with prolactin in the prepubertal period but these were positively correlated after puberty. This change in the relationship between prolactin and the hypothalamic and ovarian hormones after puberty suggests that certain modifications in the physiology of the hypothalamo-pituitary axis are associated with the onset of puberty.

These authors stated that their results indicate no deficiency in circulating levels of pituitary or hypothalamic hormones but that prepubertal anestrus is characterized by a lack of a cyclic pattern

of release of LH, which appears gradually and is apparently mediated by the action of progesterone.

Other factors of postpartum anestrus

There are no reports in the literature for plasma testosterone values for cows during the postpartum interval and to the knowledge of this reviewer only one report exists for testosterone levels in cycling cows. Shemesh and Hansel (1974) found that testosterone levels varied between 10 and 50 pg/ml during the estrous cycle and tended to vary with estrogen levels indicating an ovarian source. The possibility of an adrenal source was mentioned. Rice and Savard (1966) reported that androgens are the principal steroids secreted by human ovarian stroma during the follicular phase of the menstrual cycle and during pregnancy.

The adrenals may be involved in postpartum anestrus. Wagner et al. (1969) showed that adrenal cortisol and progesterone were lower in nursed compared to milked cows and Wagner and Oxenreider (1971) in a series of experiments suggested that blood levels of cortisol increased in response to milk removal. They further postulated that this cortisol increase may be due to increased oxytocin levels at this time and subsequent jugular infusions of oxytocin into non-lactating cows resulted in rises of plasma cortisol although carotid infusion of oxytocin did not elicit this response.

Adrenal involvement in the onset of puberty in female rats has been demonstrated. Gorski and Lawton (1973) showed that adrenalectomy

at 18 or 25 days of age delayed puberty in relation to controls and sham controls and that this delay was inhibited by autotransplantation of the adrenals. Adrenalectomy on day 35 did not delay puberty indicating that a period exists between day 25 and 35 during which adrenal secretions play a role in the maturation of the brain-pituitary ovarian axis.

The adrenals have been suggested by several research groups as a source of progesterone in postpartum cows (Corah et al., 1974; Echternkamp and Hansel, 1973; Wagner and Oxenreider, 1971) and in prepubertal heifers (Gonzalez-Padilla et al., 1975). Balfour and Comline (1957) have determined that the level of progesterone in bovine adrenal venous blood is 10 to 50 times less than the corresponding corticoid levels, but 10 to 100 times greater than the concentration of progesterone in arterial blood collected at the same time.

Another factor which may be involved in postpartum anestrus as suggested by McClure (1968) is hypoglycemia. Oxenreider and Wagner (1971) found that lowered energy intake and increased lactational stress delayed postpartum follicular growth and ovulation and decreased plasma glucose levels during the first 8 week postpartum. The data did not indicate where if at all, hypoglycemia exerts its influence, but it was mentioned that since brain tissue depends on glucose for energy, hypoglycemia may depress hypothalamic function, thus resulting in a loss of ovarian activity.

Wagner et al. (1969) found no effect of suckling or length of postpartum interval (slaughter at 7, 14 and 30 days postpartum) on pituitary TSH levels or on thyroid cell height in cows on normal diets, but in thyroprotein fed cows both pituitary TSH and thyroid cell height were depressed and the interval to first estrus increased by 20 days compared to controls.

Lactation and the Postpartum Interval

As previously discussed, suckled cows have a longer interval from parturition to first estrus than cows not suckled or milked (Graves et al., 1968; Oxenreider, 1968; Saiduddin et al., 1968; Oxenreider and Wagner, 1971). Also, the interval is increased by high milk production (Morrow et al., 1966; Saiduddin et al., 1968) and by nursing or 4X daily milking as compared to 2X daily milking (Clapp, 1937; Wiltbank and Cook, 1958). Suckling decreased the interval from parturition to uterine involution in some studies (Lauderdale et al., 1968; Riesen et al., 1968) but not in others (Wagner and Hansel, 1968; Wiltbank and Cook, 1958).

Mammary-Ovarian Relationships

The mechanism by which suckling alters postpartum ovarian activity is not known, but it probably is some combination of factors involved with the increased nutrient requirements at this time and a direct effect of intensity, frequency and means of milk removal. Lactating cows require more energy than non-lactating cows and it has been

demonstrated that insufficient nutrient intake at this time increases the postpartum interval (Dunn et al., 1968; Wiltbank et al., 1962), and decreases blood glycogen and ovarian follicular activity (Oxenreider and Wagner, 1971). However, Short et al. (1972) has shown a direct effect of the suckling stimulus independent of nutrition. Studies involving mastectomized cows (Short et al., 1972), sheep (Short et al., 1973), pigs (Peters et al., 1969) and rats (Inglebrecht, 1935) demonstrate the involvement of the mammary gland on reproductive activity.

Short et al. (1972) compared postpartum interval between mastectomized, intact non-suckled and intact suckled Angus cows. Rations were adjusted depending on lactational status to cause the cows to maintain body weight over the experimental period, thus isolating the effect of suckling and mastectomy from nutritional effects. Removing calves at birth shortened the postpartum interval and mastectomy shortened the interval even further with days from parturition to estrus averaging 65, 25 and 12 for the suckled, intact non-suckled and mastectomized groups, respectively. These treatments had no significant effect on the interval from calving to conception as cows with shorter postpartum intervals required more services per conception reflecting that the early estruses were infertile.

Results from fall lambing postpartum sheep (Short et al., 1973) indicate that suckling had little effect on the postpartum interval to

estrus compared to non-suckling and mastectomy, but suckling decreased ($P < .01$) the number of services required per conception. This decrease in services per conception probably reflects a more favorable uterine environment in suckled ewes compared to non-suckled ewes as it was further demonstrated in this experiment that suckling reduced uterine debris (7 vs. 27 g, $P < .01$) and debris free uterine weight (41 vs. 64 g, ($P < .01$)).

Peters et al. (1969) showed that mammillectomized and intact sows had less follicular development when baby pigs were present. This would indicate a psychological effect of the presence of pigs on the mammillectomized sows as they could neither lactate nor be suckled. It should be noted that suckling inhibits follicular development in sows (Lauderdale et al., 1965; Peters et al., 1969) while there appears to be no effect of lactation on follicular development in dairy cows (Saiduddin et al., 1968).

Sectioning of the spinal cord behind the first lumbar vertebra in lactating rats completely paralyzes that portion of the body that bears the last three pairs of nipples. If the cranial nipples are covered so as to force the young to suck the denervated nipples, the young starve. When healthy pups from a normal lactating mother are transferred to the denervated mother they also starve. If two of the innervated glands are exposed to suckling, lactation continues in

all the glands, irrespective of the nerve supply (Inglebrecht, 1935 as quoted by Grosvenor and Mena, 1971).

Effect of Suckling on Postpartum Physiology

Some of the effects of suckling on pituitary and blood hormone levels were discussed in the endocrinology section of this review and it appears that suckling has little effect on hormone levels with the exceptions of prolactin, oxytocin and corticoids.

Pituitary hormone levels. Suckling did not affect pituitary activity of LH in the postpartum bovine (Wagner et al., 1969) nor LH and FSH activity in postpartum cows (Saiduddin, et al., 1968) and fall lambing postpartum ewes (Short et al., 1973). Suckling caused a decrease in pituitary prolactin activity in postpartum dairy cows (Riesen et al., 1968).

Blood hormone levels. Tribble et al. (1974) and Connor et al. (1974) found no differences in patterns or levels of blood serum progesterone, 20 B-hydroxyprogesterone, 17-hydroxyprogesterone and LH between suckled and non-suckled beef heifers. They suggested that luteinization of a follicle may be necessary to elicit the resumption of ovarian cycles and estrus. If this is true, suckling may act by inhibiting the luteinization process.

Han and Moody (1974) found elevated blood plasma prolactin levels in suckled cows compared to non-lactating cows and they theorized that prolactin production may interfere with the hypothalamic

cyclic LH release mechanism.

Role of oxytocin and corticoids. Wagner and Oxenreider (1971) suggested that elevated blood levels of cortisol in responses to oxytocin may inhibit estrual activity and Peters et al. (1969) has demonstrated that oxytocin injection in intact and mastectomized sows inhibits follicular development. However, Short et al. (1972) cited unpublished data that demonstrated that daily injections of 40 IU oxytocin for 35 days postpartum did not effect postpartum interval in either intact or mastectomized cows. Also Cameron and Fosgate (1964) were unable to show any effect of oxytocin on postpartum reproduction in cows.

Techniques for Control of the Postpartum Interval

Effective control of the postpartum interval would enable producers to increase the number of cows breeding and conceiving early in the breeding season. Attempts to shorten the postpartum interval utilizing various nutritional regimes, early weaning, early breeding and exogenous hormone treatment have met with varying and often discouraging results.

Nutrition

Wiltbank et al. (1962) studied reproductive performance of mature Hereford cows on a high and low level of daily prepartum energy (9 and 4.5 lb TDN) and a high and low level of postpartum

energy (16 and 8 lb TDN) in a 2 x 2 factorial design. Postpartum interval to first estrus was 48, 43, 65 and 52 days, postpartum interval to conception was 51, 58, 75 and 62 days, the interval from first breeding to conception was 6, 17, 12 and 14 days and pregnancy rate was 95, 77, 95 and 20% for cows fed the high-high, high-low, low-high and low-low rations, respectively. Onset of postpartum estrus was delayed when the TDN intake was limited to one-half the recommended N.R.C. allowance either before or after calving. Also, the interval from first breeding to conception was longer in cows limited to one-half the N.R.C. recommended level of TDN after calving than for cows fed the recommended level. There was no apparent effect of nutrition on uterine involution.

Wiltbank et al. (1964) fed varying postpartum levels of energy to mature Hereford cows ranging from 50 to 150% of daily N.R.C. recommended allowances. Although the feeding of a higher than recommended level of TDN after calving did not decrease the interval from calving to first estrus, it did appear to increase follicular development and to exert a favorable influence on conception. The interval to estrus and conception was longer in cows receiving more or less than 100% N.R.C. recommended allowances but pregnancy rates were highest in cows receiving the high levels of postpartum energy.

Dunn et al. (1969) conducted an experiment to determine the effects of two precalving (8.7 and 17.3 megcal/day) and three

postcalving (14.2, 27.3 and 48.2 megcal/day) levels of energy intake upon the reproductive performance of 2-year-old Hereford and Angus heifers nursing their first calves. The onset of estrus was delayed in cows receiving the low level of energy before calving, while conception was directly related to the postcalving energy level. Eighty-seven percent of the cows fed the high energy level after calving were pregnant compared with 72 and 64% of those fed the moderate and low energy levels, respectively. The low pregnancy rate in the cows on low postpartum energy occurred because 30% of the Herefords and 9% of the Angus failed to show estrus.

Early Weaning

Several experiments have been conducted to investigate the possibility of applying early weaning as a means of improving reproductive performance. Early weaning may well be a feasible means of improving beef production providing that increased reproductive rate and decreased cow feed cost will cover increased costs that may occur due to decreased calf weights and increased feed requirements of the calves.

Smith and Vincent (1972) in an experiment involving both early weaning and exogenous hormone treatment were able to show that early weaning between 28 and 32 days postpartum shortened the interval to first estrus and tended to improve conception in beef cows. However, conception rates were unexplainably low in all the cows irrespective

of treatment and the hormone treatment (progestogen implant SC-21009 + FSH-P injections) improved conception in suckled cows but was detrimental in the early weaned cows (significant interaction, $P < .01$).

Laster et al. (1973b) conducted an experiment to determine the influence of weaning 8 days prior to a 42-day breeding period on postpartum reproduction in different ages and breeds of beef cattle. Early weaning increased the percentage of cows exhibiting estrus from calving until the end of the breeding season and increased the percentage of cows showing estrus the first 21 days of the breeding season in 2- and 3-year-olds but not in mature cows. Postpartum interval to first estrus was not significantly different between the early weaned and nonweaned groups, but those cows not showing estrus by the end of the breeding season were not included in the calculations.

Early weaning increased overall conception in the 42-day breeding season by 25.9% in the 2-year-old cows, 15.6% in the 3-year-old cows and 7.9% in cows 4 years old and older. The increased percentage of the cows from the weaned group that conceived during the 42-day breeding period was the result of the effects of weaning on the occurrence of estrus rather than on conception rate among those inseminated.

Bellows, et al. (1974) showed that early weaning at 3 or 10 days compared with 35 days shortened the postpartum interval to first estrus ($P < .01$) in beef cows regardless of whether they had given

birth to single calves or hormone induced multiple calves. Subsequent evaluation of the performance of the early weaned calves indicated that they made satisfactory gains under conditions of artificial rearing with cold milk or cold milk replacer. It was also demonstrated that inoculation of the calves rumens with rumen content from mature animals resulted in improved gain.

Early Breeding

Tradition has discouraged the use of early breeding as a means to shorten the calving interval because of supposed detrimental effects on subsequent reproductive performance (Casida et al., 1968). However, the literature reveals only limited evidence for detrimental effects (Williams, 1943; Hofstad, 1941; Trimberger, 1954) and very few experiments examine the long term effects of early breeding.

Olds and Cooper (1970) have demonstrated that shortening of the calving interval can be accomplished for many cows by breeding earlier and Whitmore et al. (1974) found no indication of a cumulative detrimental effect of early breeding (breeding at first postpartum estrus vs. breeding at first estrus after 74 days postpartum). The first-estrus breeding group had fewer days open than the later-estrus breeding group but required more services per conception reflecting the relative infertility of the first postpartum estrus. These authors stated that early breeding in these Holstein cows shortened the calving interval thereby increasing reproductive efficiency.

Exogenous Hormone Treatment

Many experiments have been conducted in an effort to alter postpartum reproductive activity by administering hormones including estrogens, progestins, gonadotrophins, other pituitary hormones and hypothalamic releasing hormones. Early work in this area indicated the possibility of terminating anestrus with progesterone and estrogen (Ulberg and Lindley, 1960) and pituitary hormones (Cole and Miller, 1933; Casida et al., 1943).

Subsequent research using various combinations and levels of estrogens and progestins have met with varying degrees of success. A large single injection of progesterone suspended in a starch emulsion (2.2 mg/kg body weight) delayed postpartum ovulation and estrus in beef cows when administered 14 days after calving (Foote et al., 1960b). However, smaller doses seemed to have an opposite effect and Foote and Hunter (1964) were able to show a decrease in the interval from calving to uterine involution, estrus and ovulation in beef cows receiving progesterone and estrogen alone or in combination. Progesterone was given at the rate of 50 mg per day from 12 through 23 days postpartum and estrogen was given on day 25. Treatment with progesterone alone did not influence conception while cows given both hormones or estrogen alone had lowered fertility at first estrus. However, the two groups given estrogen had the shortest interval to conception.

Foote and Saiduddin (1964) found that progesterone followed by estrogen decreased the interval from calving to conception from 61 to 46 days when compared to progesterone alone. Saiduddin et al. (1968) treated Hereford cows with daily progesterone injections (50 mg/day) followed by 10 mg of estradiol 2 days after the last progesterone injection. Different groups of cows received progesterone at different stages following calving (5 to 15, 14 to 23, 18 to 33, or 23 to 33 days postpartum) and estradiol was given to a portion of the cows from each group. Cows given estradiol alone or following progesterone treatment resumed ovarian activity earlier after calving than untreated or progesterone treated cows. The earlier treatment caused earlier ovulation but cows treated later tended to have the shortest interval to conception. Although one injection of 10 mg estradiol was as effective alone as when preceded by 10 to 51 daily injections of 50 mg progesterone in hastening ovarian activity, conception occurred earliest in cows given both hormones. Most treatments did not affect interval to uterine involution, although some tended to reduce it.

Saiduddin et al. (1968) gave 50 mg of progesterone per day to cows from day 5 through 15 postpartum and a 10 mg injection of estradiol on day 17 postpartum and found that it nonsignificantly reduced the interval from calving to first estrus and significantly reduced the interval to first ovulation and conception ($P < .05$). It did not, however, affect the interval to uterine involution.

Hill et al. (1972) used daily feeding of 10 mg chloradinone acetate (CAP) for varying length of time coupled with a .5 mg injection of estradiol-17 β given 72 hr after the last CAP feeding. The mature Hereford cows were fed CAP either 0, 20, 30 or 40 days starting on the day of calving and these treatments were not effective in reducing the interval to first estrus compared to controls except in the group fed for 10 days (51 vs. 61 days; $P < .01$). Treatment had no effect on interval to conception but uterine involution was delayed in the 40 day feeding group compared to the controls (85 vs. 80 days; ($P < .01$). Melengestrol acetate (MGA) simulated follicular development and decreased days open in postpartum Holstein cows (Britt et al., 1973).

Gonzalez-Padilla et al. (1975b) were able to induce puberty in beef heifers using various progestin-estrogen treatment schedules but were unable to show a definite effect on inducing estrus in postpartum beef cows. However, adequate controls were not employed. Twenty-six cycling and 32 anestrus lactating cows were given 6 mg of estradiol valerate plus 3 mg of norgestomet (I.M.) and 1 or 2 implants containing 6 mg of norgestomet each. Implants were removed after 9 days. Within 5 days after implant removal, 96 and 87% of the cycling and anestrus cows were detected in heat and pregnancy rates after a 28-day breeding period were 85 and 84%, respectively.

The ovaries of lactating cows are responsive to exogenous

gonadotrophic hormones (Casida, et al., 1943; Foote et al., 1966; Oxenreider, 1968) but responses reported have been variable at the same and different stages postpartum. Casida et al. (1943) found that exogenous pituitary gonadotrophins caused earlier development of larger follicles than controls and accelerated the appearance of estrus when cows were injected early in the postpartum interval. Foote et al. (1966) gave single injections of HCG to Hereford cows between 5 and 15 days postpartum and noted that the treatment shortened the interval from parturition to estrus and ovulation. HCG caused immediate ovulation in some cows. Oxenreider (1968) induced ovulation and CL development with PMS and HCG in four cows at 8¹/₂ days postpartum and estrus occurred approximately 20 days after the induced ovulations.

Brown et al. (1972) gave gonadotrophins to beef cows after progestogen and estrogen treatments to determine their influence on reproductive activity when administered at various stages postpartum. DHPA (16 α -17-dihydroxyprogesterone acetophenide) was fed for 9 days and estradiol valerate was injected on the second day of feeding to three treatment groups. On the day after last feeding, one of these groups received a second injection of estradiol valerate and another received 1000 IU PMS followed by 750 IU HGG at the time of insemination. These groups were compared to nontreated control. DHPA feeding was initiated 5 to 10, 10 to 15, 20 to 25, 30 to 35 or 40 to 45 days

postpartum.

Estrogen treatment following DHPA feeding had the greatest effect in reducing the interval from parturition to first estrus, first ovulation and conception, especially when administered early in the postpartum period. This treatment reduced the interval to first estrus, ovulation and conception by 12, 16 and 13 days, respectively. Gonadotrophin treatment following DHPA feeding did not significantly decrease the interval from calving to estrus, ovulation or conception but here also it appears that the response was greatest when treatment occurred early.

Smith and Vincent (1972) looked at the effect of gonadotrophin-progestogen-estrogen treatments in conjunction with early weaning. One-half of the early weaned (28 to 32 days postpartum) and one-half of the suckled cows were implanted with 6 mg SC-21009 at 30 days postpartum which were removed after 10 days. Each implanted cow received 2.5 mg FSH-P per day the last 4 days of implantation and 1 mg estradiol-17B on the last day. The effects of early weaning were previously discussed. The hormone treatment increased the proportion of cows exhibiting estrus and reduced the interval from parturition to first estrus. An interaction was detected ($P < .01$) between early weaning and hormone treatment on first service conception rate and interval from calving to conception. Hormone therapy reduced the conception rate to first service of early-weaned cows and lengthened

the interval to conception, but in the suckled cows, increased the conception rate to first service and shortened the interval to conception.

Other hormones have been used in attempts to alter postpartum physiology including growth hormone (GH), oxytocin and gonadotrophin releasing hormone (GnRH). Ovine GH administered to six Angus cows at the rate of 12.5 U.S.P. per day from 2 to 8 days postpartum shortened the interval from calving to ovulation, estrus, uterine involution and conception by 4, 24, 1 and 21 days, respectively. Only the interval to estrus was significantly shortened ($P < .05$) (Oxenreider, 1968). Cameron and Fosgate (1964) injected 200 U.S.P. units of oxytocin daily for 42 days in postpartum cows but were unable to affect postpartum reproduction.

Britt et al. (1974) administered 100 ug of GnRH or saline to 20 lactating Holstein cows on day 14 postpartum. Serum LH increased from 1.9 ng/ml at the time of GnRH administration to 15 ng/ml 4 hr later and then returned to basal levels. There was no acute response of progesterone to GnRH but the chronic response was similar to that observed during normal estrus cycles indicating that ovulation and luteinization had occurred. Rectal palpation verified that GnRH treatment was followed in one day by a silent ovulation and that the cows continued to cycle to day 65 when the study was terminated. The incidence of silent estrus was also high in second and third ovulations

with 50 and 40% of the cows in the GnRH treatment group showing first estrus at the second and third ovulations, respectively. In the controls, 25, 62 and 13% of the cows showed their first estrus at the first, second and third ovulation, respectively. It appears that GnRH has great potential as an agent to control postpartum reproduction and fertility.

Induced Parturition and the Postpartum Interval

It has been demonstrated that exogenous glucocorticoids can be used to control and synchronize calving under intensified and under range conditions (Adams, 1969; Adams and Wagner, 1970; Carroll, 1974; LaVoie and Moody, 1973a; Wagner et al., 1974; Winter et al., 1974). Induction of early parturition affects the postpartum interval by increasing the amount of time from calving to the breeding season which could improve reproductive efficiency, but it also increases the incidence of retained placenta to as much as 80% (Adams, 1949; Adams and Wagner, 1970; LaVoie and Moody, 1973a; Carroll, 1974; Jöchle, 1971; Wagner et al., 1974; Winter et al., 1974).

One report has been published which gives data on the effect of induced parturition on the postpartum interval. Bailey et al. (1973) has shown a decrease ($P < .05$) in the days from parturition to first estrus in cows induced to calve with a long acting corticoid as compared to controls. Cows induced to calve with long acting

corticoids have a low incidence of retained placenta but also have a high incidence of calf loss (Bailey et al., 1973; Welsh et al., 1973). These are interesting results and they indicate that the postpartum interval to first estrus is shorter in cows experiencing induced parturition without retained placenta, but a more practical approach would be to assess the effect of short acting corticoids which result in retained placenta, but also, live calves.

Retained placenta has traditionally been associated with metritis, infertility and long postpartum intervals, but in most studies retained placenta has occurred in conjunction with pathological conditions and dystocia (Morrow et al., 1966; Morrow, 1971). Also it has been an accepted practice to manually remove retained placental membranes (Wetherill, 1965) and this has been shown to be detrimental to fertility with or without antibiotic support (Banerjee, 1963, as quoted by Wetherill, 1965).

Another point to be made is that most cows have some degree of uterine infection following calving which raises the question as to whether micro-organisms in the postpartum uterus are a normal or abnormal situation. Elliott et al. (1968) reported that 93% of bovine uteri were infected from parturition to 15 days postpartum, 78% between 16 and 33 days, 50% between 31 and 45 days and 9% between 45 and 60 days. Thirty organisms were isolated, including streptococcus, staphylococcus, micrococcus, corynebacterium, pseudomonas and escherichia.

Wagner et al. (1974) induced parturition with dexamethasone and flumethasone in 294 cows and compared reproduction between cows with retained placenta (54%) to those without (46%). All cows with retained placenta were treated systemically with penicillin and streptomycin the day after calving but no attempt was made to remove the placenta manually nor was any intrauterine therapy used. There was essentially no difference in days open (81 vs. 82), services per conception (1.23 vs. 1.35) or calving interval (365.2 vs. 365.6) between those cows with retained placenta compared to those with no retained placenta.

Other field trials with beef cattle have demonstrated that reproductive performance is comparable between cows experiencing corticoid induced and natural parturition (Carroll, 1974; LaVoie and Moody, 1973b; Poncelet et al., 1974). Poncelet et al. (1974) reported pregnancy rates of 74 and 75% for range beef cows experiencing induced and natural parturition, respectively. Bellows (personal communication) has observed decreased fertility in cows following induced parturition.

In the above mentioned studies no attempts were made to manually remove placental membranes but all cows with retained placenta were treated with antibiotics. In a study by Lauderdale (1972) it was stated that cows were not treated for retained placenta, and although not explicitly stated, this is assumed to mean that they did not receive antibiotics. It was found that pregnancy rate and days open

in 11 flumethasone treated cows all of which had retained placenta was comparable to non-induced control cows with a very low incidence of retained placenta.

CHAPTER 3

HORMONE ASSAYS

The theory behind the radioimmunoassay (RIA) is the immunological response of the rabbit, in this instance, to develop an antibody to the injected foreign protein - steroid complex. This antiserum can be used to bind radioactive and unlabeled steroid in a competitive manner. Unlabeled, known amounts of steroid can be measured which in turn allow unknowns to be estimated through comparison to this standard curve.

Progesterone Assay

Materials

Progesterone was obtained from Sigma Chemical Company, St. Louis and used without further purification. All chemicals used were reagent grade. The solvents used for extraction were spectrophotometric grade, obtained from American Scientific Chemical, Portland.

Antisera to progesterone. The antisera to progesterone-11 α -bovine serum albumin (#337) and progesterone-6-B-bovine serum albumin (#869) were prepared, treated and donated by Drs. Niswender and Nett, (Niswender, 1973), Colorado State University, Fort Collins. Each antiserum was diluted to 1:50 in a 1:400 dilution of stripped normal rabbit serum (NRS). Stripped 1:400 NRS was prepared by adding 1 ml of buffer A, a 0.1M phosphate buffer (PH 7) containing 5.38 gm NaH₂PO₄, 16.35 gm Na₂HPO₄, 9 gm NaCl, 1 gm Na Azide and 1 gm Knox Gelatin in

1 liter double distilled water. Eight grams of ethanol washed Florisil for every 100 ml of 1:400 NRS was added, and the resulting mixture was placed on a magnetic stirrer for 2 hours. Following the stirring, the supernate was placed in large disposable test tubes and centrifuged at 2000 RPM for 30 minutes, after which the supernatant was decanted into storage bottles and stored at 4°C until used. By means of a pipette, 2.4 ml aliquots of the 1:50 dilution of antisera were transferred to small glass vials and frozen in a dry ice: pentane bath. The vials were stored in a freezer awaiting further dilution. The final concentration determination for the antiserum consisted of a dilution study comparing zero aliquots and zero standards over a wide range of dilutions. The zero aliquot represented total counts and the zero standard represent bound counts. Therefore, the dilution binding approximately 50% of the total counts could be identified for the assay procedure.

^3H - progesterone. Progesterone 1, 2, 6, 7 - ^3H (N), (SA 105 Ci/mM) was obtained from New England Nuclear, Boston. To remove the free tritium, 50 uCi was run through a 3 inch column packed with LH-20 Sephadex. The eluting solvent used was benzene:methanol (90:10 V:V). Approximately 0.5 ml was collected in each of 80 tubes. Ten ul from each tube was transferred to a scintillation vial. Counting fluid was added, the vials counted, and the results plotted on a graph with GPM versus tube number. The solvent in the peak tube and the tube

immediately preceding it was transferred to a 125 ml Nalgene bottle and dried down under a stream of nitrogen. Fifty ml of Buffer A were added to the bottle and 100 ul was counted. This was the stock solution from which the dilutions used for the assay and the recovery solutions were obtained. For the assay solution, an aliquot of the stock solution was diluted to approximately 12,000 cpm/200 ul and for the recovery solution, an aliquot was diluted to approximately 2400 cpm/100 ul. The solutions were stored at 4°C until needed.

Progesterone standards. A stock solution of progesterone was prepared and concentrations of 2560, 1280, 640, 160, 80, 40, 20 and 10 pg. per 100 ul were prepared by serial dilution. The progesterone was dissolved and the standards diluted with double distilled ethanol. They were kept at 4°C until needed.

Buffers. Buffer A was prepared as described earlier. Buffer B was the same as Buffer A, except it contained 5% Knox Gelatin. Buffer A and charcoal (dextran-coated charcoal) was prepared with 500 ml Buffer A, 2.5 gm Dextran T-80, and 1.25 gm charcoal "novit a" with fines removed. The fines were removed by washing six times with double distilled water and decanting after the charcoal settled for 5 minutes. The washed charcoal was dried in an oven at 100°C.

Scintillation fluid. The scintillation fluid was prepared with 21 grams of 2,5-Diphenyloxazole, 0.9 gram Phenylloxazolyphenyl and 300 grams Napthalene, which was dissolved in 3 liters of p-Dioxane.

Serum samples and lab standards. The serum samples and lab standards were kept solidly frozen until needed. The lab standards were two previously assayed samples, one a high value, one a low, which were run with every assay so that comparisons could be made between individual assays.

Radioimmunoassay procedures

Pre-extraction. An extraction tube was labeled for each serum sample, lab standard, and water blank. An Eppendorf pipette was used to transfer 0.5 ml of sample, lab standard, or double distilled water into each tube. To account for procedural losses, 100 ul of ^3H -progesterone (2400 cpm/100 ul) for recovery was added to every extraction tube. The tubes were lightly mixed with a vortex mixer at #2 speed and placed in the refrigerator to incubate overnight. One-hundred ul of ^3H -progesterone for recovery were also added directly to 4 scintillation vials, which were capped and put aside until the end of the assay when scintillation fluid was added. These 4 vials were used to determine a value for total counts (recovery).

Extraction. A second tube (16x125 mm) was labeled for each extraction tube and 1 ml of double distilled water was added to each. The extraction tubes were removed from the refrigerator and 3 ml of benzene:hexane (1:2 V:V) were added to each. The tubes were vortexed for 30 seconds at #5 speed, and placed in the freezer for 30 to 60 minutes, at which time the tubes were removed from the freezer and the

solvent layer was poured into the corresponding tube containing water. These tubes were then mixed for a few seconds and placed in the freezer until the water layer froze. The solvent layer was poured off into a corresponding scintillation vial and all the steps above were repeated for the second extraction.

Pre-assay steps. After the solvent from the second extraction was in the scintillation vials, the vials were completely dried down under a stream of nitrogen, and 1 ml of double distilled ethanol (room temperature) was added to each vial. The vials were immediately capped to prevent evaporation, and the ethanol was carefully rinsed around in the vials.

Two 300 ul aliquots were taken from each scintillation vial by means of an Eppendorf pipette, and placed in duplicate disposable assay tubes (12x75 mm). The aliquots in the assay tubes were dried in the nitrogen bath, and the scintillation vials were air dried for recovery. These recovery vials were used to determine the procedural losses. The amount of ^3H -progesterone for recovery extracted from the serum samples was determined, and this percent was used to later adjust the values obtained for the unknowns.

Duplicate standard curves were prepared by using a Micromedic to aliquot 100 ul of each standard concentration to corresponding assay tubes. The Micromedic Automatic Pipetor was used, and each 100 ul aliquot was flushed from the pipetor with 200 ul of doubly distilled

ethanol. The zero aliquots and zero standards were included as part of each standard curve; however, 100 ul of doubly distilled ethanol was pipetted into these assay tubes instead of progesterone. The unknown samples, lab standards, water blanks and standard curves were evaporated under nitrogen in a 45°C sand bath.

The samples were arranged in small metal test tube racks such that the unknown samples, blanks, and lab standards had a standard curve before and after them. (The sequence of treatments in the assay progressed in the same direction throughout the assay to allow for more uniformity.)

Assay additions. The anti-progesterone and ^3H -progesterone for assay were brought to room temperature, and the Micromedic Pipetor was set up to make the additions. First, each assay tube received a 200 ul aliquot of the dilute antisera, followed by gentle agitation (2 seconds on a Vortex set at 1.5) and then a 30 minute incubation period at room temperature to allow the progesterone and antisera to equilibrate. Next, 200 ul ^3H -progesterone for assay was added, followed by another 2 second agitation. The sample racks were then placed in a refrigerator at 4°C for 14 to 18 hours.

Final additions. It was critical to keep the assay tubes cold throughout the rest of the assay, so the final additions were made by taking only one rack of 20 tubes from the refrigerator at a time. The additions were chilled to 4°C and the racks returned to the refrigerator

immediately following the additions.

One-hundred ul of Buffer B were added to each tube using a 250 ul Hamilton syringe. This was followed by the addition of 1 ml of Buffer A and charcoal to all tubes except the zero aliquots. One ml of Buffer A was added to the zero aliquot (total count) tubes.

After the additions of the buffers, all samples were agitated by shaking the racks, and the tubes were arranged in 4 chilled centrifuge buckets which were placed in a refrigerated centrifuge at 4°C. A 10 minute time interval elapsed before the samples were centrifuged at 2500 RPM for 10 minutes.

One centrifuge bucket at a time was removed from the cold and 300 ul aliquots were transferred into counting vials. A 300 ul Eppendorf pipette was used to aliquot the samples, being careful to avoid contamination with charcoal.

Fifteen ml of scintillation fluid was distributed to all counting vials - the 4 total counts for recovery, the recovery vials, the standard curve and sample vials - and to four empty vials, to get a value for background. The vials were then counted on a Beckman liquid scintillation LS-100 system for 5 minutes.

Calculations. Calculations were done using the basic computer program listed in appendix table 9-1. The raw data (CPM) from the counter printout tape was first corrected for background by subtracting the average background CPM from each value.

The percent recovery for each sample, water blank or lab standard was calculated by dividing the CPM for the corresponding recovery vial into the average obtained for total count for recovery and multiplying by 100.

The average percent bound for each progesterone level of the standard curve was calculated and plotted against the corresponding picogram level.

By comparing the average standard curve to the percent bound values obtained for the unknowns, a value was obtained, which was expressed in picograms per 0.3 ml of unknown serum. This particular value was then adjusted by dividing it into the percent recovery for that particular sample, to adjust for procedural losses. This value, corrected for recovery, was adjusted again by multiplying by 6.66. Since only 0.5 ml was extracted, and only 0.3 ml was counted, this was done to express the answer as picograms per ml of unknown serum.

Testosterone Assay

Materials and procedures were the same for the testosterone assay except testosterone was used in place of progesterone and anti-testosterone in place of progesterone antibody. Anti-testosterone -3-BSA (667) was used as a dilution of 1:2400 and was donated by Dr. G. D. Niswender.

CHAPTER 4

BLOOD PLASMA PROGESTERONE AND TESTOSTERONE LEVELS IN POSTPARTUM BEEF COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI

Introduction

Following parturition in the cow the corpus luteum of pregnancy regresses and ceases to secrete progesterone (Labhsetwer et al., 1964; Gomes and Erb, 1965; Oxenreider, 1968; Stabenfeldt, 1968; Wagner and Hansel, 1969); and a period of time ensues in which no functional palpable corpus luteum exist on the ovary (Callahan et al., 1971; Connor et al., 1974). This time interval is terminated when a new corpus luteum develops in response to the first ovulatory surge of LH. Several experiments have found that elevated levels of progesterone exist before the first ovulatory surge of LH in the prepubertal heifer (Gonzales-Padilla et al., 1975a) and in postpartum cows (Donaldson et al., 1970; Erb et al., 1971; Henricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974; Corah et al., 1974; Tribble et al., 1974). This rise in progesterone prior to the first postpartum ovulation may play a role in the reestablishment of estrous cycles in the postpartum cow.

The physiological role of testosterone in the female is obscure and very few workers have attempted to measure it in the cow. Shemesh and Hansel (1974) found blood plasma testosterone levels between 10 and 50 pg/ml in cycling cows but no experiments have been reported for levels in postpartum anestrus cows.

The stimulus provided by the nursing of a calf increased the interval from parturition to first postpartum estrus when compared to milked cows (Clapp, 1937; Wiltbank and Cook 1958) and to non-lactating cows (Graves et al., 1968; Oxenreider, 1968; Saiduddin et al., 1968; Oxenreider and Wagner, 1971; Short et al., 1972). Experiments comparing 2X to 4X daily milkings (Clapp, 1937) indicated that the degree of increased postpartum interval varies with the level (frequency) of milk removal.

Objectives of this study were to quantify daily blood plasma levels of progesterone to characterize the progesterone profile during the postpartum interval, and to measure plasma testosterone to determine what levels exist in the anestrous period. Another objective was to determine the effect of three levels of suckling stimuli on the levels of these hormones and on the occurrence and intensity of estrous behavior.

Materials and Methods

Twelve Angus and six Hereford mature cows all on the same prepartum nutrition and management scheme were randomly assigned in equal numbers to three treatment groups. The first group was dried-off (calves removed) 3 days postpartum and did not lactate thereafter (NON-LACTATING). The cows in the second group were suckled by their calves 2X daily for one-half hour every 12 hr (SUCKLED 2X), and the cows in the third group were with their calves continuously and were

suckled ad libitum (SUCKLED AD LIBITUM) (table 4-1).

At parturition the cows were placed in one of three consecutive lots according to treatment group and calves from the SUCKLED 2X group were kept in an adjacent pen. Cows were removed from the experiment after they had experienced an observed estrous cycle of greater than 17 days with the exception of one cow which was inadvertently bred and conceived on her first postpartum estrus.

To minimize the effects of nutrition on the postpartum interval a level of energy was fed that was calculated to maintain postpartum body weight over the experimental period and was adjusted for lactational status. The two lactating groups received 6.7 kg TDN daily while the NON-LACTATING group received 5.1 kg TDN daily (table 4-1). All cows were weighed within one week prior to calving, within 48 hr after calving and weekly thereafter until the end of the experiment.

The cows were observed 2X daily for the detection of estrus and a sterile bull and Kmar¹ heat patches were employed as heat detection aids. The bull was kept separate from the cows and was allowed to spend one-half hour in each lot of cows 2X daily at approximately a 12 hr interval. Intensity of estrous behavior was scored as follows: 0=no estrous behavior observed, 1=slight estrous behavior, 2=active, non-standing estrous behavior, 3=very active but non-standing estrous

¹Kmar, Inc., Steamboat Springs, Colorado.

TABLE 4-1. EXPERIMENTAL DESIGN

	Cows dried off 3 days postpartum (NON-LACTATING)	Calves allowed to suckle twice daily (SUCKLED 2X)	Calves continuously with cows (SUCKLED <u>AD LIBITUM</u>)
No. of cows			
Angus	4	4	4
Hereford	2	2	2
Total	6	6	6
Postpartum ration (TDN, kg)			
Alfalfa hay	4.5	4.5	4.5
Concentrate ^a	.6	2.2	2.2
Total	5.1	6.7	6.7

^a 50% oats and 50% barley.

behavior and 4=standing estrous behavior. In addition the vulva and vagina of each cow scoring >0 but <4 was inspected as an aid to determine if the cows were actually in heat. The vaginal membranes of cows in estrus tend to be red, inflamed and covered with a highly viscous mucus compared to anestrus cows whose membranes are relatively pale and dry (Roberts, 1971). Rectal palpation of ovaries was conducted at weekly intervals for the determination of corpus luteum development.

Jugular blood samples were obtained in heparinized tubes via daily venipuncture and were stored in a refrigerator until centrifuged the following day. Plasma was stored at approximately -20°C until assayed. Progesterone was assayed in all plasma samples from each cow from parturition through the first postpartum estrus and in samples taken on days 9, 10 and 11 of the first estrous cycle. Three cows, each from one treatment group, with similar length postpartum intervals and with expressed estrus at first ovulation, were selected to have their plasma analyzed for testosterone. Testosterone was analyzed at 3 day intervals from parturition through the first postpartum estrus.

Radioimmunoassay procedures for progesterone and testosterone were modifications of assays as used in the laboratory of Dr. H. D. Hafs (Louis et al., 1973 and personal communication). Anti-progesterone -6B-BSA (869), anti-progesterone -11 α -BSA (337), and anti-testosterone -3-BSA (667) were donated by Drs. G. D. Niswender and T. M. Nett. It was necessary to change from progesterone antibody #869 to #337 after

several assays had been completed because of an apparent degradation of the former antibody.

Progesterone assays were done by time blocks of about 11 days each, i.e., all samples from a particular 11 day unit of time in relation to the first observed postpartum estrus were done in consecutive assays. All assay reagents were from the same lot within a block. Time blocks, in the order they were completed were -10 through 0 days, - 21 through -11 days, parturition through -20 days and 0 through 11 days in relation to the first observed postpartum estrus. One cow from each treatment was included in an assay when possible. A standard plasma sample containing approximately 6 ng of progesterone was included with each assay and the values were used to adjust the samples values of each assay to the standard to minimize between assay variation. All samples for testosterone were analyzed within one assay.

In the progesterone assay recovery of progesterone was calculated using triated progesterone and recoveries of greater than 100% were often found. The highest recovery obtained within each assay was set at 100% and all other recoveries within the assay were adjusted accordingly. data were analyzed by the least squares method as given by Harvey (1960). Non-detectable plasma progesterone levels were assigned a value for statistical analysis. This value was .05 ng/ml which was the average of 0 ng/ml and .1 ng/ml which was the sensitivity of the assay. Two analyses were run; one including three cows with silent first ovulation

and the other excluding these cows. Regression analysis of progesterone levels for the first 19 days postpartum, the last 19 days postpartum and the first 11 days of the first observed estrous cycle was done by fitting orthogonal polynomials to the data. Time of ovulation was not determined in this experiment; therefore, regressions analysis of progesterone levels the last 18 days postpartum were conducted with the cows aligned on first observed estrus regardless of whether they had shown evidence of a previous silent ovulation.

Regression analysis of testosterone levels was conducted from parturition through the first postpartum estrus and the regression analysis of body weights included weights from one week prior through six weeks post parturition.

Results and Discussion

Individual cow data on postpartum weight changes, the occurrence and intensity of estrus, corpus luteum development and blood plasma progesterone levels are found in the Appendix, Figures 9-1 through 9-18. Examination of the individual palpation and plasma progesterone data reveals that two cows (235 and 615) in the NON-LACTATING group and one cow (634) in the SUCKLED AD LIBITUM group had their first ovulation in the absence of observed estrous behavior (silent estrus). Two cows (929 and 628) in the SUCKLED 2X group had short first observed estrous cycles of 8 and 12 days, respectively. In cows with expressed first

estrus there appears to be an elevation or spike of plasma progesterone that occurs 2, 3 or 4 days prior to the first estrus and prior to a palpable corpus luteum. Exception to this is found in two cows (142 and 715) from the NON-LACTATING group and the two cows (929 and 628) in the SUCKLED 2X group which had short first estrous cycles.

Average body weight for all cows and all weights recorded from within 7 days prior to estrus through 6 weeks postpartum was 521.2 ± 3.5 kilograms. There was no significant regression of body weight over this time period either within treatment groups or across all cows (figure 4-1). Weight changes from within 48 hr postpartum through the week of the first observed estrus were -2, +12 and -8 kg for the NON-LACTATING, SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively, (table 4-2). Weight changes were essentially the same when analyzed with the silent estrus cows excluded (table 4-3).

In the analysis including cows with silent first ovulation (table 4-2) it was found that the intensity score for first estrus and/or ovulation was $1.5 \pm .5$ reported in the SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively. Intensity of first observed estrus was also lower ($P < .05$) in the NON-LACTATING group compared to the two lactating groups but intensity of the second observed estrus was unaffected by group.

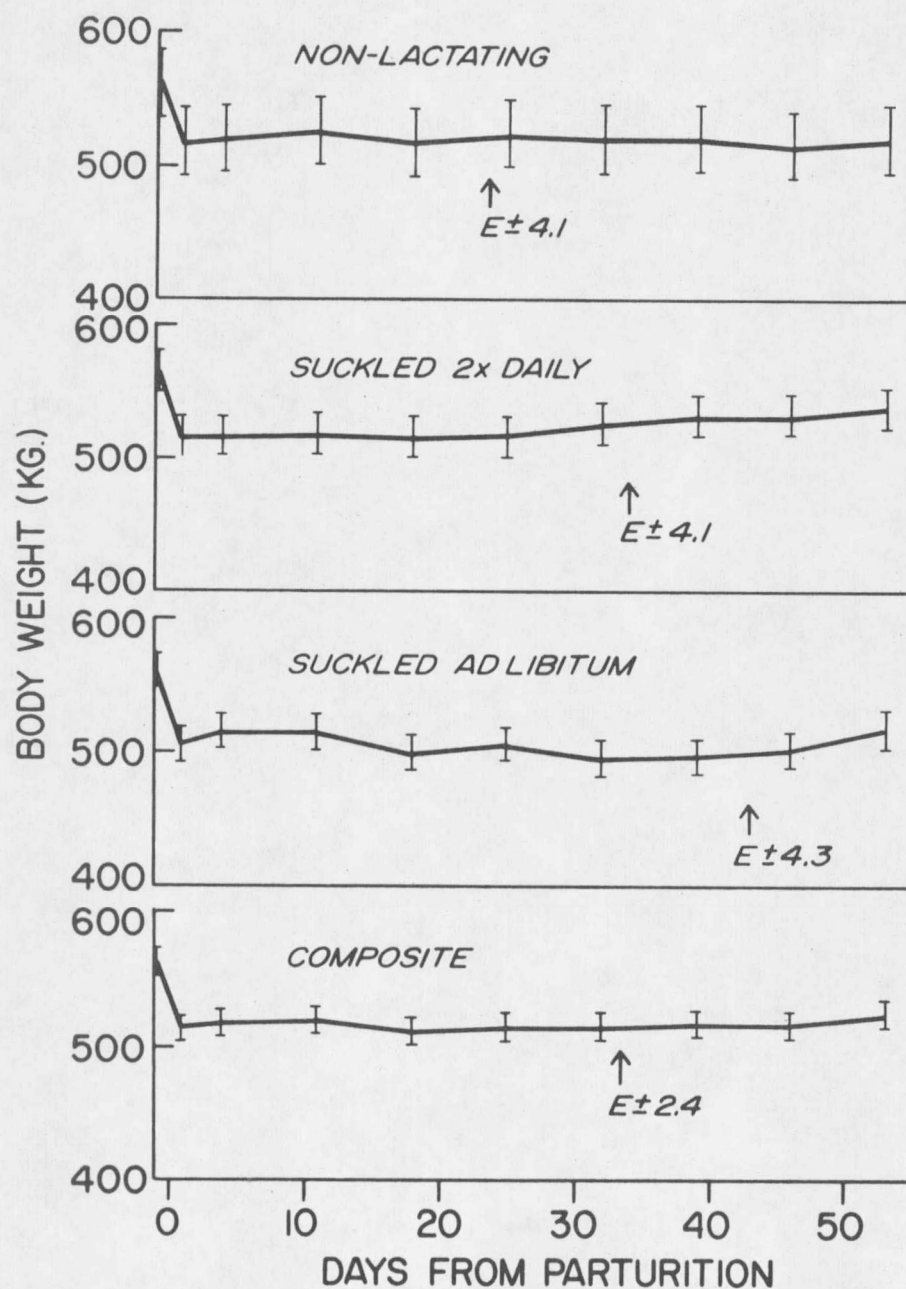


Figure 4-1. Body weights of cows following parturition with the average day of first observed postpartum estrus depicted by $E \pm S.E.$

TABLE 4-2. EFFECT OF THREE LEVELS OF SUCKLING STIMULI ON BEHAVIORAL ESTRUS AND PLASMA PROGESTERONE LEVELS IN POSTPARTUM BEEF COWS INCLUDING THREE COWS WITH SILENT FIRST OVULATION^a

Measurement	NON- LACTATING	SUCKLED 2X DAILY	SUCKLED <u>AD LIBITUM</u>
No. of cows	6	6	6
Body wt within 7 days prior to parturition (kg)	562(19)	565(19)	561(19)
Body wt within 48 hr postpartum (kg)	518(19)	515(19)	507(19)
Body wt within 7 days of first observed estrus (kg)	516(18)	527(18)	499(18)
Incidence of silent (0) 1st estrus (%)	33	0	17
Intensity of 1st estrus and/or ovulation ((0-4)	1.5(.5) ^b	3.8(.5) ^c	3.2(.5) ^c
Intensity of 1st observed estrus (1-4)	2.5(.5) ^b	3.8(.5) ^c	3.2(.5) ^c
Interval from parturition to 1st observed estrus (days)	24(4) ^b	34(4) ^{bc}	43(4) ^c
Intensity of 2nd observed estrus (1-4)	3.0(.4)	3.5(.4)	3.2(.5) ^h
Length of 1st observed estrous cycle (days)	19(2)	18(2)	21(2) ^h
Incidence of non-standing (1-3) estrus behavior (%)	58	25	33
Blood plasma progesterone levels (ng/ml)			
Avg of days 6, 7 and 8 prior to 1st observed estrus	2.5(1.2)	.3(1.2)	1.5(1.2)
Avg of days 2, 3 and 4 prior to 1st observed estrus	1.7(.7)	.9(.7)	2.6(.7)
Highest level occurring on day 2, 3 or 4 prior to 1st observed estrus	2.6(1.2)	1.4(1.2)	4.6(1.2)
Avg 1st 19 days postpartum (n=86, 96 and 107)	.5(.1) ^d	.1(.1) ^e	.2(.1) ^e
Avg last 19 days postpartum (n=87, 101 and 110)	1.4(.2) ^d	.3(.2) ^e	1.2(.2) ^d
Avg of days 0, 9, 10 and 11 of 1st observed estrous cycle (n=24, 15 and 23)	2.6(.4)	3.4(.5)	2.5(.4)

^a All averages and percentages are least squares means. Figures in parenthesis are standard errors.

^{bc} Means on the same lines with different superscripts differ significantly, $P < .05$.

^{de} Means on the same lines with different superscripts differ significantly, $P < .01$.

^h Average for 5 cows.

TABLE 4-3. EFFECT OF THREE LEVELS OF SUCKLING STIMULI ON BEHAVIORAL ESTRUS AND PLASMA STEROID LEVELS IN POSTPARTUM BEEF COWS EXCLUDING THREE COWS WITH SILENT FIRST OVULATION^A

Measurement	NON- LACTATING	SUCKLED 2X DAILY	SUCKLED AD LIBITUM
No. of cows	4	6	5
Body wt within 7 days prior to parturition (kg)	543(19)	565(16)	549(17)
Body wt within 48 hr postpartum (kg)	500(20)	515(16)	498(18)
Body wt within 7 days of first estrus (kg)	501(19)	527(15)	492(17)
Intensity of first estrus (1-4)	2.2(.4) ^b	3.8(.3) ^c	3.8(.3) ^c
Interval from parturition to first estrus (days)	20(4) ^b	34(3) ^c	38(4) ^c
Intensity of 2nd estrus (1-4)	3.0(.5)	3.5(.4)	3.3(.5) ^h
Length of 1st estrous cycle (days)	18(2)	18(2)	22(2) ^h
Incidence of non-standing (1-3) estrus behavior (%)	63	25	30
Blood plasma progesterone levels (ng/ml)			
Avg of days 6, 7 and 8 prior to first estrus	.4(.3)	.3(.2)	.6(.2)
Avg of days 2, 3 and 4 prior to first estrus	.7(.4) ^f	.9(3) ^f	2.0(.4) ^g
Highest level occurring on day 2, 3 or 4 prior to first estrus	.9(.8) ^b	1.5(.7) ^b	3.9(.7) ^c
Avg first 19 days postpartum (n=51, 96, 90)	.4(.02) ^d	.1(.01) ^e	.1(.01) ^e
Avg last 19 days postpartum (n=50, 101, 92)	.4(.1) ^{bc}	.3(.1) ^b	.5(.1) ^c
Avg of days 0, 9, 10 and 11 of 1st estrous cycle (n=16, 15 and 19)	2.1(.5)	3.5(.5)	2.8(.4)
Blood plasma testosterone levels (ng/ml) of 3 cows (1/treatment)			
Avg parturition through first estrus (n=10, 10 and 10)	.4(.1) ^d	.1(.1) ^e	.1(.1) ^e

^a All averages and percentages are least squares means. Figures in parenthesis are standard errors.

^{bc} Means on the same lines with different superscripts differ significantly, $P < .05$.

^{de} Means on the same lines with different superscripts differ significantly, $P < .01$.

^{fg} Means on the same lines with different superscripts differ significantly, $P < .08$.

^h Average for 4 cows.

The first observed estrous cycle was 18 ± 2 , 18 ± 2 and 21 ± 2 days in the NON-LACTATING, SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively. The postpartum interval to first observed estrus was 10 ($P > .05$) and 19 ($P < .05$) days shorter in the NON-LACTATING group as compared to the SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively.

Results for estrus intensities and interval lengths were similar when the data were analyzed excluding the cows with silent first ovulation. The intensity score for first estrus was lower ($P < .05$) in the NON-LACTATING group compared to the two lactating groups and the score for the second estrus was again unaffected by group. The proportion of first and second estruses that were characterized by non-standing behavior (score > 0 but < 4) was 63, 25 and 30% in the NON-LACTATING, SUCKLED 2X and SUCKLED AD LIBITUM group, respectively. The interval from parturition to first estrus was 20 ± 4 , 34 ± 3 and 38 ± 4 days for the NON-LACTATING, SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively, with the NON-LACTATING group being significantly different ($P < .05$) from the other two groups. The length of the first estrous cycle was unaffected by treatment.

Patterns of changes in plasma progesterone the first 19 days postpartum were described by quartic equations in analyses including and excluding cows with silent first ovulation ($P < .01$). In the analysis excluding cows with silent estrus, plasma progesterone levels averaged $.8 \pm .1$ ng/ml at parturition, ranged between $.1 \pm .1$ and $.3 \pm .1$

ng/ml from day 2 through 17 and increased to .4 ng/ml at day 18 postpartum. In the analysis including cows with silent first ovulation progesterone levels were similar ranging from $.1 \pm .2$ and $.9 \pm .2$ ng/ml and the pattern of changes were similar with the exception of a .8 ng/ml peak of progesterone that occurred on day 7 post partum (figures 4-2 and 4-6). Regression analysis of individual treatment groups revealed similar patterns of changes in progesterone (figures 4-3 through 4-5 and 4-7 through 4-9).

Average plasma progesterone levels over the first 19 days postpartum were higher ($P < .01$) in the NON-LACTATING group compared to the two non-lactating groups. Average levels of progesterone were $.4 \pm .02$, $.1 \pm .01$ and $.2 \pm .01$ ng/ml in the NON-LACTATING or SUCKLED 2X and SUCKLED AD LIBITUM group, respectively, when the analysis excluded the cows with silent first ovulation. Means were similar but variation was larger when the analysis was done including those cows with silent first ovulation.

Plasma progesterone levels the last 18 days postpartum followed different patterns of changes depending on whether cows with silent first ovulation were included in the analysis.

In the analysis including cows with silent first ovulation, patterns of changes in plasma progesterone the last 18 days of the postpartum interval were described by a cubic equation ($P < .01$) (figure 4-2). Elevated levels between .5 and 4 ng/ml occurred over an

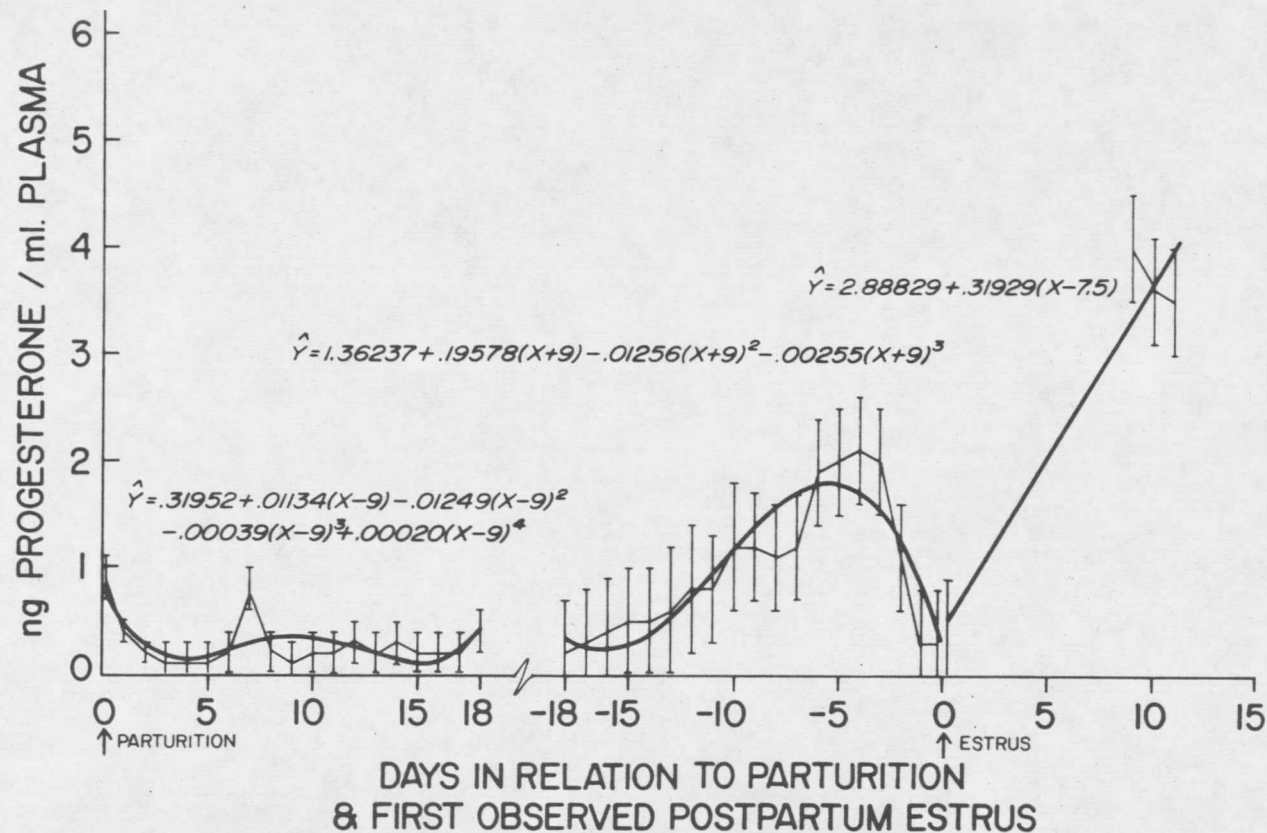


Figure 4-2. Mean blood plasma progesterone levels and regression equations across all treatments (including cows with silent first ovulation) from 0 to 18 days postpartum 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle.

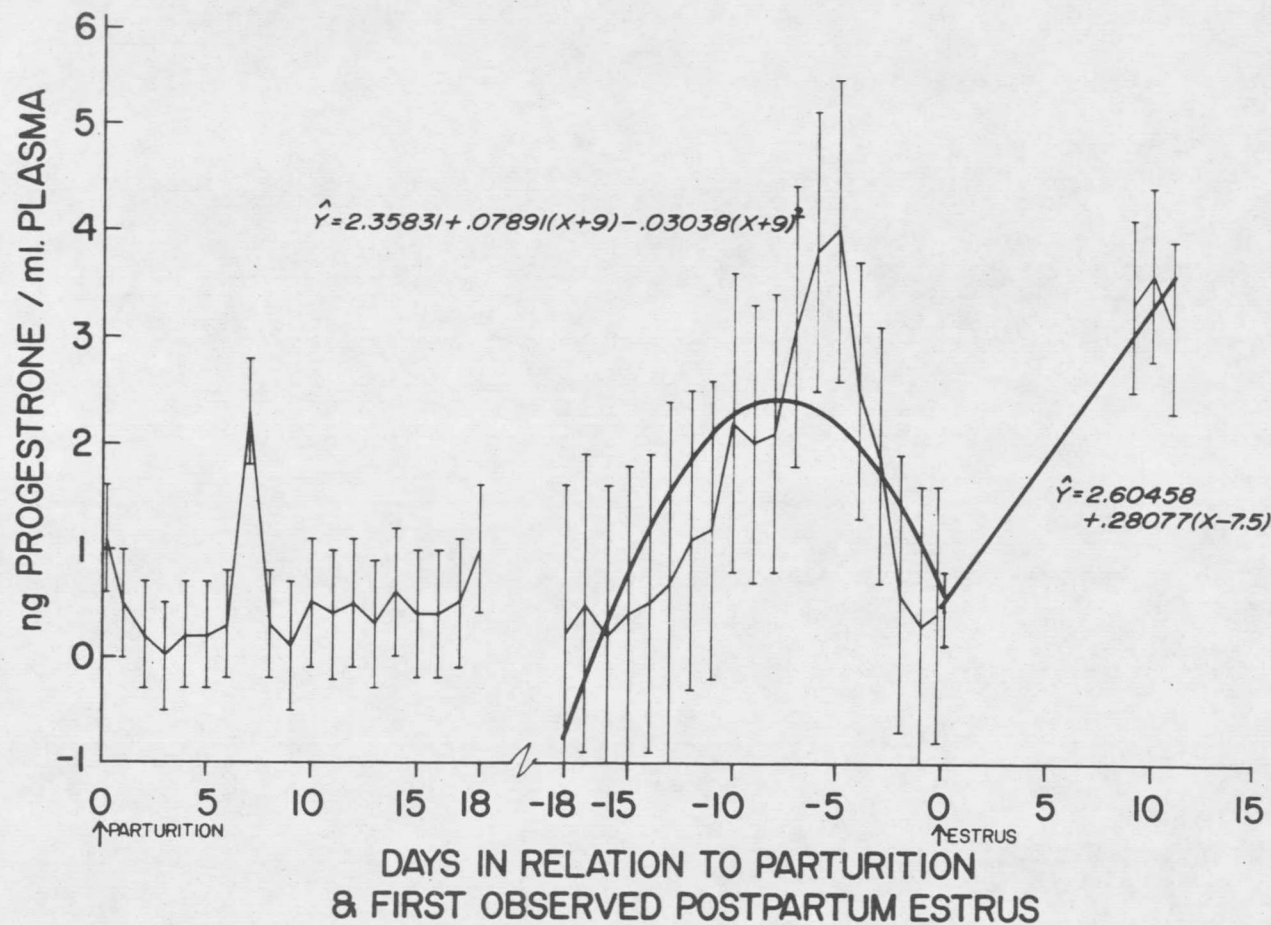


Figure 4-3. Mean blood plasma progesterone levels and regression equations for the NON-LACTATING treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle.

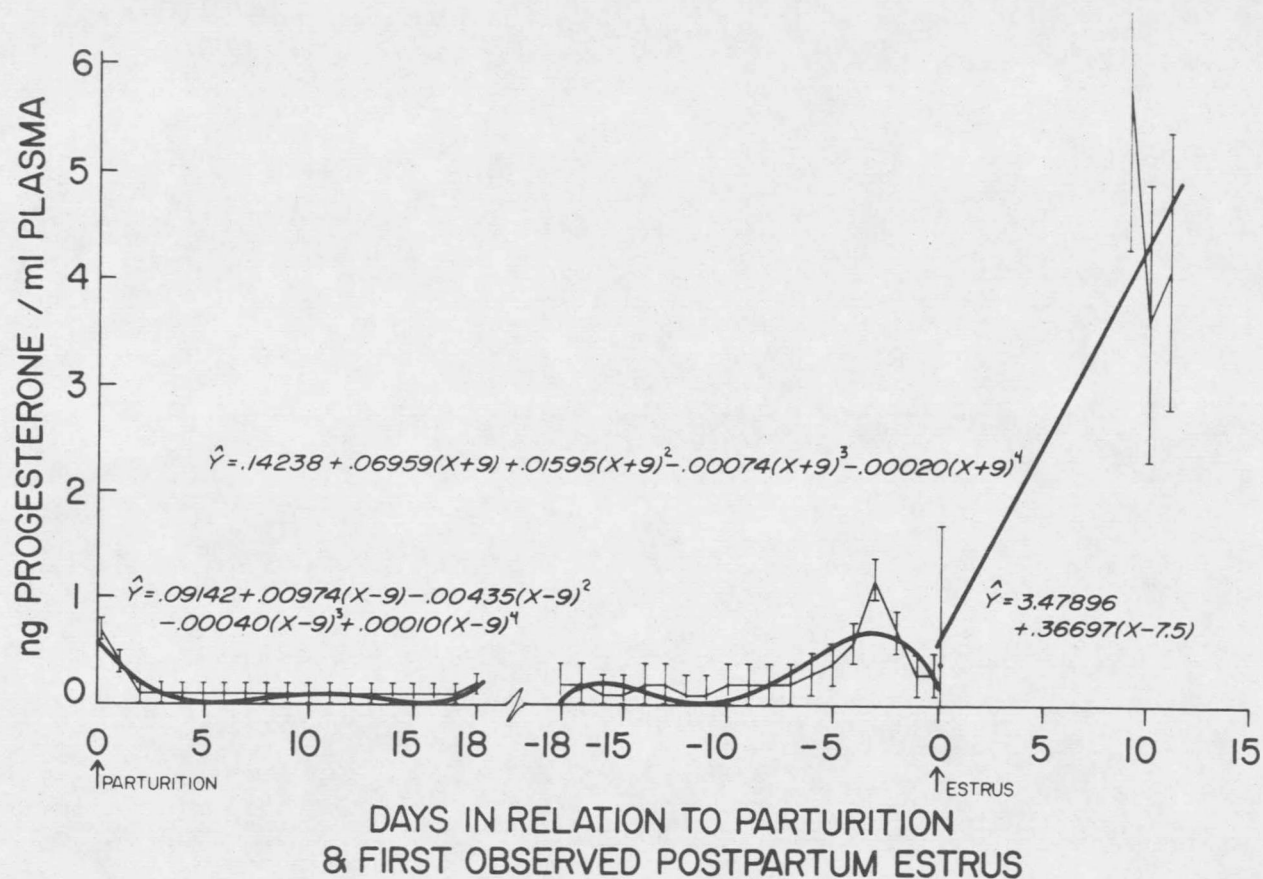


Figure 4-4. Mean blood plasma progesterone levels and regression equations for the SUCKLED 2X treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle.

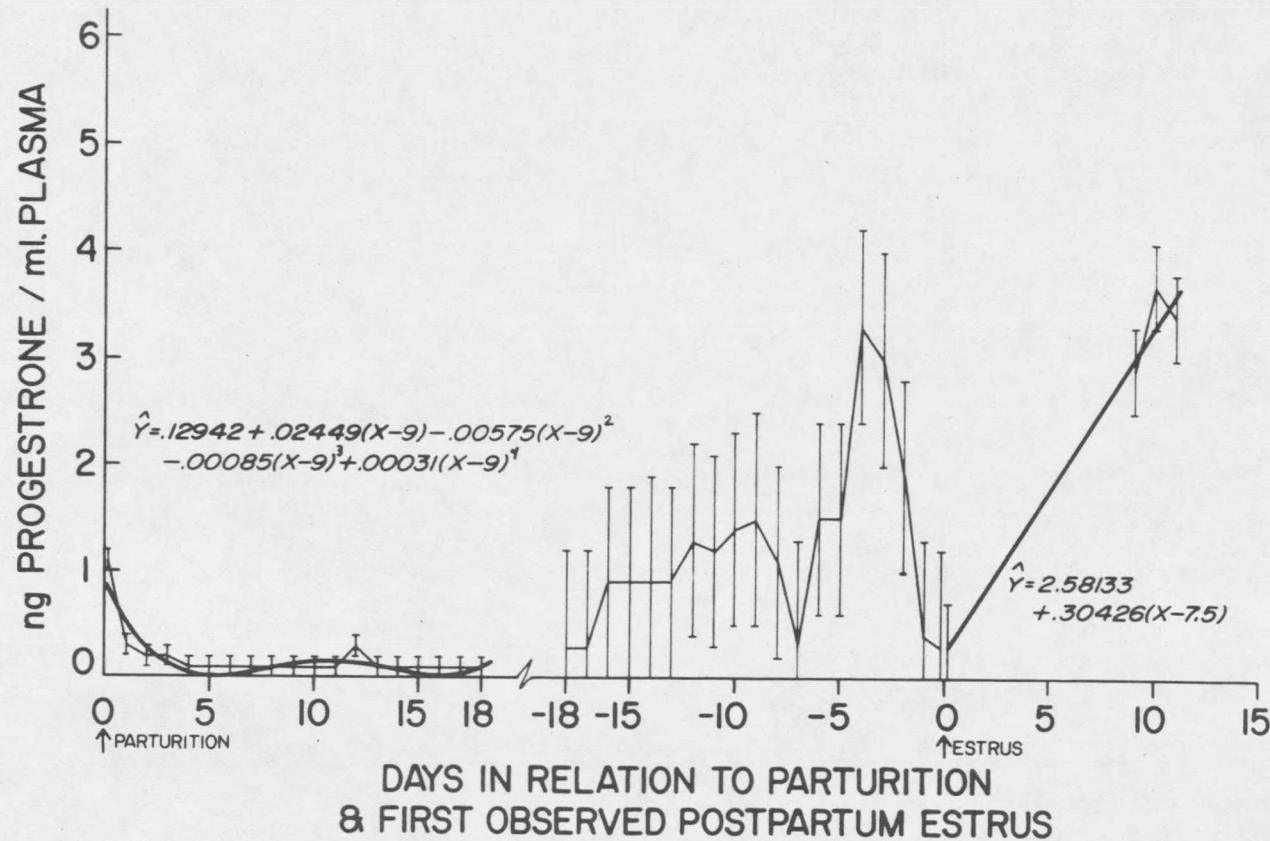


Figure 4-5. Mean blood plasma progesterone levels and regression equations for the SUCKLED AD LIBITUM treatment (including cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first observed estrus and from 0 through 11 days of the first observed estrous cycle.

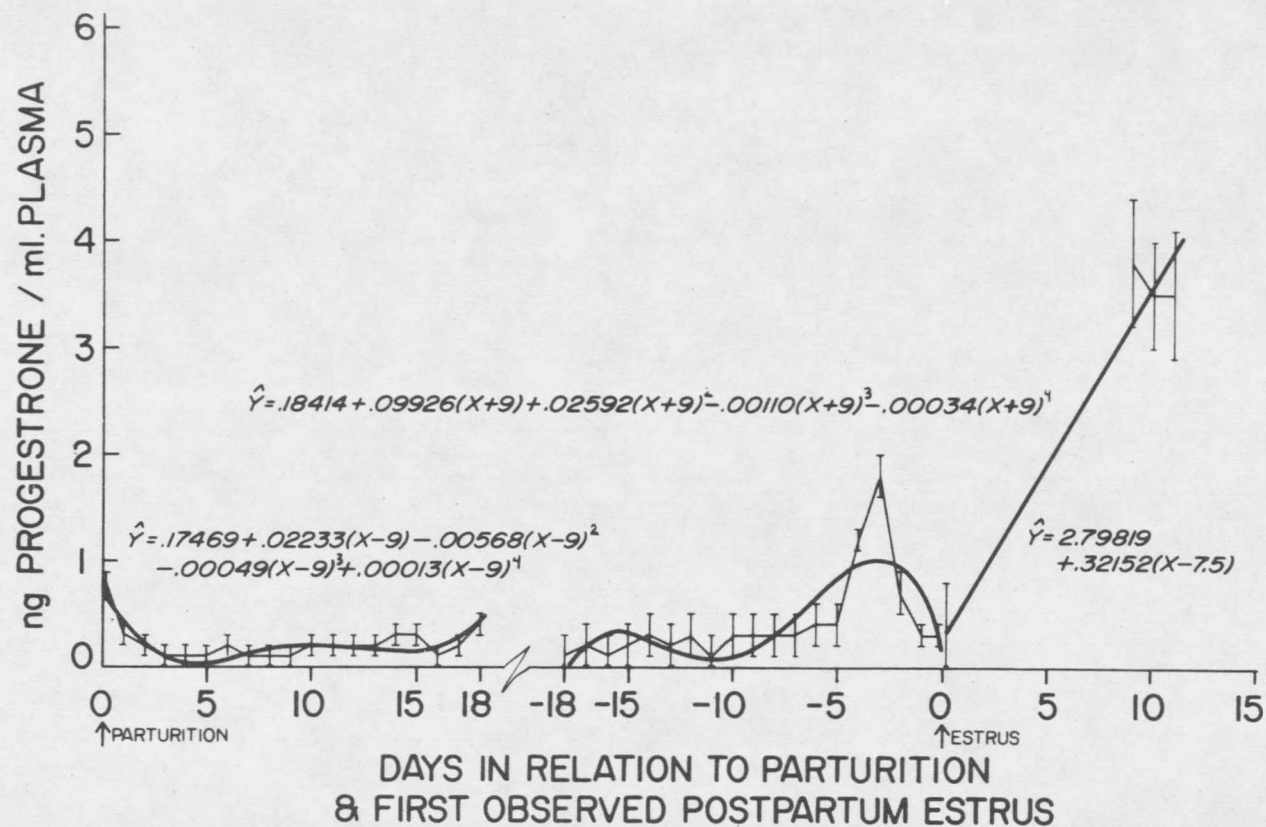


Figure 4-6. Mean blood plasma progesterone levels and regression equations across all treatments (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle.

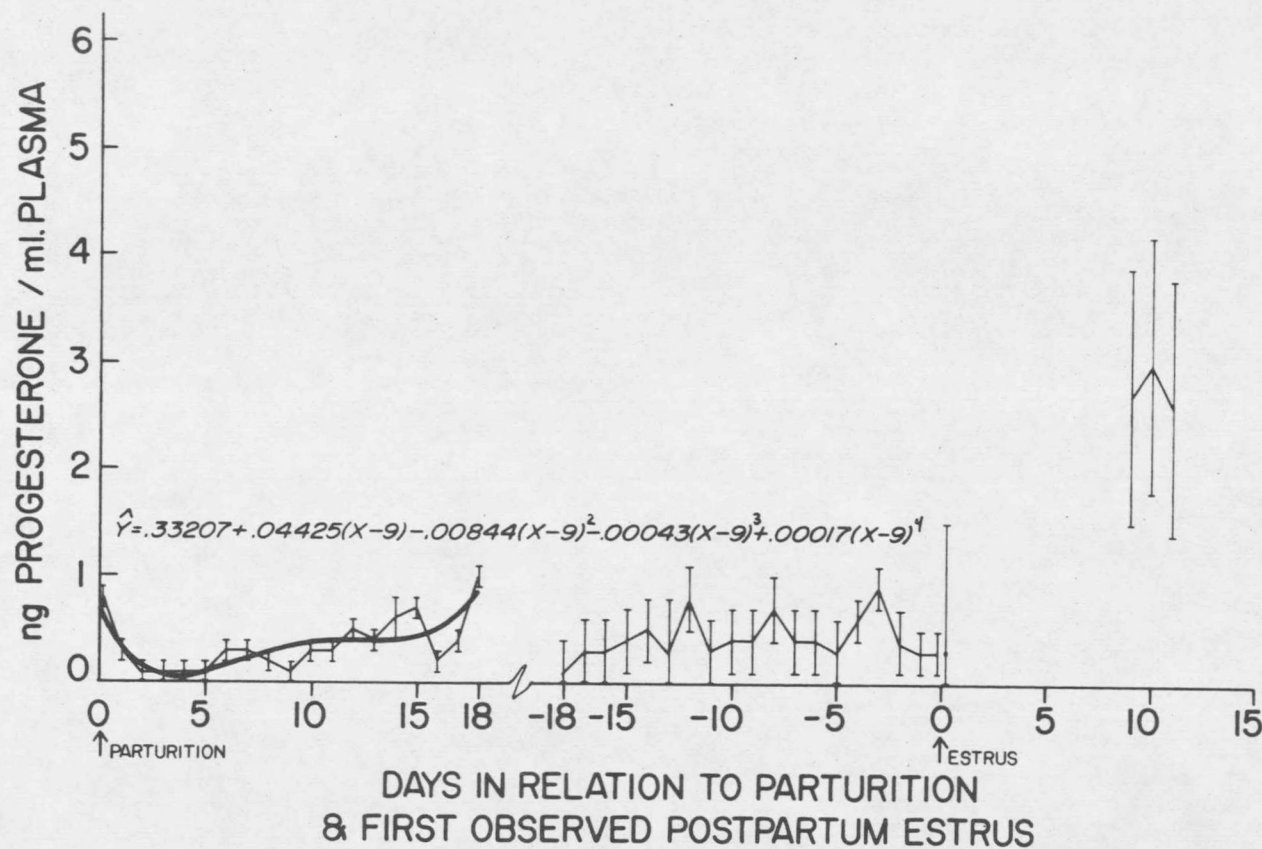


Figure 4-7. Mean blood plasma progesterone levels and regression equations for the NON-LACTATING treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle.

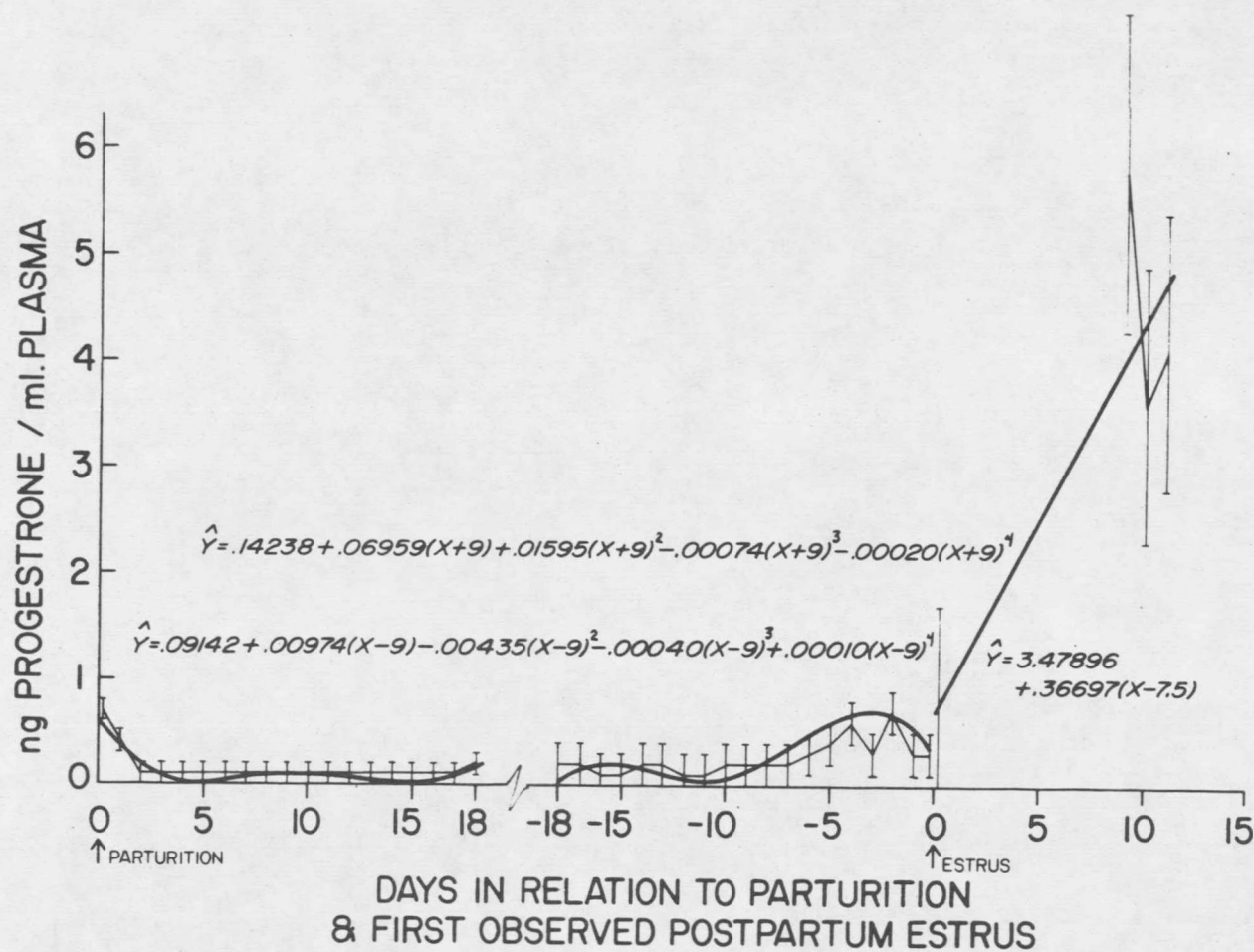


Figure 4-8. Mean blood plasma progesterone levels and regression equations for the SUCKLED 2X treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle.

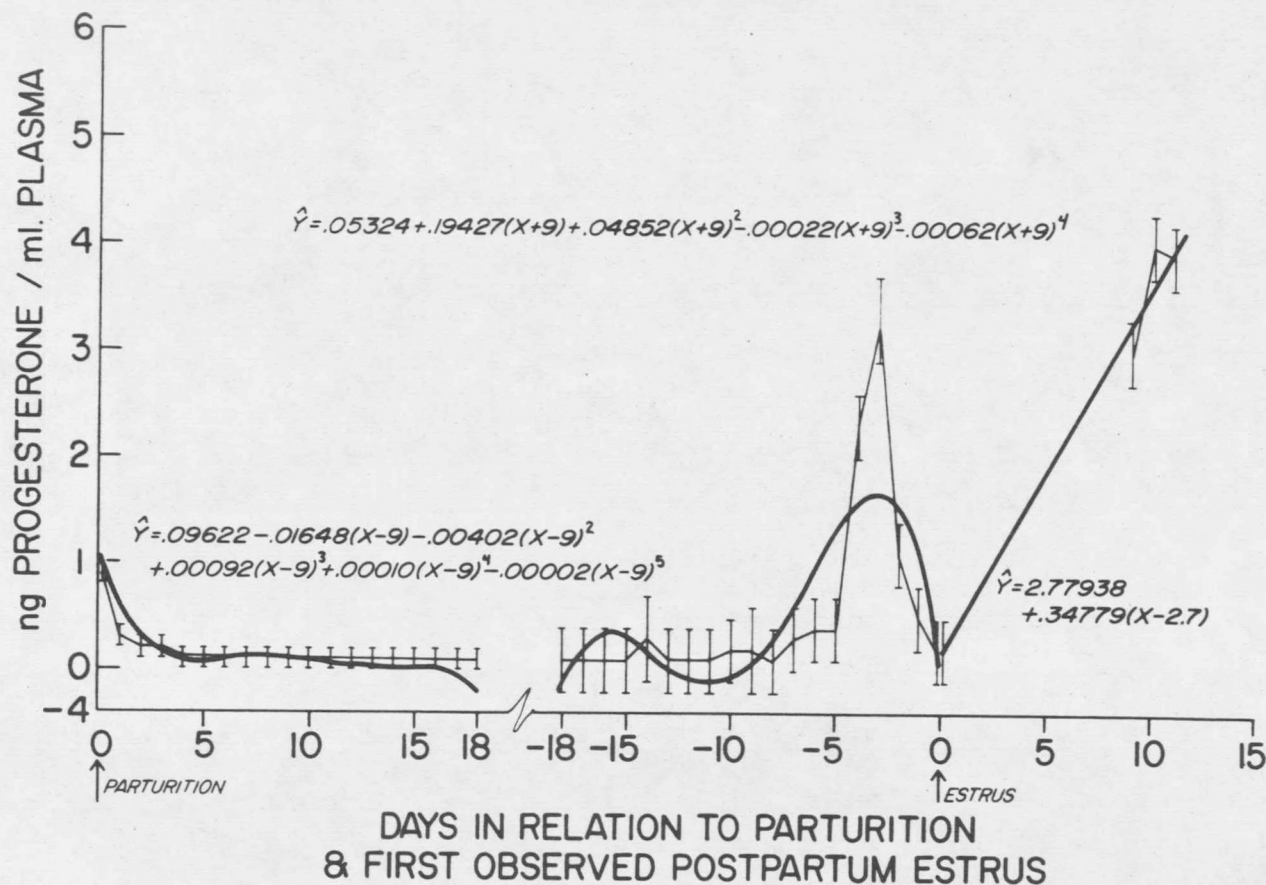


Figure 4-9. Mean blood plasma progesterone levels and regression equations for the SUCKLED AD LIBITUM treatment (excluding cows with silent first ovulation) from 0 to 18 days postpartum, 18 through 0 days prior to first estrus and from 0 through 11 days of the first estrous cycle.

extended period from 15 through 2 days prior to first observed estrus in the NON-LACTATING AND SUCKLED AD LIBITUM group which contained cows with silent first ovulation. The pattern was different in the SUCKLED 2X group where levels ranged between $.1 \pm .2$ and $.4 \pm .2$ ng/ml with slight elevation of $.6 \pm .2$ and $.7 \pm .2$ ng/ml occurring on days -4 and -2 in relation to first observed estrus (figures 4-2 through 4-5).

Average plasma progesterone levels over the 18 days prior to the first observed estrus were higher ($P < .01$) in the NON-LACTATING AND SUCKLED AD LIBITUM groups compared to the SUCKLED 2X group with levels of $1.4 \pm .2$, $.3 \pm .2$ and $1.2 \pm .2$ reported for the NON-LACTATING, SUCKLED 2X and SUCKLED AD LIBITUM groups, respectively. Anestrous progesterone levels (average of values from days 6, 7 and 8 prior to first observed estrus) pre-estrous levels (average of values from days 2, 3 and 4 prior to first observed estrus) and the highest level attained within days 2, 3 and 4 prior to first estrus were nonsignificantly lower in the SUCKLED 2X group compared to the other two groups (table 4-2).

In the analysis excluding cows with silent first ovulation, patterns of changes in plasma progesterone the last 18 days of the postpartum interval were described by a quartic equation ($P < .01$). Average plasma progesterone levels ranged between $.1 \pm .2$ and $.4 \pm .2$ ng/ml from 18 through 5 days, increased rapidly to $1.8 \pm .2$ ng/ml at 3 days and returned to $.3 \pm .1$ ng/ml by 1 day prior to the first estrus (figure 4-6). The SUCKLED 2X and SUCKLED AD LIBITUM groups showed the

same pattern of changes in progesterone over this time period (figures 4-8 and 4-9) but the NON-LACTATING group had no significant regressions and the pre-estrus progesterone peak seen in the other two groups was not as evident (figure 4-7).

Average plasma progesterone levels over the 18 days prior to first estrus was highest in the SUCKLED AD LIBITUM group ($.5 \pm .1$ ng/ml) which was significantly higher ($P < .05$) than the SUCKLED 2X group ($.3 \pm .1$ ng/ml) but not significantly different from the NON-LACTATING group. There was no significant difference in anestrus progesterone levels (average of day 6, 7 and 8 prior to first estrus) between treatment groups but pre-estrus progesterone levels (average of days 2, 3 and 4 prior to first estrus) were higher ($P < .08$), and peak levels within day 2, 3 and 4 prior to first estrus were higher ($P < .05$) in the SUCKLED AD LIBITUM group compared with the NON-LACTATING and SUCKLED 2X groups (table 4-3).

Plasma progesterone levels increased linearly ($P < .01$) from estrus through days 9, 10 and 11 of the first observed estrous cycle and there were no differences detected in levels due to treatment (figures 4-2 and 4-6 and tables 4-2 and 4-3).

Plasma testosterone levels (table 4-3) measured in one cow from each treatment group were described by a quartic equation ($P < .05$) over the time interval from parturition to first postpartum estrus.

Testosterone levels were low over the postpartum period, varying between $.1 \pm .1$ and $.4 \pm .1$ ng/ml (figure 4-10). Levels were higher ($P < .01$) in the cow from the NON-LACTATING group compared to the cows from the SUCKLED 2X and SUCKLED AD LIBITUM groups.

Significant correlations included a positive relationship between the length of the first estrous cycle and the postpartum interval ($r = .59$; $P < .05$), a negative relationship between length of the first estrous cycle and the incidence of non-standing estrus ($r = -.60$; $P < .05$), a positive relationship of progesterone levels 4 to 2 days prior to first estrus and the length of the postpartum interval ($r = .69$; $P < .01$) and a positive relationship between progesterone levels 4 to 2 days prior to first estrus and the length of the first estrous cycle ($r = .55$; $P < .05$) (table 4-4).

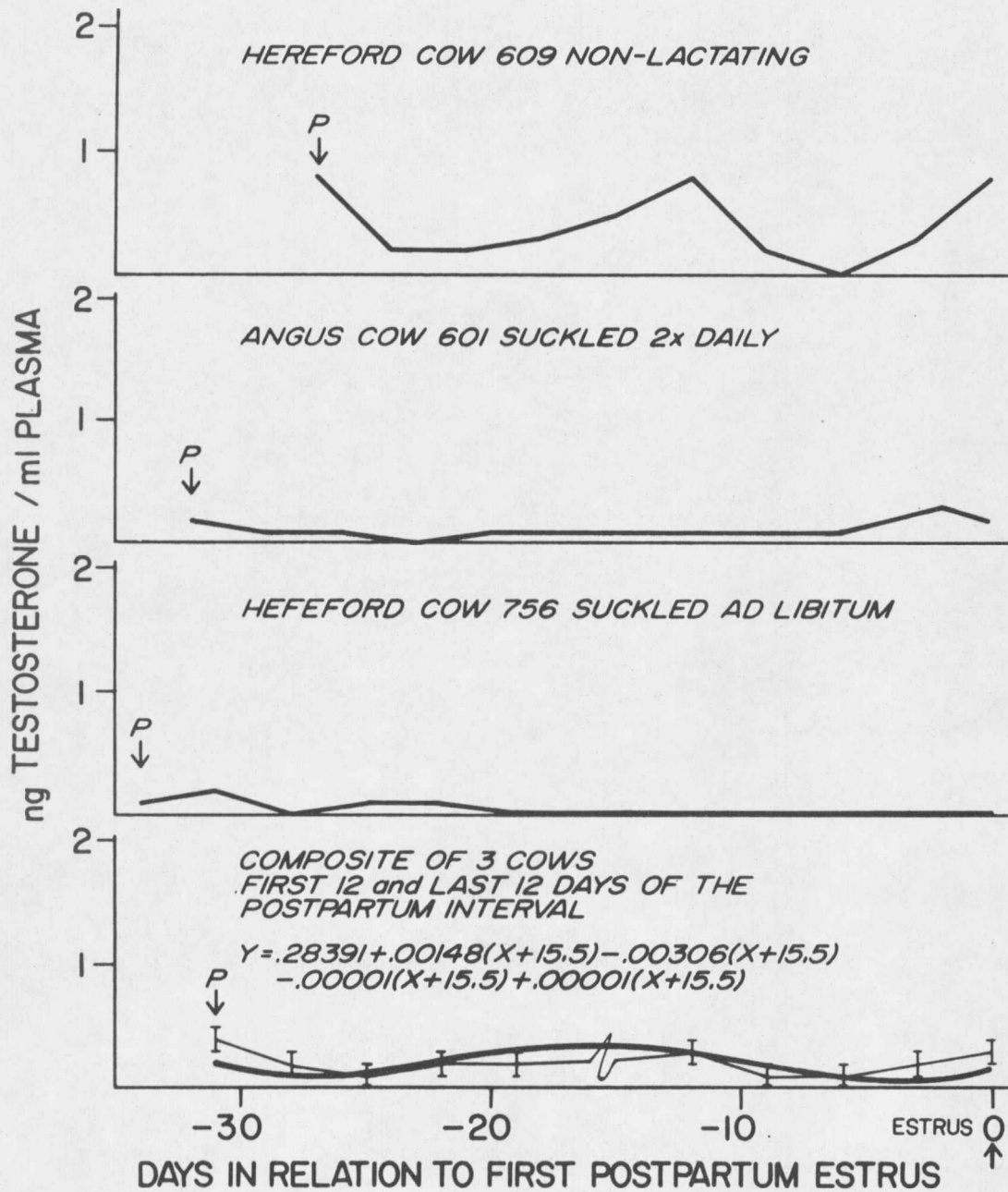


Figure 4-10. Blood plasma testosterone levels in three cows, one from each treatment group, from parturition through the first postpartum estrus.

TABLE 4-4. RESIDUAL CORRELATIONS BETWEEN BEHAVIORAL ESTRUS, BODY WEIGHT AND BLOOD PLASMA PROGESTERONE IN POSTPARTUM COWS WITH EXPRESSED FIRST ESTRUS

Trait	Trait code	Trait code							
		1	2	3	4	5	6	7	8
Postpartum interval	1								
Intensity of first estrus	2	.25							
Intensity of second estrus	3	.25	.54*						
Incidence of non-standing estrus	4	-.32	-.75**	-.78**					
Length of first estrous cycle	5	.59*	.36	.40	-.60*				
Body weight, 48 hr postpartum	6	-.14	.28	.15	-.18	-.02			
Body weight at first estrus	7	-.12	.41	.18	-.28	-.09	.85**		
Anestrous progesterone level ^a	8	.38	.24	.07	.03	-.06	-.21	-.19	
Pre-estrous progesterone level ^b	9	.69**	.32	.33	-.17	.55*	.06	.06	.54*

a. Average progesterone of days 6, 7 and 8 prior to first estrus.

b. Average progesterone of days 2, 3 and 4 prior to first estrus.

* $P < .05$.

** $P < .01$.

Discussion

Progesterone assay. Because of the large number of plasma samples to be assayed it was necessary to analyze the samples in a series of different assays over a relatively long time span. This may cause concern that different samples were not comparable due to inherent differences between assays and because of the varying time lapse between different assays.

Desirable comparisons could be made because of the order in which samples were assayed and because of the inclusions of a laboratory standard. Plasma samples were divided into three periods: (1) parturition through 18 days postpartum, (2) 18 days pre-estrus through first postpartum estrus and (3) first postpartum estrus through 11 days post-estrus. Assay reagents, including antibody, were homogenous within these periods. Plasma progesterone values within these periods were comparable because they were done within the same block of assays as described in Materials and Methods and because of correction to a laboratory standard to remove between assay variation. Comparisons were made within these periods but not between the periods.

Triated progesterone was used as an internal standard to monitor procedural losses during extraction. However, many (28.7%) of the individual recovery factors were greater than 100% with some being as high as 151%, although 90% of the recoveries greater than 100% were less than 120 percent. Limited data from an experiment

aside indicated that this was due to an interaction between the assay reagents and the speed of the vortex used to mix the samples. Total count tubes were not vortexed in this experiment or in the assays. When samples were mixed at a low vortex speed, normal recovery values were observed (>100%), but when the mixing speed was high, values of greater than 100% were obtained. It is postulated that variation in electrical current to the vortex and in hand pressure applied by the technician accounted for the erratic appearance of recoveries greater than 100 percent.

In reality, recoveries of greater than 100% are not possible and progesterone values of samples with recoveries of greater than 100% are decreased when adjusted. For these reasons, the highest recovery obtained within each assay was set at 100% and all other recoveries within the assay were adjusted accordingly.

It is unlikely that the high recoveries (>100%) or the method of correcting the recoveries to 100% or less significantly changed the relationship of the progesterone value in one sample compared to another. The contention that events termed "physiological" are not artifacts induced by the high recoveries is strengthened by the fact that the recoveries of greater than 100% appear to occur at random within an assay.

Postpartum body weights. The energy levels of 5.1 kg of TDN for non-lactating cows and 6.7 kg TDN for lactating cows were adequate to

cause the cows to maintain their 48 hr postcalving body weights through the first postpartum estrous cycle (tables 4-2 and 4-3 and figure 4-1). Short et al. (1972) has stated that this will remove the effects of nutrition on postpartum reproduction allowing the evaluation of varying levels of suckling stimuli independent of nutrition. However, it can be visualized that in a situation where body maintenance, lactation and reproduction are in competition for nutrients that nutrients may be diverted to maintenance and lactation leaving less available for reproduction. This possibility should be considered when interpreting the effects of nutrition on reproduction based on changes in body weights.

Occurrence and intensity of estrus. Suckling increased the interval from parturition to first postpartum estrus compared to non-suckling (table 4-3) which agrees with previous studies (Graves et al., 1968; Oxenreider, 1968; Saiduddin et al., 1968; Oxenreider and Wagner, 1971; Short et al., 1972). However, allowing calves to suckle ad libitum did not significantly increase the postpartum interval compared to allowing the calves to suckle only twice daily. Assuming the suckling ad libitum results in more stimulation than suckling 2X daily one would expect the cows from the SUCKLED AD LIBITUM group to have longer postpartum intervals much the same as 4X daily milking increased postpartum interval compared to 2X daily milking

(Clapp, 1937). Possibly, suckling ad libitum does not result in a greatly increased amount of total daily stimulation compared to suckling 2X daily.

Suckling increased the interval to first estrus and at the same time increased the intensity of the first postpartum estrus compared to non-suckling (table 4-2 and 4-3). However, the correlation between the intensity of first estrus and the postpartum interval was not significant ($r=.25$; $P<.10$; table 4-4) which indicates that at least part of the effect of suckling on estrus intensity is direct and not an indirect effect of suckling on the postpartum interval. Non-standing estrus behavior occurred more in the suckled groups compared to the NON-LACTATING group although the differences were not significant (table 4-3). Saiduddin et al. (1968) has shown that the incidence of non-standing estrous behavior decreased as postpartum interval increased and this relationship is also apparent in this study, although not significant ($r=-.32$; $P>.10$; table 4-4). However, the incidence of non-standing estrous behavior was negatively associated with the length of the first estrous cycle. The above observations further substantiate the irregularity of early postpartum cycles in cows.

Three cows had silent ovulations approximately one cycle length prior to their first observed estrus as evidenced by the development of corpora lutea and by increases in plasma progesterone in a pattern

similar to that seen in cycling cows following expressed estrus (Christensen et al., 1971). Two of these cows were in the NON-LACTATING group and both showed evidence of ovulation prior to 25 days postpartum (figures 9-2 and 9-6). The other cow was in the SUCKLED AD LIBITUM group and did not show evidence of ovulation until after 50 days postpartum (figure 9-17).

A rather high proportion of cows are reported to have silent ovulations especially at the first postpartum ovulation (Casida and Wisnicky, 1950; Kidder et al., 1952; Menge et al., 1962; Casida et al., 1968b; Pope et al., 1969; Donaldson et al., 1970; Callahan et al., 1971; Wagner and Oxenreider, 1971; Connor et al., 1974), and the incidence of silent ovulation increases as ovulation occurs earlier following parturition (Casida and Wisnicky, 1950; Kidder et al., 1952; Trimberger and Fincher, 1956; Menge et al., 1962; Labhsetwar et al., 1963).

Short et al. (1972) has hypothesized that the high incidence of quiet ovulations reported in the literature was a result of failure to detect estrus rather than no estrus being expressed. This hypothesis was based on the observation that when bulls were used to detect estrus no silent ovulations were present, but in previous studies bulls had not been used. However, the present study also used bulls and in addition utilized vaginal inspection and KMAR heat detection patches as aids in detection of estrus and yet three silent ovulations were noted. In every case where estrous activity was detected, KMAR

patches had changed color, but in no cases of silent ovulation had the patches changed color.

Blood plasma progesterone. Elevated levels of plasma progesterone were observed in most of these cows prior to first postpartum estrus and ovulation which supports the findings of Donaldson et al. (1970), Erb et al. (1971), Hendricks et al. (1972), Echterkamp and Hansel (1973) Arije et al. (1975), Corah et al. (1974) and Tribble et al. (1974). This is also consistent with findings in pre-pubertal heifers where elevated levels of progesterone were found prior to the first ovulatory surge of LH (Gonzalez-Padilla et al., 1975).

The postpartum plasma progesterone profile was characterized by low levels of less than 14 ng/ml following parturition until 2 to 4 days prior to estrus when values increased and peaked 3 days prior to estrus at $1.8 \pm .2$ ng/ml then returned to $.3 \pm .1$ ng/ml one day prior and on the day of estrus (figure 4-6).

This dramatic and short-lived pre-estrus progesterone peak was evident in all but four of the cows that had expressed first estrus. Two of these cows were from the NON-LACTATING group and had short intervals to estrus of 9 and 18 days (figures 9-1 and 9-3). In all cows with expressed first estrus the average level of plasma progesterone on days 2, 3 and 4 prior to first estrus was positively correlated with postpartum interval ($r=.69$; $P<.01$). These observations indicate that either the pre-estrus progesterone peak was absent when

the postpartum interval was short or that it was of such short duration that it was not detected in daily samples.

The other two cows which did not show evidence of a progesterone peak prior to an expressed first estrus were in the SUCKLED 2X group and both had short first estrous cycles of 8 and 12 days (figures 9-10 and 9-12). There was no increase in progesterone during these short cycles suggesting that a functional corpus luteum was not formed. Corah et al. (1974) also noted that progesterone remained low during short first cycles in three postpartum heifers, but they did not report the absence of a rise in progesterone prior to first estrus in these heifers.

Two of the three cows (figures 9-2, 9-6 and 9-17) with silent first ovulations exhibited a peak in plasma progesterone prior to the detection of a corpus luteum and before the rise in progesterone associated with the first cycle. This indicated that a progesterone peak occurred prior to first ovulation without estrus. One of these cows (figure 9-6) exhibited a dramatic progesterone peak rising from a non-detectable level to 10 ng/ml and returning to .3 ng/ml on days 6, 7 and 8 postpartum, respectively. Such a short lived rise in progesterone could easily be missed in daily samples and this may explain the absence of a detected pre-estrus progesterone peak in several cows. Corah et al. (1974) reported that the pre-estrus rise in progesterone was absent in heifers that did not settle when bred

on their first postpartum estrus but was present in heifers that did settle on this estrus. However, blood samples were collected only every third day.

Two different profiles of postpartum plasma progesterone have been reported in the literature. In one of these progesterone is present in a cyclic pattern prior to the first observed estrus, i.e., a gradual rise that is maintained approximately the length of an estrous cycle. Both Tribble et al. (1974) and Hendricks et al. (1972) have noted this pattern. The other pattern finds progesterone low during most of the postpartum period with a small but abrupt increase just prior to the first estrus. This pattern has been reported by Tribble et al. (1974), Arije et al. (1974), Corah et al. (1974) and is the pattern seen in the present study.

In the present study and in the study by Corah et al. (1974) the only cows that showed extended levels of elevated progesterone prior to first estrus were those with verified silent ovulations. This suggests that undetected ovulations had occurred in the studies where extended elevated levels of progesterone were observed prior to estrus. However, the study by Tribble et al. (1974), in which both patterns of progesterone secretion were noted, was accompanied by a seemingly adequate ovarian palpation program (Connor et al., 1974) and silent ovulations should have been detected. Perhaps these two patterns of postpartum progesterone are different manifestations of the same

phenomenon.

The two statistical analyses, one including and one excluding cows with silent first ovulation, illustrated the erroneous pattern of postpartum progesterone that would have been concluded had the silent ovulations not been detected. An extended period of elevated progesterone occurred during the 18 days prior to first expressed estrus when cows with silent first ovulations were included in the analysis (figure 4-2), but when these three cows were removed, the resultant analysis showed that progesterone levels were low during this period with the exception of elevated levels that occurred on days 4, 3 and 2, prior to the first estrus (figure 4-6).

The source of the pre-estrual progesterone secretion has not been determined, but since there are apparently no functional corpora lutea present it is likely that either luteinized follicles or adrenal glands may be responsible.

Connor et al. (1974) have postulated that the source of this progesterone must be from a luteinized follicle. This postulation was based on the following observations: (1) No estrus, ovulation or corpus luteum was detected prior to the increase in progestins, (2) both 17-hydroxyprogesterone, found only in follicular tissue (Cupps, 1968) and 20-B-hydroxyprogesterone, found only in luteal tissue (Savard and Telegdy, 1965), were found prior to estrus with ovulation, (3) it had previously been reported that luteinization begins before ovulation (Lobel and Levy (1968; Short, 1962, (4) Short (1962)

quantified both 17-hydroxyprogesterone and 20-B-hydroxyprogesterone from luteinized follicles, and (5) an increase in ovarian size prior to first estrus was accompanied by an increase in plasma progestin levels. However, as eluded to previously, there may be some question as to whether or not silent ovulation had already occurred.

The above argument, although convincing, does not rule out the possibility of an adrenal source. Progesterone in bovine adrenal blood is 10 to 100 times greater than the concentration of progesterone in arterial blood collected at the same time (Balfour and Comline (1957)). The present study does not provide additional information as to the source of the pre-estruual progesterone rise but the abrupt peak in progesterone would suggest an adrenal source as opposed to a luteinized follicular source where one might expect a more gradual sustained increase.

The major difference between the suckled and non-suckled groups was the length of the postpartum interval and not differences in progesterone levels or patterns of release. However, differences in progesterone levels between treatment groups were noted. Average progesterone levels the first 18 days postpartum were highest in the NON-LACTATING group ($P < .01$). However, the average postpartum interval was only 20 ± 4 days in this group and the higher levels were probably a reflection of the pre-estrus progesterone rise (table 4-3).

Progesterone levels the first 18 days prior to estrus did not appear to be a function of increased suckling stimuli as the average progesterone levels in the suckled groups over this period of time were not significantly different from the NON-LACTATING group. However, there was a difference ($P < .05$) in the average progesterone levels the 18 days prior to estrus between the SUCKLED 2X and SUCKLED AD LIBITUM groups. The explanation for this difference is not known.

Suckling increased the postpartum interval to estrus ($P < .05$) and therefore it is reasonable to assume that it also increased the interval from parturition to the occurrence of the pre-estrus progesterone peak. The trend for the pre-estrus progesterone peak to increase as the length of the postpartum interval increased ($r = .69$; $P < .01$) is reflected in the fact that the pre-estrus progesterone peak and the postpartum interval were significantly higher ($P < .05$) in the SUCKLED AD LIBITUM group compared to both the SUCKLED 2X and NON-LACTATING groups.

The intensity of first estrus was lowest ($P < .05$) in the NON-LACTATING group and the pre-estrus progesterone peak was also lowest in this group ($P < .05$) indicating that a relationship exists between these parameters. However, the correlation of .32 was not significant. Pope et al. (1969) has reported mean peak progesterone levels of 9 ng/ml prior to ovulations with estrus compared to 3.7 ng/ml preceding quiet ovulations in postpartum and cycling cows ($P < .007$). This indicates that the level of pre-estrus progesterone affects the expression

of estrus.

Blood plasma testosterone. Testosterone was measured in plasma from three cows to determine what levels exist in the postpartum cow. A hypothesis of the mechanism controlling postpartum anestrus is that a steroid may inhibit the LH surge center. If such a steroid could be shown to exist at relatively high concentrations during the postpartum interval this would support such a hypothesis. However, blood plasma testosterone levels were low in these postpartum cows as they are in cycling cows (Shemesh and Hansel, 1974) indicating that testosterone probably has little if any effect on the events following parturition.

Physiology of anestrus. The following model, although not supported by all the evidence, helps explain anestrus and the initiation of estrous cycles following parturition in the cow. Although the ovary will be implicated as the site at which the message to resume estrous cycles originates, the hypothalamus will still be presented as the key to the control of the resumption of cyclic activity.

Two considerations are of primary importance to this proposed model. The first of these is the fact that following regression of the corpus luteum of pregnancy (parturition) ovulation and new corpus luteum development do not immediately occur (Labhsetwar et al. 1964); Gomes and Erb, 1965; Oxenreider, 1968; Stabenfeldt, 1968; Wagner and Hansel, 1969) as happens following regression of a cyclic corpus luteum, and a period of time ensues in which no functional corpus luteum exists on the ovary (Callahan et al., 197; Connor et al., 1974).

The second consideration is the assumption that there is no deficiencies of pituitary and circulating gonadotrophins and that the cause of acyclicity is the absence of cyclic LH release. Measurement of LH and FSH in pituitaries and LH in blood of postpartum cows support this view point (Labhsetwar et al., 1964; Saiduddin and Foote, 1964; Wagner et al., 1969; Han, 1973; Arjie et al., 1974; Tribble et al., 1974). The development of follicles between 5 to 30 days postpartum (Casida and Venyke, 1936; Labhsetwar et al., 1964; Wagner and Hansel, 1969; Connor et al., 1974; Arjie et al., 1974) indicated that blood FSH levels are adequate to cause follicular growth.

Follicles, growing in this environment of low progesterone produce estrogen, but this estrogen may not be able to elicit an LH surge in the absence of adequate amounts of progesterone. The fact that progesterone is low in relation to estrogen in postpartum and prepubertal anestrus cows (Arjie et al., 1974; Gonzalez-Padilla et al., 1975b) and that the first elevation in LH is always preceded by an elevation in progesterone in prepubertal heifers (Gonzalez-Padilla et al., 1975b) is indicative that estrogen is unable to elicit an LH surge in the absence of the proper level of progesterone in postpartum cows. Furthermore, it has been shown that small amounts of progesterone are needed in order for the estrogen evoked LH surge to occur in rats (Motta et al., 197).

It is postulated that one or several of the follicles growing in this low progesterone environment eventually become over mature and

luteinize under the effect of LH at tonic levels. Evidence that luteinization of follicles occurs in postpartum cows was given by Connor et al. (1974) and was previously discussed.

This postulated luteinization is either immediately regressed via the uterine luteolytic factor or is maintained approximately the length of an estrous cycle before it is regressed. The occurrence of one or the other of the two patterns in different studies would explain why two different patterns of pre-estrus progesterone would be expected as seen in this study and by Corah et al. (1974) and Arije et al. (1974). However, in the case where regression of the luteinized follicle would not occur until approximately one cycle length later, a gradual, sustained progesterone profile would be expected as seen by Tribble et al. (1974).

It is further postulated that this rise and fall in progesterone in combination with the already existing estrogen provides the proper hormonal milieu to cause an LH surge center located in the hypothalamus. This LH surge would then cause ovulation and corpus luteum development and thus ovarian cycles would be resumed.

This hypothesis, that postpartum anestrus is a result of the hypothalamus waiting for a message from the ovary is consistent with findings of Britt et al. (1974) who showed that cyclic activity would continue at normal intervals when ovulation was induced with GnRH at 14 days postpartum in dairy cows. However, this does not exclude

the possibility that the message for resumption of estrous cycles is mediated at the hypothalamo-hypophyseal level.

Han (1973) has reviewed the literature and has himself provided evidence that postpartum anestrus is caused by the inability of the hypothalamo-hypophyseal complex to properly stimulate the ovary and that resumption of estrus cycles take place when changes occur in the hypothalamus. More specifically he contends that prolactin production during lactation is antagonistic to the ability of the hypothalamus to elicit an LH surge. The gradual decrease of prolactin production that occurs as lactation progresses finally becomes low enough to allow cyclic gonadotrophin production and release.

The present model is compatible with that hypothesis and although it contends that the message for resumption of estrous cycles originates at the ovary in the cow it is probably correct that the ultimate control of the anestrus period is at the level of the hypothalamus. A message from the ovary that falls on an insensitive hypothalamus cannot secure a response. Such things as suckling, lactation and a negative energy balance may have their primary effect on the hypothalamus, rendering it insensitive to messages from the ovary. This would explain long postpartum intervals even after the early development of follicles as shown by Arije et al. (1974).

In summary, this model of postpartum anestrus suggests that follicles become over mature in a progesterone deficient environment

and luteinize. The combination of estrogen and falling progesterone produced by regression of these luteinized follicles elicit an LH surge via the hypothalamus causing ovulation and corpus luteum development and thus estrous cycles are resumed. Due to the circumstances of suckling, lactation and/or negative energy balance the hypothalamus may be insensitive to the ovarian messages and postpartum anestrous will continue until hypothalamic sensitivity is restored.

CHAPTER 5

USE OF PROGESTERONE AND ESTROGEN TO CONTROL POSTPARTUM REPRODUCTION IN DAIRY MANAGEMENT

Introduction

Attempts to shorten the interval from parturition to first estrus, ovulation and conception with progestins and/or estrogens have been made with varying levels of success (Ulberg and Lindley, 1960; Foote and Hunter, 1964; Saiduddin et al., 1968; Hill et al., 1972; Ulberg et al., 1974).

The objective of this study was to determine if progesterone and estrogen given in single doses could be used in a routine dairy management system to improve reproductive performance.

Materials and Methods

The cows used in this study were from the Montana State University dairy herd, and with the exception of the experimental conditions imposed upon them, were managed accordingly.

Fourteen and 18 Holstein cows were assigned to trial A (TRIAL A) in 1972 and 1973 and trial B (TRIAL B) in 1973 and 1974, respectively, and within trial were assigned to treatment groups. Cows in TRIAL A were assigned to a control group (CONTROL) or a steroid-treated group (TREATED) by pairing in order of calving. Each pair consisted of a CONTROL and a TREATED cow with similar calving dates. Cows in TRIAL B were assigned prior to calving by expected calving date and 305-day

milk production to a corn oil-treated group (CORN OIL) and a steroid-treated group (STEROID).

In both trials steroid treatment consisted of a single 20 mg intramuscular injection of progesterone in corn oil, followed in 48 hr by a single 2 mg intramuscular injection of estradiol-17B. In TRIAL A the control cows (CONTROL) did not receive any injections, but in TRIAL B the control cows (CORN OIL) received corn oil at the appropriate volumes and times consistent with the steroid treatments. The time of progesterone treatment for cows in TRIAL A ranged from 30 to 90 days postpartum with a mean of 48 days postpartum (table 5-1). In TRIAL B all cows received their first injection (progesterone or corn oil) at 30 days postpartum (table 5-2).

In TRIAL A each TREATED-CONTROL pair was palpated per rectum on the same day at 0, 2, 4 and 12 days post-progesterone treatment to detect ovulation and corpus luteum development. In TRIAL B all cows were palpated per rectum at 10, 20, 30 through 38 and 44 days postpartum to assess ovarian size and activity. Ovary size was estimated by palpating the ovary while concurrently observing a chart with various sizes of ovaries depicted upon it.

Breeding management consisted of starting artificial insemination at 60 days postpartum and continuing until the cow did not return to estrus. Pregnancy and correct conception dates were verified by observing the date of subsequent parturition.

Daily and total milk production has been calculated from 3 to 50 days postpartum for the cows in TRIAL B.

The data were analyzed by least squares analysis (Harvey, 1960), except number of cows in estrus by 60 days postpartum number of cows in estrus by 82 days postpartum and first service conception rate, which were analyzed using a chi-square test.

Results and Discussion

TRIAL A

The interval from parturition to the first postpartum estrus was shorter ($P < .07$) in the TREATED group than in the CONTROL group, with 52 ± 8 and 76 ± 8 day intervals being recorded, respectively. Six out of seven cows exhibited estrus prior to 60 days postpartum in the TREATED group, compared with two out of seven in the CONTROL group ($P = .10$). At 82 days postpartum six out of seven cows had exhibited estrus in the TREATED group, compared with four out of seven in the CONTROL group ($P = .56$) (table 5-1).

There were no significant differences in the first service conception rates of 43 and 14%, conceptions per service of 0.7 ± 0.1 and 0.7 ± 0.1 , pregnancy rates of 100 and 100% and days open of 113 ± 24 and 127 ± 24 days for the CONTROL and TREATED groups, respectively (table 5-1).

TRIAL B

The interval from parturition to the first postpartum estrus was 60 ± 11 and 35 ± 2 days ($P < .01$) for the CORN OIL and STERIOD groups, respectively, and all ten cows in the STERIOD group had exhibited estrus by 60 days postpartum with five out of eight cows in the CORN OIL group ($P = .13$) (table 5-2).

There were no significant differences in the first service conception rates of 50 and 50%, conceptions per service of .72 and .69, pregnancy rates of 100 and 100% and days open of 105 and 128 days for the CORN OIL and STERIOD groups, respectively, (table 5-2).

There was no significant difference in ovary diameter at 10, 20, 30 through 38 or 44 days postpartum, nor was there any significant difference in daily milk production from day 3 through day 50 postpartum (table 5-2). Total milk production from day 3 through day 50 postpartum was 1348 ± 42 and 1433 ± 64 kg for the CORN OIL and STERIOD groups, respectively, (table 5-2).

Discussion

The interval from parturition to first postpartum estrus was 24 days shorter ($P < .07$) in the TREATED group compared to the CONTROL group in TRIAL A, and was 25 days shorter ($P < .01$) in the STERIOD group compared to the CORN OIL group in TRIAL B. This demonstrates that steroid treatment did shorten the interval from parturition to first estrus (tables 5-1 and 5-2).

The tendency toward early postpartum estrus in both TRIAL A and TRIAL B is seen in the increased number of cows first exhibiting estrus prior to 60 days postpartum (tables 5-1 and 5-2). Reproductive performance in either trial was not improved by steroid treatment as measured by first service conception rate, conceptions per service and pregnancy rate (tables 5-1 and 5-2). The first service conception rate of the TREATED group in TRIAL A was rather low with 14% compared to 43% for the CONTROL group (table 5-1).

Steroid treatment did not reduce the interval from parturition to conception in TRIAL A with the TREATED group showing 14 more days open than the CONTROL group or in TRIAL B with the STERIOD group showing 23 more days open than the CORN OIL group (tables 5-1 and 5-2). Other research has indicated that when the interval from parturition to first estrus is shortened by progestin and/or estrogen treatments (Foote and Hunter, 1964; Saiduddin et al., 1968; Hill et al., 1972), early weaning (Short et al., 1972) or mastectomy (Short et al., 1972; LaVoie et al., 1974) that the interval to conception may not be shortened. A notable exception is found in the work by Saiduddin et al. (1968) where two different progesterone + estrogen treatments shortened the interval to conception but had no effect on the interval to estrus. Ulberg et al. (1974) has found that feeding MGA to early postpartum dairy cows has a favorable effect on reproductive rate.

In TRIAL B there was no significant difference in ovary diameter on days 10, 20, 30 through 38 and 44 postpartum, but ovary diameter was consistently smaller in the STEROID group than in the CORN OIL group from day 30 through day 44. Possibly this was due to negative feedback by the exogenous steroids on the pituitary.

TABLE 5-1. REPRODUCTIVE PERFORMANCE OF STEROID-TREATED COWS (TRIAL A)

Measurement	CONTROL mean ^a $\pm$ SE	TREATED mean ^a $\pm$ SE
No. of cows	7	7
Average date of calving ($\pm$ days)	Jan. 25 $\pm$ 14	Jan. 24 $\pm$ 14
Interval from parturition to treatment (days)	--	48 $\pm$ 6
Interval from parturition to first estrus (days)	76 $\pm$ 8 ^b	52 $\pm$ 8 ^b
No. of cows in estrus by 60 days postpartum	2 ^c	6 ^c
No. of cows in estrus by 82 days postpartum	4	6
First service conception rate (%)	43	14
Conceptions per service	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1
Pregnancy rate (%)	100	100
Days open	113 $\pm$ 24	127 $\pm$ 24

^a Least squares means.

^b P<.07

^c P<.10

TABLE 5-2. REPRODUCTIVE PERFORMANCE OF STEROID-TREATED COWS (TRIAL B)

Measurement	CORN OIL mean ^a $\pm$ SE	STEROID mean ^a $\pm$ SE
No. of cows	8	10
Average date of calving	Dec. 15 $\pm$ 6	Dec. 18 $\pm$ 5
Interval from parturition to treatment (days)	30	30
Interval from parturition to first estrus (days)	60 $\pm$ 11**	35 $\pm$ 2**
No. cows in estrus by 60 days postpartum	5 ^b	10 ^b
First service conception rate (%)	50	50
Conceptions per service	.72	.69
Pregnancy rate (%)	100	100
Days open	105	128
Ovary diameter at various stages postpartum (mm)		
10 days	13.3 $\pm$ 1.2	13.3 $\pm$ 1.1
20 days	15.8 $\pm$ 1.1	15.9 $\pm$ 0.7
30 days	18.1 $\pm$ 1.1	15.9 $\pm$ 1.0
31 days	17.7 $\pm$ 1.2	15.7 $\pm$ 1.0
32 days	18.9 $\pm$ 1.3	16.6 $\pm$ 1.1
33 days	19.1 $\pm$ 1.2	15.9 $\pm$ 1.0
34 days	19.4 $\pm$ 1.3	15.9 $\pm$ 1.2
35 days	24.0 $\pm$ 1.5	19.0 $\pm$ 1.4
36 days	19.5 $\pm$ 0.8	17.6 $\pm$ 0.7
37 days	19.2 $\pm$ 1.2	17.7 $\pm$ 1.0
38 days	18.5 $\pm$ 1.5	18.0 $\pm$ 1.4
44 days	19.4 $\pm$ 1.3	18.7 $\pm$ 1.1
305-day milk production from previous lactation (kg)	7097 $\pm$ 394	7459 $\pm$ 352
Total milk production through 50 days postpartum (kg)	1348 $\pm$ 42	1433 $\pm$ 64

^a Least squares means.

^b P=.13

**P<.01

CHAPTER 6

EFFECT OF MASTECTOMY ON POSTPARTUM REPRODUCTION IN BEEF COWS

Introduction

Recent experiments have shown that mastectomy will shorten the interval from parturition to the first postpartum estrus in beef cows (LaVoie and Moody, unpublished observations; Short et al., 1972) and that this mastectomy effect is independent of nutrition (Short et al., 1972). No experiments have been reported which show the effect of mastectomy on the interval from parturition to conception. If mastectomized cows could conceive early in the postpartum period, it would be possible to have calving intervals of 10 or 11 months.

This study was designed to determine if mastectomized beef cows could be managed to conceive within 82 days after parturition, which would result in calving intervals of less than 365 days.

Materials and Methods

Three 2-year-old Angus cows pregnant with their first calf were mastectomized in 1971, and three Hereford and four Angus x Hereford open yearlings were mastectomized in the spring of 1972. The surgical technique was as described by Short et al. (1972).

The animals were bred during the summer of 1972 and the cows and six of the heifers calved in 1973. They were then observed two times daily for estrus, and were artificially inseminated from the second

postpartum estrus until conception occurred or until a total of six services were reached. Pregnancy testing was done by rectal palpation from 60 to 100 days after breeding.

The Montana State University beef herd served as a control group, but it should be noted that while the nutritional management was consistent between the beef herd and the mastectomized cows, the breeding schemes were not consistent. Also, other experiments were imposed on the mastectomized group, and the two groups were often kept in separate pastures.

Results and Discussion

The interval from parturition to the first postpartum estrus ranged from 8 to 51 days and averaged 30 days for the mastectomized cows. The interval from parturition to conception ranged from 22 to 218 and 57 to 122 days for the mastectomized group and the MSU beef herd, respectively, and averaged 76 and 79 days for the two groups, respectively (tables 6-1 and 6-2).

The mastectomized cows had 0.38 conceptions per service, resulting in an 89% pregnancy rate compared with 0.59 conceptions per service and a 74% pregnancy rate for the MSU beef herd (table 6-1).

Five mastectomized cows first bred on their second postpartum estrus conceived, resulting in a first service conception rate of 56% compared to 62% for the MSU beef herd. One mastectomized cow was

mistakenly bred on her first postpartum estrus, but did not conceive until bred on her second postpartum estrus. Of the remaining mastectomized cows, one was in estrus four times, bred three times and conceived; another was in estrus eight times, bred five times and conceived; one was in estrus nine times, bred six times and did not conceive (tables 6-1 and 6-2).

Discussion

These data show rather low fertility in mastectomized cows where breeding was started on the second postpartum estrus. Several workers have shown that early postpartum heats are infertile (Casida et al., 1968) and this is suggested in the low conceptions per service and first service conception rate seen in this experiment. The pregnancy rate was higher in the mastectomized cows (89%) compared with the MSU beef herd (74%), but this was probably due to the longer breeding season imposed on the mastectomized group.

The interval from parturition to the first postpartum estrus in the mastectomized cows was 30 days, which is 18 days greater than reported by Short et al. (1972); but less than postpartum intervals generally reported for cows suckling calves. Casida et al. (1968) found that various researchers had reported postpartum intervals ranging from 46 to 104 days.

The interval from parturition to conception in the mastectomized cows was similar to that of the MSU beef herd (76 vs. 79 days), and

this interval was not greatly shorter than 83 days, indicating that mastectomy had little effect in reducing calving interval to less than 365 days.

The interval from parturition to the first postpartum estrus is shortened by inhibiting lactation (Short et al., 1972), but inhibiting lactation does not appear to affect the interval from parturition to conception. Possibly lactation is necessary for proper uterine involution creating an environment conducive to a high fertilization and embryo survival rate. Short et al. (1973) have shown that uterine debris is significantly greater in non-suckled ewes compared with suckled ewes killed at 20 days postpartum.

TABLE 6-1. REPRODUCTIVE PERFORMANCE OF MASTECTOMIZED COWS

Measurement	Mastectomized group	M.S.U beef herd
No. of cows	9	66
Interval to first estrus (days)	30	--
Interval to conception (days)	76 ^a	79 ^b
Conceptions per service	0.38	0.59
First service conception rate (%)	56	62
Pregnancy rate (%)	89	74

TABLE 6-2. INDIVIDUAL PERFORMANCE OF MASTECTOMIZED COWS

Cow No.	Breed ^a	Interval to first estrus (days)	Interval to conception (days)	Number of heats	Number of services	Pregnancy ^b test
925	A	28	49	2	1	+
949 ^c	A	51	76	2	2	+
964	A	22	86	4	3	+
157	H	8	22	2	1	+
140	AxH	42	62	2	1	+
132	AxH	30	51	2	1	+
131 ^d	AxH	27	218	8	5	+
111	H	20	40	2	1	+
109 ^d	H	22	--	9	6	-

^a A = Angus, H = Hereford.

^b + = pregnant, - = open.

^c 949 was bred on her first postpartum estrus.

^d 131 and 109 were not bred on two different occasions when they were scheduled to be bred. Both cows were bred on their last observed estrus.

CHAPTER 7

EFFECT OF FLUMETHASONE INDUCED CALVING ON THE POSTPARTUM INTERVAL OF BEEF COWS

Introduction

Several studies report the effects of induced parturition on subsequent fertility (LaVoie and Moody, 1973; Carroll, 1974; Poncelet et al., 1974; Wagner et al., 1974) but none have reported the effect of corticoid induced parturition on the postpartum interval per se.

Reproductive performance of cows following corticoid induced parturition is reported equal to that of control cows regardless of the high incidence of retained placenta associated with the induced parturitions (LaVoie and Moody, 1973; Carroll, 1974; Poncelet et al., 1974). Wagner et al. (1974) found that fertility was equal between cows with or without retained placenta after induced parturition. These studies do not account for the fact that induced parturition increased that interval from calving to the breeding season, and therefore, may not reflect the actual effect of induced parturition and/or retained placenta on fertility.

The objectives of this study were to test the effect of flumethasone induced parturition on the postpartum interval and to determine the effect on fertility independent of differences in the interval from parturition to the breeding season.

Materials and Methods

Thirty-one pluriparous cows, 15 of which were Herefords bred to Hereford, Angus and Holstein bulls in a ratio of 3:1:1, and 16 Angus all bred to Angus bulls, were assigned to a non-treated group (NT) or a group receiving a total of 5 mg of flumethasone in either a single dose or two 2.5 mg doses at a 12 hr interval. Injections were given 17 days prior to expected calving date to induce parturition 15 days prior to the end of a 281 day calculated gestation.

Fifty percent of the cows receiving a single 5 mg injection and 55.5% of the cows receiving two 2.5 mg injections responded with induced parturition (calved within 90 hr post-injection) and were assigned to an induced parturition group (INDU). Cows that did not respond (calved later than 90 hr post-injection) were assigned to a non-induced parturition group (NI). Cow breed affected the success of induced parturition ($P < .01$) so that the INDU group contained two Herefords and eight Angus, and the NI group contained eight Herefords and one Angus.

The cows were observed every 4 hr to collect calving information and to determine the time of placental expulsion. All cows that did not expel the placenta by 24 hr postpartum were injected at that time with 20 cc Distrycillin¹ intramuscularly. Cows that lost their calves were fostered to dairy calves or orphans and were suckled throughout the experiment.

¹Distrycillin, E. R. Squibb and Sons, Inc. New Brunswick, New Jersey.

Calving difficulty was scored using the following method:

1=unassisted, 2=assistance with hand traction 3=assistance with mechanical puller and 4=extremely difficult pull and/or malpresentation.

The vigor of calves at birth was scored from 1 to 4 depending on their initial activity: 1=very vigorous, 2="normal" calf, 3=weak calf and 4=very weak or dead calf. All calves were weighed at birth but in addition each live calf from an induced parturition was weighed at the time normal parturition was expected to occur had parturition not been induced.

The cows were observed 1 to 2 hr twice daily from the time of calving through the breeding season for detection of estrus. AI breeding for each individual cow started 60 days postpartum and was continued for 45 days (60 to 104 days, postpartum). Pregnancy diagnosis was done by rectal palpation 70 days after the end of the breeding season.

The statistical analysis for effect of breed on induced parturition success and on conceptions per service was done by a chi-square test. All other measurements were analyzed by the method of least-squares (Harvey, 1960) and a Newman Keuls test (Snedecor, 1967) was applied when least-squares analysis indicated significant differences. An adjusted postpartum interval to first estrus and an adjusted number of times in estrus prior to 60 day postpartum were calculated by using expected normal calving date in place of actual calving date.

Results and Discussion

The results are given in table 7-1. Flumethasone caused induced parturition in two Herefords and eight Angus cows while only one Angus cow failed to respond compared with eight Herefords ($P < .01$). Parturition occurred 60 ± 20 hr and 325 ± 21 hr ($P < .01$) after initial flumethasone injection in the INDU and NI groups, respectively, and gestation length was shortened ($P < .01$) by 12 days in the INDU group compared with both the NT and NI groups. Average calving date occurred on day 86 of the year (March 27) in the INDU group which was 9 days earlier than the NT group ($P < .05$) and 14 days sooner than the NI group ($P < .01$).

Dystocia was increased ($P < .05$) as measured by calving difficulty score in the NI group compared to the NT group, but calf vigor score was not significantly affected although it was highest in the NI group. Calves from induced parturitions weighed 24 ± 2 kg compared with 32 ± 2 ($P < .05$) and 34 ± 3 kg ($P < .01$) for the NT and NI groups, respectively.

Incidence of retained placenta was 8, 100, and 0% at 24 hr ($P < .01$), 8, 80 and 0% at 72 hr ($P < .01$) and 0, 0, and 0% at one week postpartum for the NT, INDU and NI groups, respectively.

Unadjusted and adjusted postpartum intervals to first estrus were 45 ± 6 , 47 ± 6 and 40 ± 6 days, and 43 ± 6 , 32 ± 6 and 37 ± 7 days for the NT, INDU and NI groups, respectively. Unadjusted and adjusted times in estrus prior to 60 days postpartum were 1.2 ± 0.2 , 1.1 ± 0.3 and 1.3 ± 0.3 ,

and 1.4 ± 0.3 , 1.7 ± 0.3 and 1.5 ± 0.3 for the NT, INDU and NI groups, respectively.

A comparison of Hereford and Angus cows within the control group (NT) revealed that the Herefords had slightly better reproductive performance than the Angus. First service conception rates and pregnancy rates were both 80 and 43% for Herefords and Angus, respectively. Services per conception were 1.3 and 3.3, postpartum interval to first estrus and 42 and 47 days and days open were 84 and 66 days for Hereford and Angus, respectively (table 7-2).

Discussion

Parturition was successfully induced with flumethasone in two Hereford and eight Angus cows while only one Angus cow failed to respond compared with eight Herefords ($P < .01$). This suggests that a breed difference exists in the response to flumethasone. However, this experiment was not designed to determine the effect of breed on induced parturition and other factors including, but not limited to, fetal genotype, fetal size and the relative maturity of the fetus at the time of injection may be involved.

Breed difference in gestation length could be responsible for this differential of flumethasone response as Herefords have gestation lengths approximately 8 days longer than Angus cows (Wiltbank, 1974). However, the control Herefords in this study had shorter gestation than the control Angus (table 7-2). Another factor which may be important

would be a difference of body weights of responding vs. non-responding cows. Unfortunately, body weights of cows were not obtained in this experiment but records from previous years indicate that the Herefords weighed 50 to 100 kg more than the Angus.

Carroll (1974) found no significant correlation between response and cow weight using 11 cows injected with 5 mg flumethasone on day 270 of gestation. However, when 5 mg of flumethasone is given on day 264 of gestation as it was in this study, threshold levels may be attained in small cows but not in large cows. Both Bosc (1971) and Winter et al. (1974) have shown that induction success decreases as cows are treated earlier in gestation and Poncelet and Moody (unpublished data) have found a low rate of induced parturition using 3.5 mg of flumethasone approximately 17 days prior to expected parturition.

Cows in the INDU group had shortened gestation ($P < .01$), calving 9 days earlier than the NT cows and 14 days sooner than the NI cows. In a conventional management system this means that the INDU group would have more days from calving to the start of the breeding season than the other two groups. In this experiment, each cow as exposed to breeding after a definite interval following calving thereby removing the effect of variable times from parturition to the breeding season.

There were no significant differences between groups in reproductive efficiency as measured by postpartum interval to first estrus, number of times in estrus prior to 60 days postpartum, first service

conception rate, services per conception, pregnancy rate and days open. However, the INDU group had higher conception rates required fewer services per conception and were open less days than either the NT or NI groups. This indicates that induced parturition and/or retained placenta did not adversely affect fertility.

It is unlikely that the better reproduction in the INDU group compared with the NT and NI groups was due to the increased number of Angus cows in the INDU group. A comparison of Hereford and Angus cows in the NT group indicates that reproductive performance was equal between the breeds if not superior in the Hereford cows (table 7-2).

Postpartum interval to first estrus was unaffected by induced parturition with the INDU group showing a slightly longer interval of 2 and 7 days over the NT and NI groups, respectively. However, when postpartum interval to first estrus was calculated from expected normal parturition date, the resultant adjusted interval was shortest in the INDU group by 11 and 5 days over the NT and NI groups, respectively. Also the INDU group expressed estrus more times prior to 60 days postpartum than the NT or NI groups after, but not before, adjusted to expected normal parturition. In a conventional management system where breeding of all cows is started on a given day, this means that cows with induced parturition would have increased opportunity to cycle prior to breeding season and that breeding would occur

after the cow had cycled several times. Several workers as reviewed by Casida et al. (1968) have shown that fertility is low on the first postpartum estrus and increases with each subsequent cycle.

The NI group had more calving problems ($P < .05$) and had nonsignificantly higher calf vigor scores (decreased vigor) than controls. There was a negative correlation of calving difficulty score to first service conception rate ($r = .39$; $P < .05$). Jöchle (1972) found that when dexamethasone induced parturition was blocked with progesterone that the ensuing parturitions were marked with dystocia, low calf vigor and a high incidence of stillbirth. Calving difficulty has been shown to adversely affect fertility (Laster et al., 1973) and may explain the decrease in fertility in the NI group compared to the INDU group but does not explain the lowered fertility in the NT group where calving difficulty was minimal.

The weather during this calving season was cold and wet and eight calves were lost to either exposure or scours. However, the losses were evenly distributed across the groups with 2, 3 and 3 calf losses recorded in the NT, INDU and NI groups, respectively. In this study, calves from parturitions induced 15 days early were as vigorous as calves born from normal length gestations, even under these adverse conditions.

Birth weight was reduced by induced parturition which is consistent with other reports (LaVoie and Moody, 1973; Carroll, 1974; Wagner et al., 1974; Winter et al., 1974). It is felt that possibly the reduced birth weight seen with induced parturition is responsible for a reduction in weaning weight (Carroll, 1974). In this study when calves from induced parturitions were weighed on the date of their expected normal parturition it was found that they had gained 10 kg and weighed 34 kg which is comparable to the actual birth weights of 32 and 34 kg for the NT and NI groups, respectively. These calves from induced parturition gained as well as they would have in utero and can be expected to make the same weight gains that they would have had they not been born early. LaVoie and Moody (1973) found no significant difference in actual weaning weights between calves from induced parturitions compared to those from normal parturitions or in weaning weights adjusted for age from day of conception.

TABLE 7-1. REPRODUCTIVE PERFORMANCE OF HEREFORD AND ANGUS COWS FOLLOWING FLUMETHASONE INDUCED PARTURITION^a

	NT	INDU	NI
No. of cows:			
Hereford ^b	5	2	8
Angus ^b	7	8	1
Total	12	10	9
Post-injection interval to parturition (hours)	--	60 (20) ^c	325 (21) ^d
Birthdate (day of year)	95 (3) ^e	86 (3) ^f	100 (3) ^e
Gestation length (days)	278 (1) ^c	266 (1) ^d	278 (1) ^c
Calving difficulty (score 1 to 4)	1.0 (0.2) ^g	1.2 (0.2) ^{gh}	1.6 (0.2) ^h
Calf vigor at birth (score 1 to 4)	1.2 (0.2)	1.2 (0.2)	1.5 (0.2)
Calf losses (number of calves)	2	3	3
Birth weight (kg)	32 (2) ^e	24 (2) ^f	34 (3) ^e
Wt (kg) of induced calves at expected birth date ⁱ	--	34	--
Incidence of retained placenta (%)			
at: 24 hr	8 ^c	100 ^d	0 ^c
72 hr	8 ^c	80 ^d	0 ^c
1 wk	0	0	0
Postpartum interval to first estrus (days)	45 (6)	47 (6)	40 (6)
Adjusted ^j postpartum interval to first estrus (days)	43 (6)	32 (6)	37 (7)
Number times in estrus prior to 60 days postpartum	1.2 (0.2)	1.1 (0.3)	1.3 (0.3)
Adjusted ^j number of times in estrus prior to 50 days postpartum	1.4 (0.3)	1.7 (0.3)	1.5 (0.3)
First service conception rate (%)	58	90	56
Services per conception	2.1	1.1	1.8
Pregnancy rate (%)	58	90	67
Days open ^k	76 (5)	69 (5)	77 (6)

^a All means and percentages are least squares means except where indicated. Number in parenthesis are standard errors.

^b There was a difference ($P < .01$) in distribution of Hereford and Angus cows between INDU and NI groups.

^{cd} Means on the same line with different superscripts differ significantly ($P < .01$).

^{ef} These means differ as follows: NT vs INDU, $P < .05$; INDU vs NI, ($P < .01$).

^{gh} Means on the same line with different superscripts differ significantly ($P < .05$).

ⁱ Arithmetic mean. Seven calves from INDU group were weighed on the calculated day of expected normal parturition.

^j Postpartum interval to first estrus and times in estrus prior to 60 days postpartum were calculated from expected normal parturition date.

^k Calculated for pregnant cows only. N=7, 9 & 6 for NT, INDU & NI, respectively.

TABLE 7-2. REPRODUCTIVE PERFORMANCE OF HEREFORD AND ANGUS COWS IN THE CONTROL GROUP (NT)^a

	Hereford	Angus
No. of cows	5	7
Gestation length (days)	277	279
Birth wt (kg)	35	31
Postpartum interval to first estrus (days)	42	47
First service conception rate (%)	80	43
Services per conception	1.3	3.3
Pregnancy rate	80	43
Days open ^b	84	66

a All means are arithmetic means.

b Calculated for pregnant cows only. N=4 and 3 for Hereford and Angus, respectively.

CHAPTER 8

SUMMARY AND CONCLUSIONS

The bovine postpartum interval was investigated. The reports of the studies are preceded by a comprehensive literature review (CHAPTER 2) and a description of the technique of radioimmunoassay (CHAPTER 3). Four studies were conducted using techniques including radioimmunoassay, mastectomy and hormone treatment to evaluate various aspects of bovine postpartum anestrus.

In the first study (CHAPTER 4) radioimmunoassay was used to measure blood plasma progesterone in daily samples from six Hereford and 12 Angus cows to characterize the progesterone profile during the postpartum period. Cows were assigned to a NON-LACTATING, a SUCKLED 2X daily or a SUCKLED AD LIBITUM group to determine the effect of suckling on these hormones and on the occurrence and intensity of estrus. Level of TDN intake was adjusted to cause all cows to maintain their post-calving body weights over the experimental period to minimize the effects of parturition. Testosterone was measured by radioimmunoassay in every third sample from parturition through first estrus in three cows, one from each treatment group, to determine what levels exist in the postpartum cow.

Suckling increased the postpartum interval and the intensity of first estrus ($P < .05$) and two cows from the NON-LACTATING group and one from the SUCKLED AD LIBITUM group first ovulated in the absence of

estrous behavior. In analyses excluding these three cows progesterone averaged .8 ng/ml at parturition and ranged between .1 and .4 ng/ml through day 18 postpartum and was lowest in suckled cows ($P < .01$). Progesterone ranged between .1 and .4 ng/ml from 18 through 5 days, increased rapidly to 1.8 ng/ml at 3 days and returned to .3 ng/ml by one day prior to estrus. This pre-estrus progesterone peak was highest in suckled cows ($P < .05$) and was positively associated with the postpartum interval ($r = .69$; $P < .01$). The pre-estrus progesterone peak appeared to be absent in cows with short postpartum intervals or short first estrous cycles and the possibility that peaks of short duration were present but not detectable in daily samples was discussed.

Blood plasma testosterone levels in three postpartum cows were low (averaging less than .5 ng/ml) which indicates that testosterone has little effect on the events following parturition in the cow.

In the second study (CHAPTER 5), progesterone and estrogen given in single doses of 20 and 2 mg, respectively, to dairy cows from 30 to 90 days postpartum, tended to shorten the interval from parturition to first postpartum estrus; but did not shorten the interval from parturition to conception nor improve reproductive performance as measured by first service conception rate, conception per service and pregnancy rate. The steroid treatment did not affect ovary diameter at 10 through 44 days postpartum or milk production from 3 through 50 days postpartum.

In the third study (CHAPTER 6), nine mastectomized beef cows showed rather low fertility as measured by first service conception rate and conceptions per service as compared to the Montana State University beef herd. The pregnancy rate of the mastectomized groups was high due to an extended breeding season. Mastectomy did not appear to affect the interval from parturition to conception when compared to the MSU beef herd, and did not substantially shorten the interval to less than 82 days; indicating that mastectomy had little effect in reducing calving interval to less than 365 days.

In the fourth study (CHAPTER 7), 31 Hereford and Angus cows were assigned to a non-treated group (NT) or a group receiving 5 mg of flumethasone 17 days prior to expected calving date to test the effect of flumethasone induced parturition on the postpartum interval and fertility. Flumethasone treated cows that calved within 90 hr after injection were assigned to an induced parturition group (INDU) while the remainder were assigned to a non-induced parturition group (NI). Cow breed affected ($P < .01$) induced parturition success so that INDU group contained 2 and 8 and NI group contained 8 and 1 Herefords and Angus, respectively. This apparent breed effect may have resulted from other factors such as differences in stage of gestation at time of injection or differences in body weight. AI breeding for each individual cow started 60 days postpartum and continued 45 days.

Induced parturition shortened gestation ($P < .01$) and decreased birth weights ($P < .05$) over both of the non-induced groups, but it was found that induced calves gained at a rate that made their weights at the day of expected normal parturition comparable to birth weights of NT and NI calves. INDU cows had 100% incidence of retained placenta while that of the two non-induced groups was close to 0%. NI cows had an increased amount of calving difficulty ($P < .05$) but calf vigor was unaffected by group.

There were no significant differences between groups in reproductive efficiency as measured by postpartum interval to first estrus, number of times in estrus prior to 60 days postpartum, first service conception rate and days open, but the INDU group had higher conception rates, required fewer services per conception, and were open less days than either the NT or NI group. When postpartum interval to first estrus and times in estrus prior to 60 days postpartum were calculated from expected normal parturition date, the INDU group had the shortest postpartum interval and showed estrus more times prior to 60 days than either the NT or NI groups.

The major conclusions of these studies were:

1. A pre-estrus blood plasma progesterone rise occurs and is associated with first postpartum estrus and ovulation, but it is difficult to determine progesterone's role in the reestablishment of postpartum estrous cycles.

2. Progesterone and estrogen treatments can be used in a dairy management situation to shorten the interval from parturition to first estrus but the interval to conception is not affected.

3. Mastectomy of beef cows does not appear to enhance early postpartum fertility, indicating that mastectomy has little effect in reducing calving interval to less than 365 days.

4. Although induced parturition did not significantly affect fertility, cows with induced parturition had a greater interval from calving to the start of breeding ($P < .01$). Induced parturition shows potential of improving fertility.

CHAPTER 9

APPENDIX

APPENDIX TABLE 9-1. BASIC COMPUTER PROGRAM FOR COMPUTATION OF
PROGESTERONE AND TESTOSTERONE CONCENTRATIONS
IN BLOOD PLASMA

```

1 OPEN 'ASSAY', TO:1, PRINT, OVER, TFILE
2 PRINT ' INPUT ASSAY #'
3 INPUT N
4 PRINT ' ASSAY DATE?'
5 INPUT D
6 PRINT ' INPUT PLASMA CORRECTION FACTOR'
7 INPUT C
8 PRINT:1,'1PROGESTERONE RIA #N; ' DATE='D
9 PRINT:1,' PLASMA CORRECTION FACTOR='C
10 :1REC ASSAY REC STD & AVG PG COREC NG AVG #
11 : REC ASSAY REC STD & AVG PG COREC NG AVG #
15 : TB TUBE CPM % SMPL SMPL CPM % PER FOR PER STD #
20 : NO. NUMBER -BK REC ID DATE -BK BND .3ML RECOV ML CUR 3
25 : ## ###-### ##### ##### ##### ##### ##### #####.## ##
30 : AVE BK= #####/4=###
35 : TC FOR RECOV=((#####+#####+#####+#####)/4)-AVG BK)*.4=###
40 : AVE CPM FOR 100% RECOV =####
41 PRINT "IMPUT 4 BKGRND CPMS"
42 IMPUT B1,B2,B3,B4
43 PRINT "IMPUT 4 TC FOR RECOV CPMS"
44 INPUT T1,T2,T3,T4
45 PRINT "IMPUT BEGIN & END LINE# FOR LST CURVE, UNKNOWNNS & 2ND CURVE"
46 INPUT U,Z,U1,Z1,U2,Z2
50 B5=(B1+B2+B3+B4)/4
55 T5=((T1+T2+T3+T4)/4)-B5)*.4
60 F=-1
65 I=0
70 DIM A(56),B(56),D(56),E(56),F(56),G(56),H(56),I(56),J(56),L(56)
75 DIM M(56),N(56),O(56),P(56),Q(56)
80 FOR K=U TO Z2
85 READ A(K),B(K),C(K),D(K),E(K)
90 F=F+2
95 F(K)=F
100 G(K)=F(K)+1
105 H(K)=(D(K)+E(K))/2-B5
110 NEXT K
115 FOR K=U TO Z
120 I(K)=' '
125 J(K)=' '
130 L(K)=' '
135 M(K)=' '
140 N(K)=' '
145 O(K)=' '

```

APPENDIX TABLE 9-1. (CONTINUED)

```

150 NEXT K
155 FOR K=U1 TO Z1
160 I=I+1
165 I(K)=I
170 J(K)=A(K)-B5
175 L(K)=J(K)/T5
180 P(K)=I
185 NEXT K
190 FOR K=U2 TO Z2
195 I(K)=I
200 J(K)=I
205 L(K)=I
210 M(K)=I
215 N(K)=I
220 O(K)=I
225 P(K)=I
230 NEXT K
235 Q(1)=(H(2)/H(1))*100
240 FOR K=U+1 TO Z
245 Q(K)=H(K)/H(2)*100
250 NEXT K
255 Q(U2)=(H(U2+1)/H(U2))*100
260 FOR K=U2+1 TO Z2
265 Q(K)=H(K)/H(U2+1)*100
270 NEXT K
275 P(1)=I
280 FOR K=U+1 TO Z
285 P(K)=Q(K)+Q(K+Z1))/2
290 NEXT K
295 A=(H(2)+H(U2+1))/2
300 FOR K=U1 TO Z1
305 Q(K)=(H(K)/A)*100
310 NEXT K
315 FOR K=3 TO 10
320 U(K)=LGT(B(K))
325 NEXT K
330 FOR K=U+2 TO Z-1
335 X=(U(K)+U(K+1))/2
340 Y1=U(K)-X
350 X2=U(K+1)-X
355 Y1=P(K)-Y
360 Y2=P(K+1)-Y
365 X3=X1*Y1+Y2*Y2

```

APPENDIX TABLE 9-1. (CONTINUED)

370 $X_4 = X_1^{**2} + X_2^{**2}$

375 $R(K) = X_3 / X_4$

380 $S(K) = Y - R(K) * X$

385 NEXT K

390 FOR K=U1 TO Z1

395 IF Q(K),P(3) THEN 435

400 IF Q(K),P(4) THEN 455

405 IF Q(K),P(5) THEN 465

410 IF Q(K),P(6) THEN 475

415 IF Q(K),P(7) THEN 485

420 IF Q(K),P(8) THEN 495

425 IF Q(K),P(9) THEN 505

430 IF Q(K),P(10) THEN 515

435 $M(K) = .10$

440 $N(K) = 10 / L(K)$

445 $O(K) = N(K) * 6.66 / 1000$

450 GO TO 520

455 $M(K) = 10 * ((Q(K) - S(3)) / R(3))$

460 GOTO 520

465 $M(K) = 10 * ((Q(K) - S(4)) / R(4))$

470 GOTO 520

475 $M(K) = 10 * ((Q(K) - S(5)) / R(5))$

480 GO TO 520

485 $M(K) = 10 * ((Q(K) - S(6)) / R(6))$

490 GOTO 520

495 $M(K) = 10 * ((Q(K) - S(7)) / R(7))$

500 GOTO 520

505 $M(K) = 10 * ((Q(K) - S(8)) / R(8))$

510 GOTO 520

515 $M(K) = 10 * ((Q(K) - S(8)) / R(8))$

520 NEXT K

525 FOR K=U1 TO Z1

530 IF Q(K)>=(3) THEN 545

535 $N(K) = M(K) / L(K)$

540 $O(K) * 6.66 / 1000$

545 NEXT K

550 PRINT:1,USING 30,B1,B2,B3,B4,B5

555 PRINT:1,USING 35,T1,T2,T3,T4,T5

560 PRINT:1,USING 40,A

570 PRINT:1

575 PRINT:1,USING 11,Z2

580 PRINT:1,USING 15,Z2

585 PRINT:1,USING 20,Z2

595 IF Z2<29 THEN 670

APPENDIX TABLE 9-1. (CONTINUED)

```
600 FOR (K=1 TO 28
601 IF M(K)=' ' THEN 605
602 PRINT:1,USING 25,I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),)(K),
M(K),N(K),)(K)*C,P(K)
603 GO TO 620
605 (PRINT:1,USING 25,I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),Q(K),
M(K)MB*J(MO(K),P(K)
620 NEXT K
625 PRINT:1,USING 10,Z2
630 PRINT:1,USING 15,Z2
635 PRINT:1,USING 20,Z2
645 FOR K=s9 TO Z2
646 IF M(K)=' ' THEN 650
647 PRINT:1,USING 25,I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),Q(K),
M(K),N(K),O(K)*C,P(K)
648 GOTO 660
650 PRINT:1,USING 25,I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),Q(K),
M(K),N(K),O(K),P(K),O(K),P(K)
660 NEXT K
665 GOTO 800
670 (FOR K=1 TO Z2
671 IF M(K)=' ' THEN 675
672 PRINT:1,USING 25, I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),Q(K),
M(K),N(K),O(K)*C,P(K)
673 GO TO 685
675 PRINT:1,USING 25,I(K),F(K),G(K),J(K),L(K)*100,B(K),C(K),H(K),Q(K),
M(K),N(K),N(K),O(K),P(K)O(K),P(K)
685 NEXT K
800 END
```

APPENDIX TABLE 9-2. LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI (CHAP. 4)

Source	d.f.	Mean squares body weight
Treatment	2	4030.00
Days	9	4037.36
Line	1	3236.32
Quad	1	9161.85
Cubi	1	1645.89
Quar	1	3186.98
Quin	1	2052.81
Days x treatment	18	296.36
Error	150	2144.64

APPENDIX TABLE 9-3. LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN NON-LACTATING COWS (CHAP. 4)

Source	d.f.	Mean squares body weight
Days	9	1086.63
Line	1	2176.25
Quad	1	751.17
Cubi	1	638.65
Quar	1	995.74
Quin	1	603.95
Error	50	3876.56

APPENDIX TABLE 9-4. LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUCKLED 2X DAILY (CHAP. 4)

Source	d.f.	Mean squares body weight
Days	9	1416.34
Line	1	87.46
Quad	1	3483.47
Cubi	1	1781.38
Quar	1	1060.47
Quin	1	702.08
Error	50	1451.95

APPENDIX TABLE 9-5. LEAST SQUARES ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGES DURING THE POSTPARTUM PERIOD IN COWS SUCKLED 2X DAILY (CHAP. 4)

Source	d.f.	Mean squares body weight
Days	9	2127.11
Line	1	3749.76
Quad	1	6297.93
Cubi	1	7.85
Quar	1	1134.72
Quin	1	761.83
Error	50	1105.39

APPENDIX TABLE 9-6. LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Trait	Source			
	Treatment		Error	
	d.f.	Mean squares	d.f.	Mean squares
Body wt within 7 days prior to parturition	2	25.39	15	2102.23
Body wt within 48 hr postpartum	2	225.17	15	2171.34
Body wt with 7 days of first observed estrus	2	1230.06	15	1929.31
Incidence of silent first estrus	2	0.1666	15	0.1444
Intensity of first estrus and/or ovulation	2	8.6667*	15	1.6778
Intensity of first observed estrus	2	3.5556*	15	0.6111
Postpartum interval to first observed estrus	2	552.72*	15	102.73
Incidence of non-standing estrous behavior	2	0.7222	15	0.6444
Progesterone, average days 6, 7 and 8 prior to first observed estrus	2	7.6089	15	8.5605
Progesterone, average days 2, 3 and 4 prior to first observed estrus	2	3.9951	15	2.7689
Progesterone highest level on day 2, 3 or 4 prior to first observed estrus	2	15.766	15	8.024

* $P < .05$

APPENDIX TABLE 9-7. LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Trait	Source			
	Treatment		Error	
	d.f.	Mean squares	d.f.	Mean squares
Body wt with 7 days prior to parturition	2	649.63	12	1463.76
Body wt within 48 hr postpartum	2	483.80	12	1562.34
Body wt within 7 days of first estrus	2	1835.42	12	1423.17
Intensity of first estrus	2	3.608*	12	0.5319
Postpartum interval to first estrus	2	404.18*	12	65.836
Incidence of non-standing estrous behavior	2	0.7417	12	0.6208
Progesterone, average days 6,7 and 8 prior to first estrus	2	0.1351	12	0.3026
Progesterone, average days 2, 3 and 4 prior to first estrus	2	2.3050**	12	0.6549
Progesterone, highest level on day 2, 3 or 4 prior to first estrus	2	12.379*	12	2.548

** P<.10

* P<.05

APPENDIX TABLE 9-8. LEAST SQUARES ANALYSIS OF VARIANCE FOR INTENSITY OF SECOND OBSERVED ESTRUS AND LENGTH OF FIRST OBSERVED ESTROUS CYCLE IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares	
		Intensity of second observed estrus	Length of first observed estrous cycle
Treatment	2	0.3794	18.669
Error	14	1.0214	16.224

APPENDIX TABLE 9-9. LEAST SQUARES ANALYSIS OF VARIANCE FOR INTENSITY OF SECOND ESTRUS AND LENGTH OF FIRST ESTROUS CYCLE IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI EXCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares	
		Intensity of second estrus	Length of first estrous cycle
Treatment	2	0.3036	20.928
Error	11	1.1136	18.955

APPENDIX TABLE 9-10. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI INCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares				
		Progesterone 0 to 18 days postpartum	d.f.	Progesterone 18 to 0 days prior first obs. estrus	d.f.	Progesterone 0 to 11 days of first obs. estrous cycle
Treatment	2	4.0059**	2	34.5898	2	4.4079
Days	18	0.8187**	18	6.5817 ^a	3	42.7155**
Line	1	0.7883	1	26.2202*	1	119.5729**
Quad	1	1.8794*	1	38.1562**	1	8.1531
Cubi	1	0.8822	1	31.0859**	1	0.4221
Quar	1	3.9834**	1	5.9893	-	--
Quin	1	1.0023	1	0.4777	-	--
Treatment X Days	36	0.4453	36	2.6931	6	2.0747
Error	232	0.3840	241	4.1161	50	3.3824

^a P<.10

* P<.05

**P<.01

APPENDIX TABLE 9-11. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN NON-LACTATING COWS INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares		d.f.	d.f.
		Progesterone 0 to 18 days postpartum	Progesterone 18 to 0 days prior first obs. estrus	Progesterone 0 to 11 days of first obs. estrous cycle	
Days	18	1.2409	6.7444	3	12.9303*
Line	1	0.0258	11.5194	1	36.4200**
Quad	1	0.1615	58.3698**	1	1.9455
Cubi	1	0.0550	27.2312	1	0.4259
Quar	1	4.6796	1.2375	-	--
Quin	1	1.3353	10.8097	-	--
Error	67	1.2432	8.2020	20	4.0400

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-12. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED 2X DAILY INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Progesterone 0 to 18 days postpartum	d.f.	Mean squares		d.f.
				Progesterone 18 to 0 days prior first obs. estrus	Progesterone 0 to 11 days of first obs. estrous cycle	
Days	18	0.0996**	18	0.4570**	3	18.0965
Line	1	.3019**	1	2.6852**	1	41.1543*
Quad	1	.8452**	1	0.0264	1	8.0967
Cubi	1	.2214**	1	1.0091*	1	5.0459
Quar	1	.3551**	1	1.3764**	1	--
Quin	1	.0128	1	0.4287	1	--
Error	77	0.0212	82	0.1839	11	6.6167

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-13. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED AD LIBITUM INCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares		d.f.	d.f.
		Progesterone 0 to 18 days postpartum	Progesterone 18 to 0 days prior first obs. estrus	Progesterone 0 to 11 days of first obs. estrous cycle	
Days	18	0.3189**	4.0775	3	14.1638**
Line	1	1.5452**	11.2989	1	41.5859**
Quad	1	1.3298**	9.2527	1	.0216
Cubi	1	1.3456**	7.1539	1	.8836
Quar	1	0.5961**	15.7521	-	--
Quin	1	0.1265	3.3072	-	--
Error	88	0.0473	4.6061	15	0.8178

**P<.01

APPENDIX TABLE 9-14. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI. EXCLUDING THREE COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares		d.f.	d.f.
		Progesterone 0 to 18 days postpartum	Progesterone 18 to 0 days prior first obs. estrus	Progesterone 0 to 11 days, of first obs. estrous cycle	
Treatment	2	0.9122**	2	1.0266	2
Days	18	0.3447**	18	2.3251**	3
Line	1	0.1651*	1	9.8258**	1
Quad	1	2.9240**	1	0.1675	1
Cubi	1	1.0522**	1	5.6470**	1
Quar	1	1.2025**	1	9.0539**	-
Quin	1	0.1149*	1	1.6181*	-
Treatment X					
Days	36	0.0726	36	0.7002	6
Error	180	0.0257	186	0.3179	38

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-15. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN NON-LACTATING COWS EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares		d.f.	d.f.
		Progesterone 0 to 18 days postpartum	Progesterone 18 to 0 days prior first estrus	Progesterone 0 to 11 days of first estrous cycle	
Days	18	0.1837**	0.1239	3	6.3661
Line	1	0.4473**	0.0769	1	17.9285
Quad	1	1.1454**	0.4236	1	0.9073
Cubi	1	0.3075*	0.0272	1	0.2625
Quar	1	0.5389**	0.3066	-	--
Quin	1	0.0154	0.0399	-	--
Error	32	0.0440	0.2233	20	5.3103

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-16. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED 2X DAILY EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Mean squares		d.f.	d.f.
		Progesterone 0 to 18 days postpartum	Progesterone 18 to 0 days prior first estrus		Progesterone 0 to 11 days of first estrous cycle
Days	18	.0996**	.4570**	3	18.0965
Line	1	.3019**	2.6851**	1	41.1543*
Quad	1	.8452**	0.0264	1	8.0967
Cubi	1	.2214**	1.0091*	1	5.0460
Quar	1	.3551**	1.3764**	-	--
Quin	1	.0127	0.4287	-	--
Error	77	.0212	0.1840	11	6.6167

* P<.05

**P<.01

APPENDIX TABLE 9-17. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAILY BLOOD PLASMA PROGESTERONE LEVELS DURING VARIOUS STAGES POSTPARTUM IN COWS SUCKLED AD LIBITUM EXCLUDING COWS WITH SILENT FIRST OVULATION (CHAP. 4)

Source	d.f.	Progesterone 0 to 18 days postpartum	d.f.	Mean squares		d.f.
				Progesterone 18 to 0 days prior first estrus	Progesterone 0 to 11 days of first estrous cycle	
Days	18	0.2050**	18	3.2815**	15.4187**	3
Line	1	1.3140**	1	15.0500**	45.0079**	1
Quad	1	1.0274**	1	.0793	0.2029	1
Cubi	1	0.6230**	1	7.4113**	1.0427	1
Quar	1	0.3477**	1	12.2558**	--	-
Quin	1	0.1739**	1	2.8490*	--	-
Error	71	0.0223	73	0.5087	0.4324	15

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-18. LEAST SQUARES ANALYSIS OF VARIANCE FOR BLOOD PLASMA TESTOSTERONE LEVELS DURING THE POST-PARTUM PERIOD IN THREE COWS SUBJECTED TO THREE LEVELS OF SUCKLING STIMULI (CHAP. 4)

Source	d.f.	Mean square for blood plasma testosterone (ng/ml)
Cow	2	0.3165**
Days	9	0.0293
Line	1	0.0001
Quad	1	0.0437
Cubi	1	0.0009
Quar	1	0.1652*
Quin	1	0.0002
Error	18	0.0259

* $P < .05$

** $P < .01$

APPENDIX TABLE 9-19. LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS REPRODUCTIVE TRAITS OF STEROID TREATED COWS (CHAP. 5, TRIAL A)

Source	d.f.	Mean squares					
		Average date of calving	Interval from parturition to first estrus	No. cows in estrus by 60 days postpartum	No. cows in estrus by 82 days postpartum	Conception per service	Days open
Treatment	1	1.79	1897.79*	1.1429**	1.7857	0.0018	728.6
Error	12	1317.28	452.76	0.1904	0.5953	0.1223	4100.3

* P<.07

**P<.10

APPENDIX TABLE 9-20. LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS REPRODUCTIVE TRAITS OF STEROID TREATED COWS (CHAP. 5, TRIAL B)

Source	d.f.	Mean squares			
		Average date of calving	Interval parturition to first estrus	Conceptions per service	Days open
Treatment	1	44.10	2975.62**	0.0016	2407.7
Error	16	262.27	437.40	0.1042	3463.2

**P<.01

APPENDIX TABLE 9-21. LEAST SQUARES ANALYSIS OF VARIANCE FOR OVARY DIAMETER AT VARIOUS STAGES POSTPARTUM IN STEROID TREATED COWS (CHAP. 5, TRIAL B)

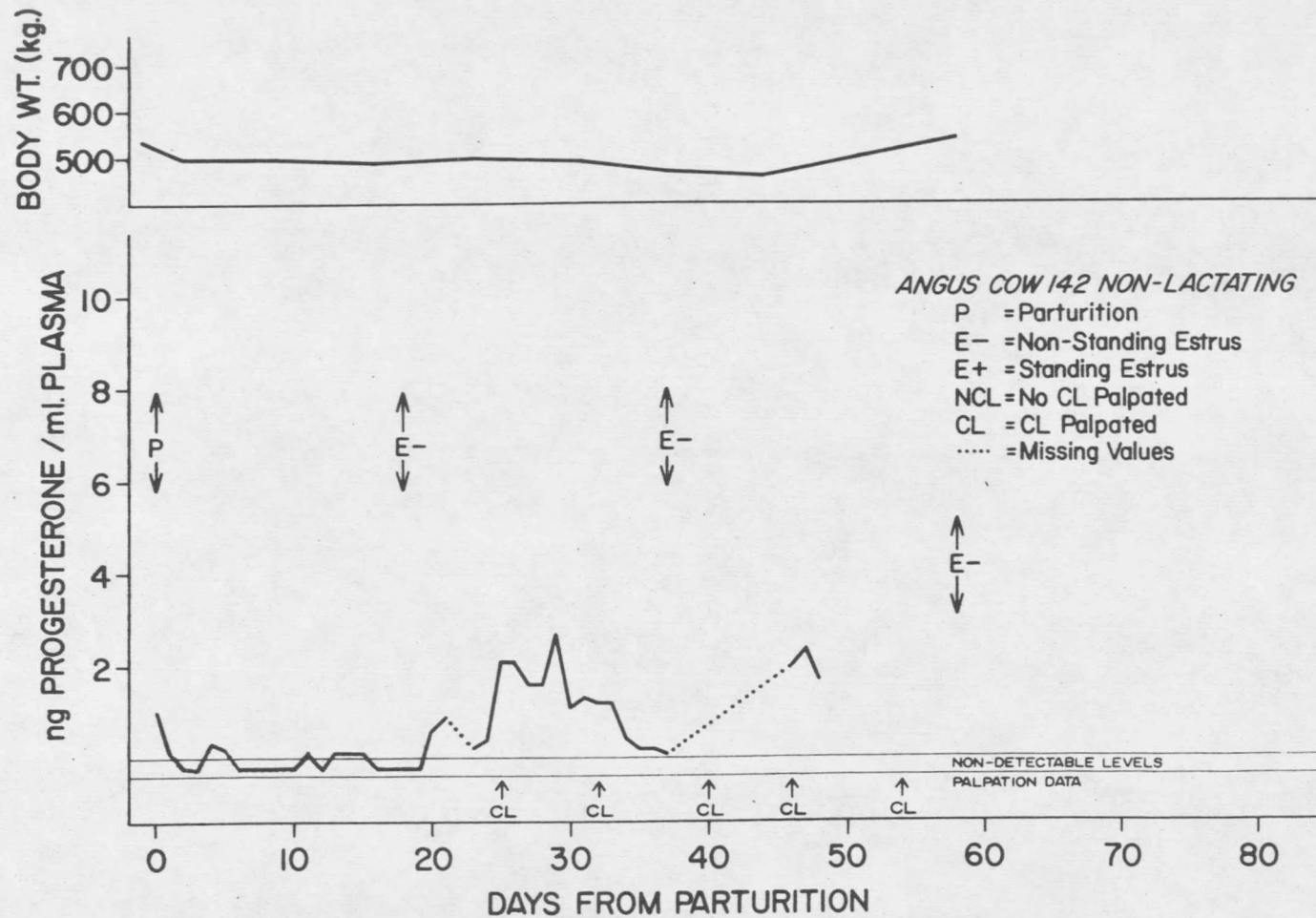
Source	d.f.	Mean squares ovary diameter											
		10 days	20 days	30 days	31 days	32 days	33 days	34 days	35 days	36 days	37 days	38 days	44 days
Treatment	1	0.0	0.0	22.0	17.6	23.3	47.7	112.2	143.7	15.2	4.3	0.9	12.8
Error	16	11.5	9.0	10.3	11.4	12.6	11.0	27.4	28.2	5.2	29.5	19.1	31.6

APPENDIX TABLE 9-22. LEAST SQUARES ANALYSIS OF VARIANCE FOR VARIOUS TRAITS IN COWS AND CALVES SUBJECTED TO FLUMETHASONE INDUCED PARTURITION (CHAP. 7)

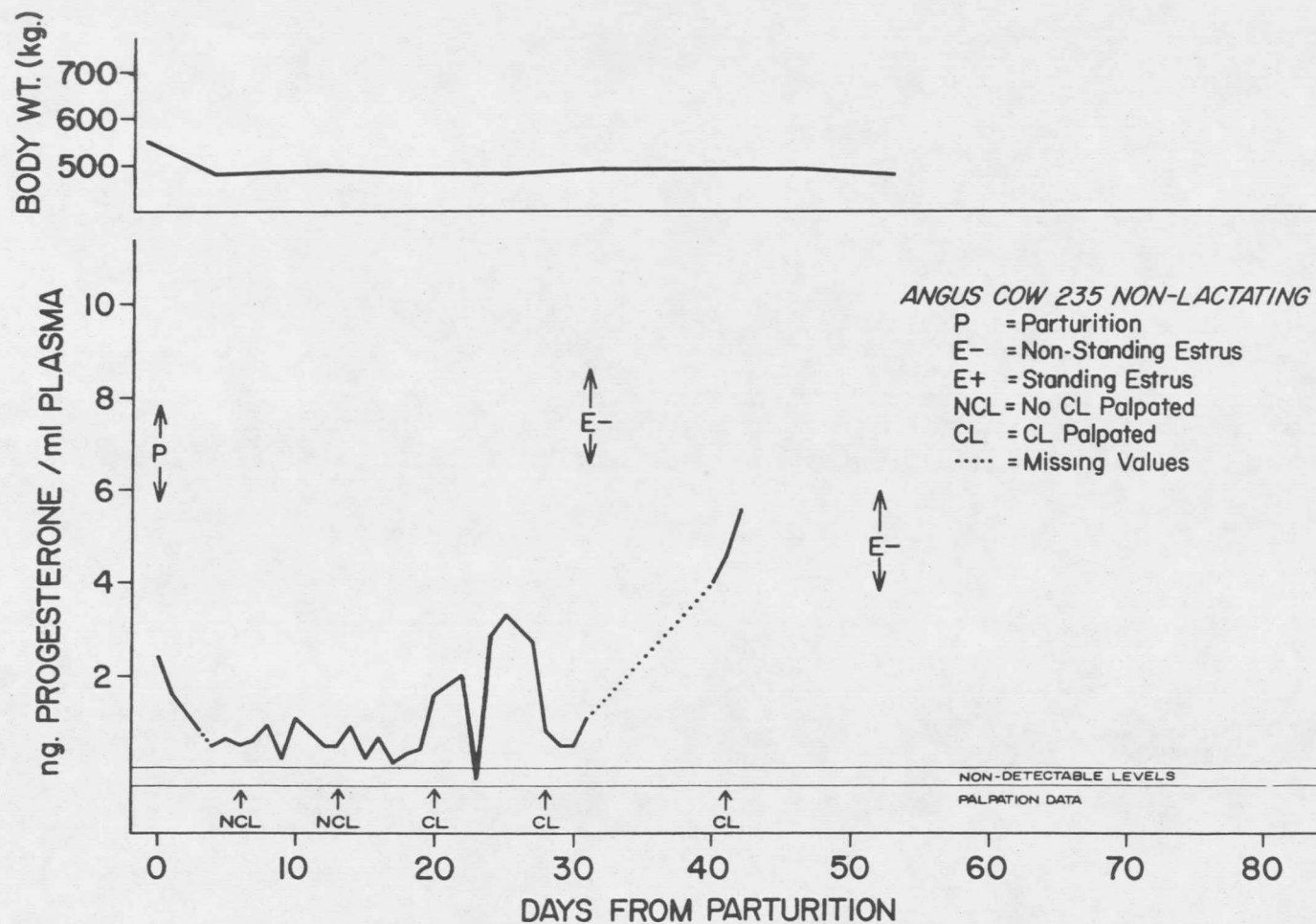
Trait	Source			
	Treatment		Error	
	d.f.	Mean squares	d.f.	Mean squares
Post-injection interval to parturition	2	334488.3**	17	3965.4
Birth date	2	465.54*	28	88.16
Gestation length	2	512.53**	28	14.78
Calving difficulty	2	0.7986	28	0.4222
Calf vigor	2	0.4491	28	0.4817
Birth weight	2	1399.2**	28	241.98
Retained placenta at 24 hr	2	3.090**	28	0.032
Retained placenta at 72 hr	2	1.935**	28	0.0898
Postpartum interval (estrus)	2	112.81	28	379.19
Adjusted postpartum interval	2	300.38	28	395.46
Times in estrus prior to 60 days postpartum	2	0.1360	28	0.6631
Adjusted times in estrus prior to 60 days postpartum	2	0.2193	28	0.7585
First service conception rate	2	0.3677	28	0.2157
Pregnancy rate	2	0.2852	28	0.2077
Days open	2	135.11	19	187.43

* $P < .05$

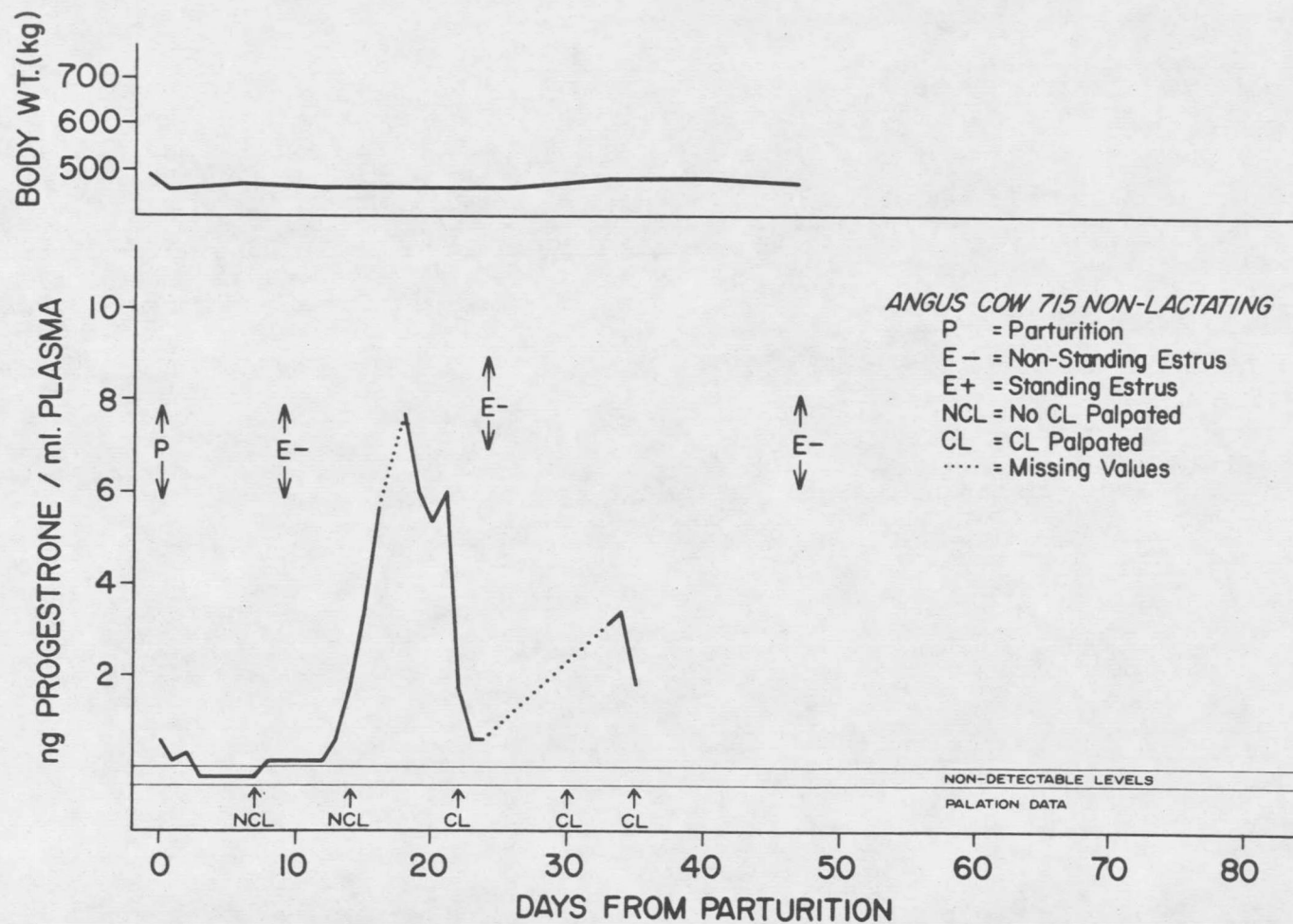
** $P < .01$



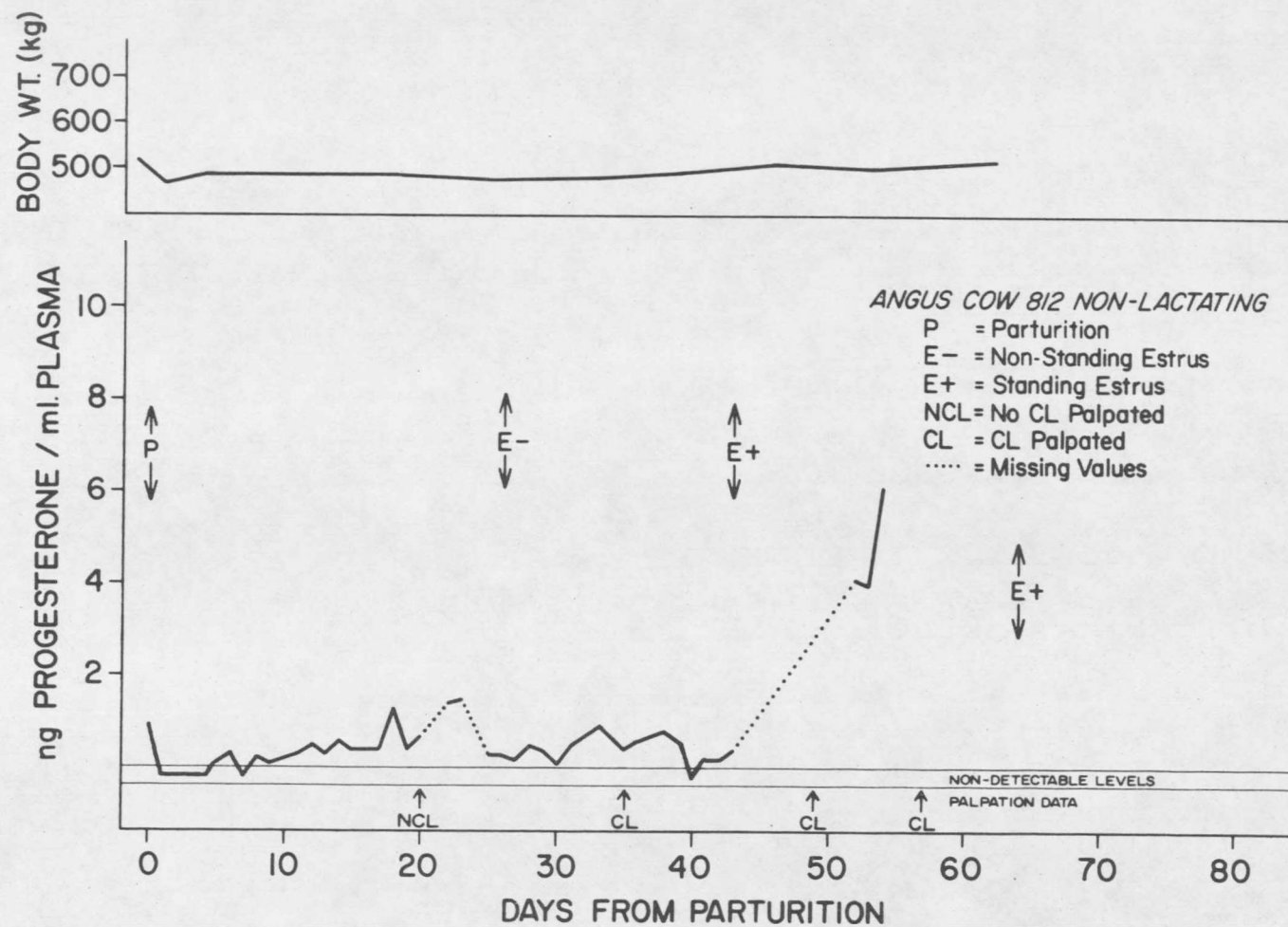
Appendix figure 9-1. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 142 (NON-LACTATING) during the postpartum period.



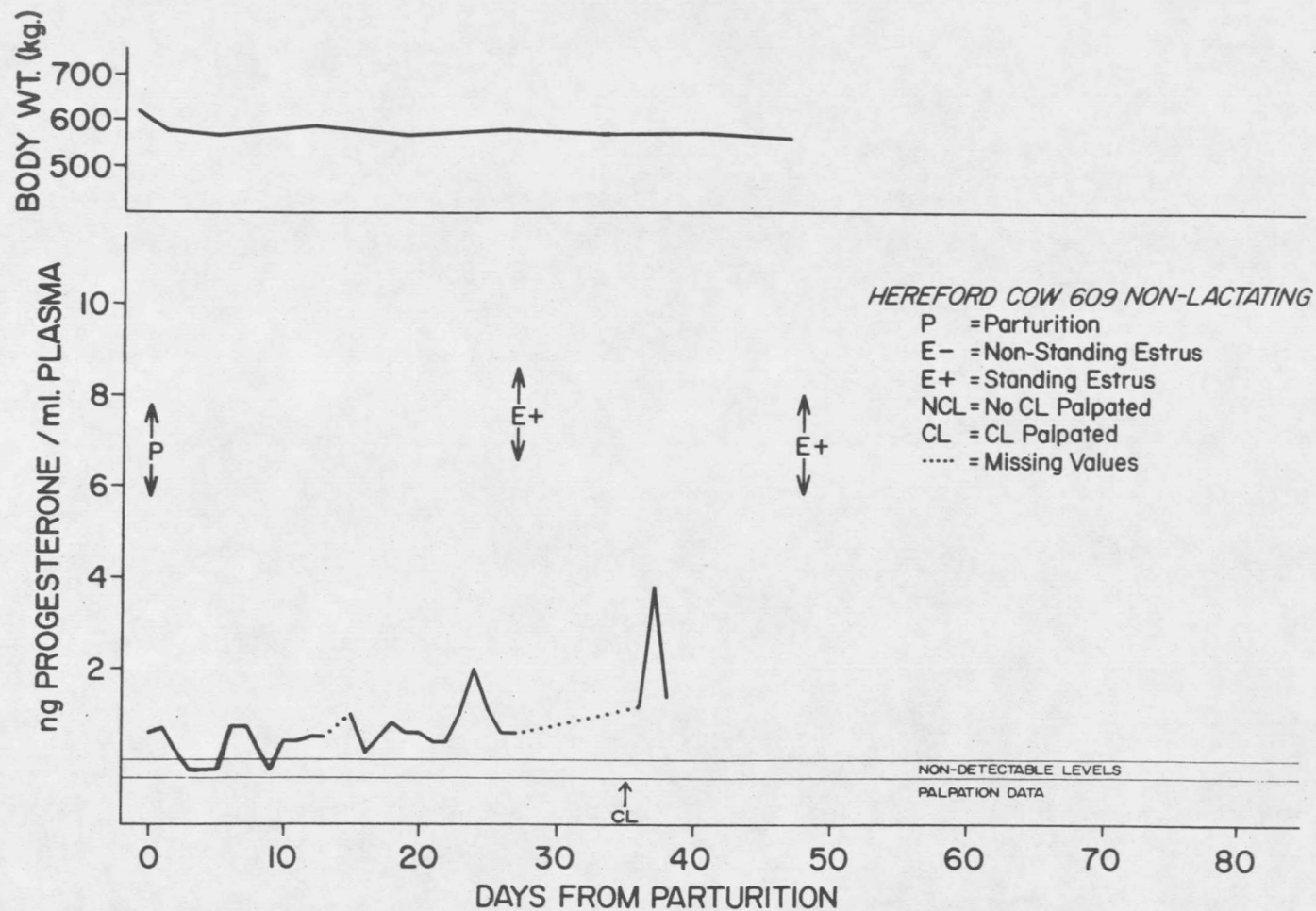
Appendix figure 9-2. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 235 (NON-LACTATING) during the postpartum period.



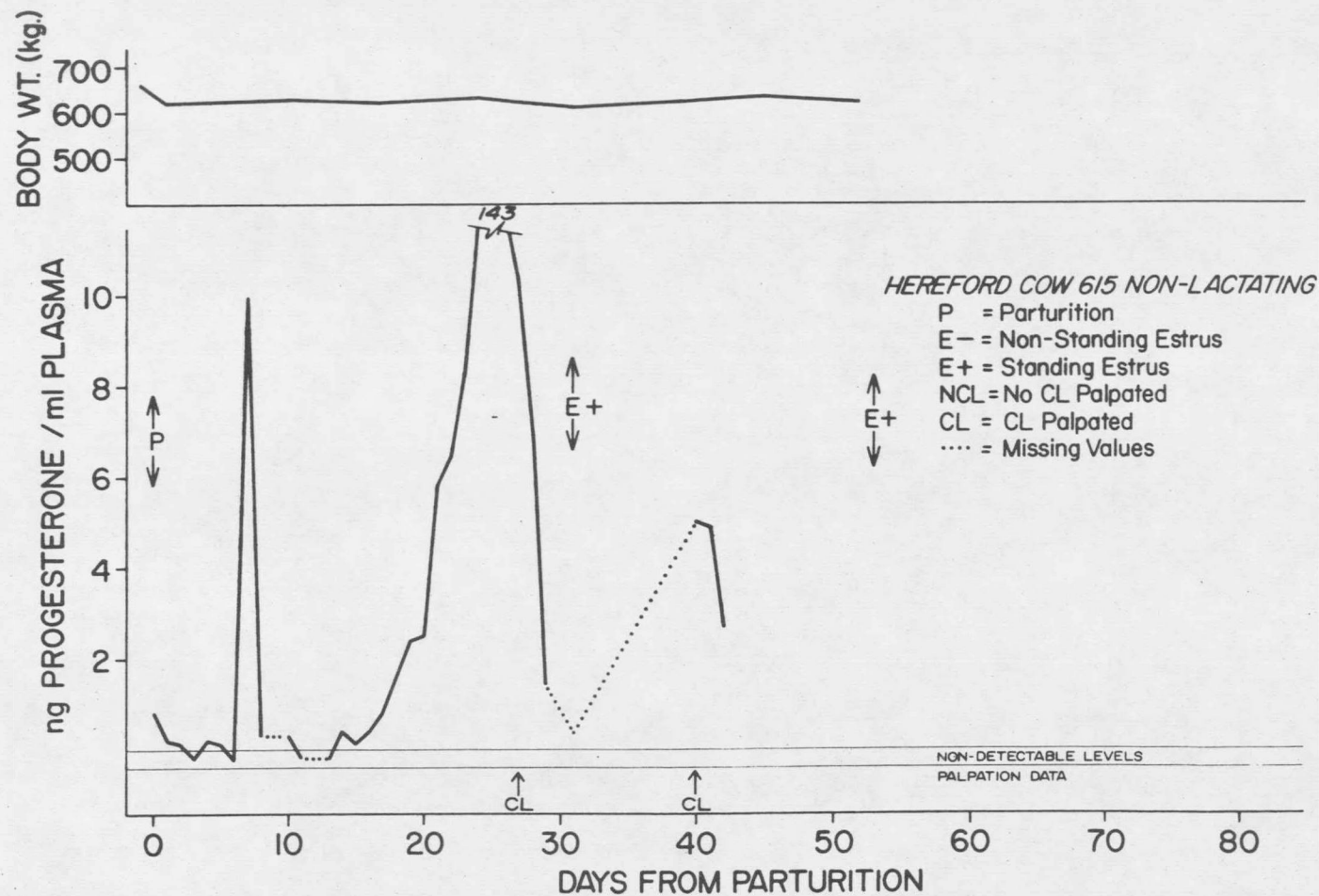
Appendix figure 9-3. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 715 (NON-LACTATING) during the postpartum period.



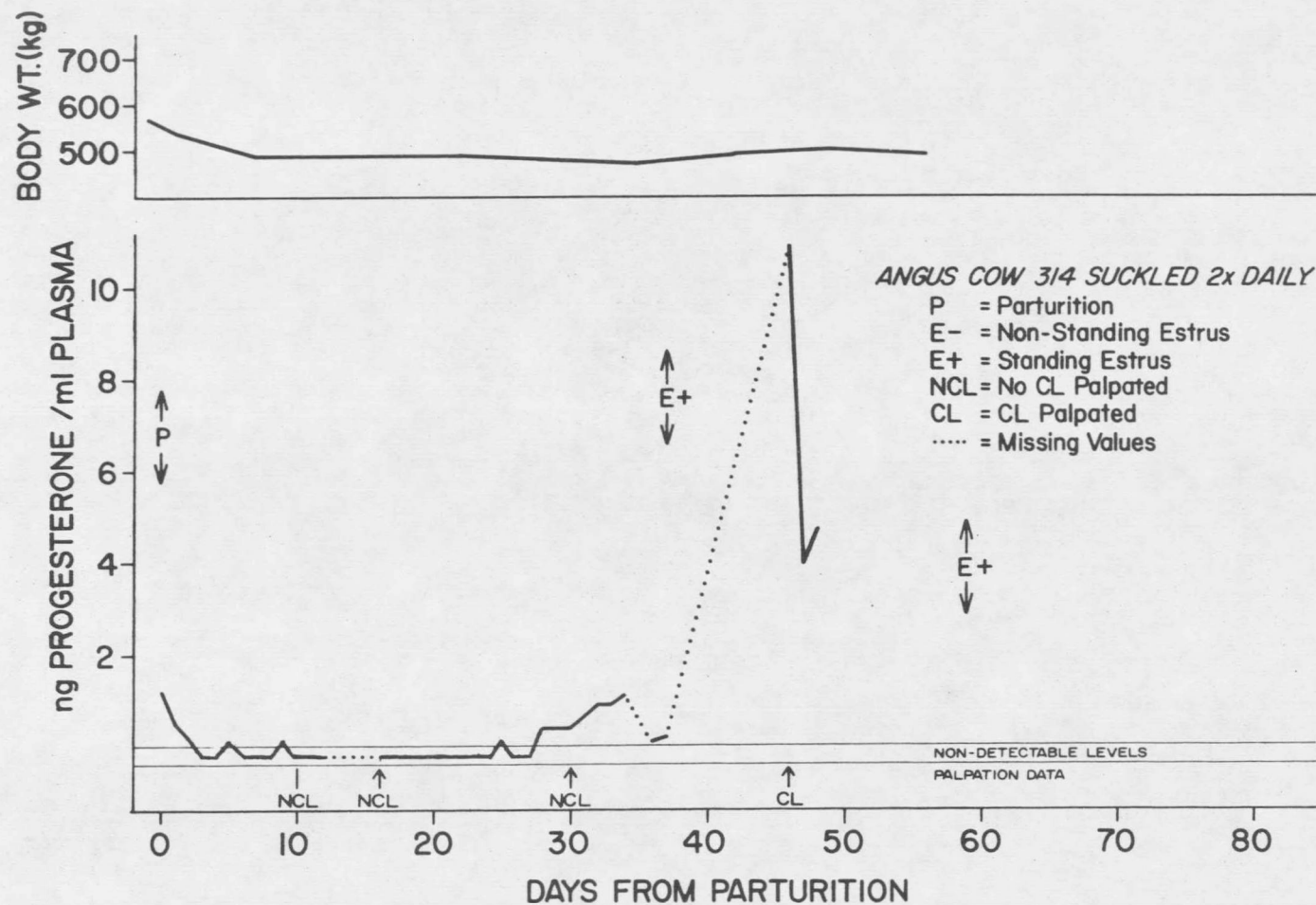
Appendix figure 9-4. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 812 (NON-LACTATING) during the postpartum period.



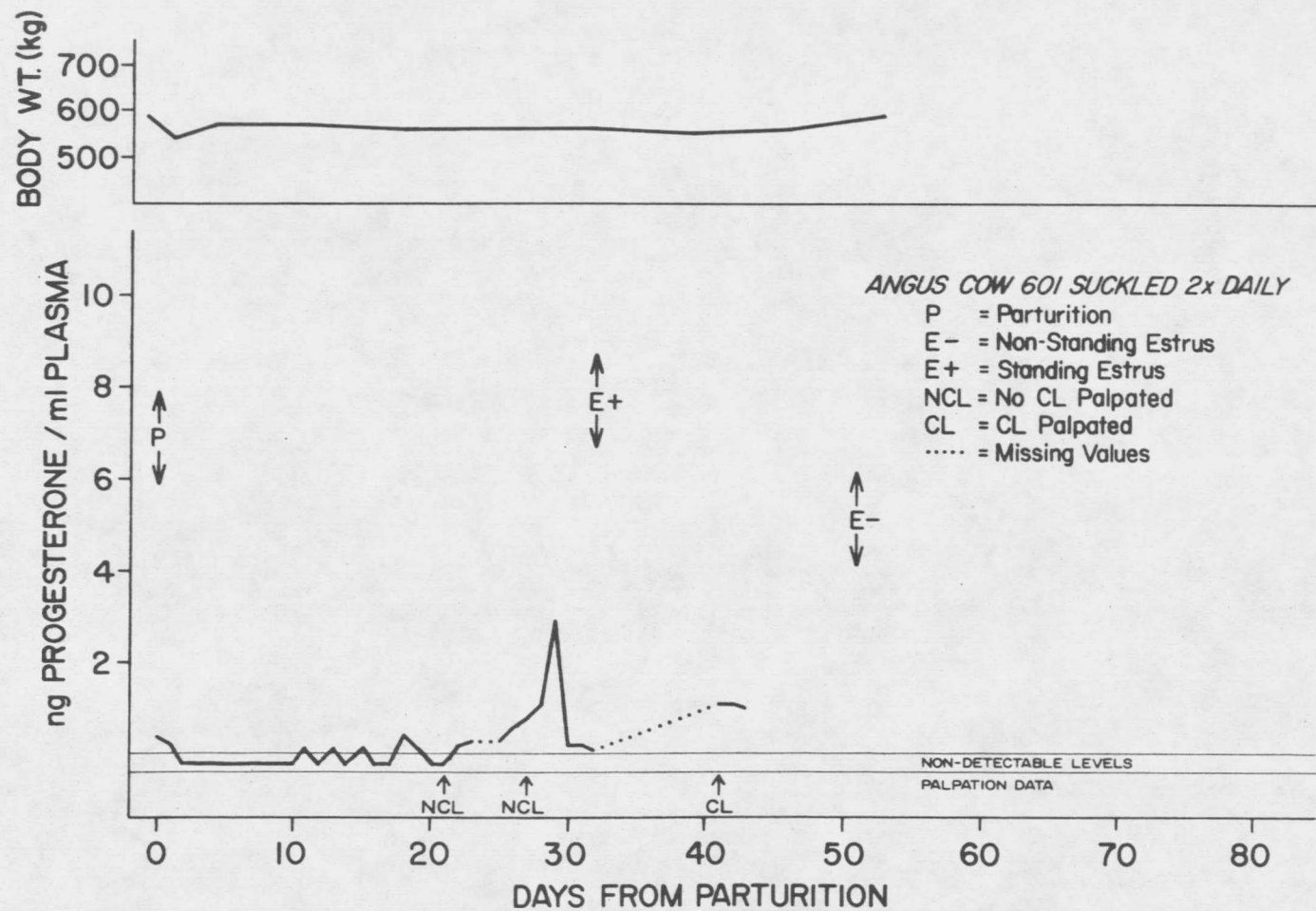
Appendix figure 9-5. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 609 (NON-LACTATING) during the postpartum period.



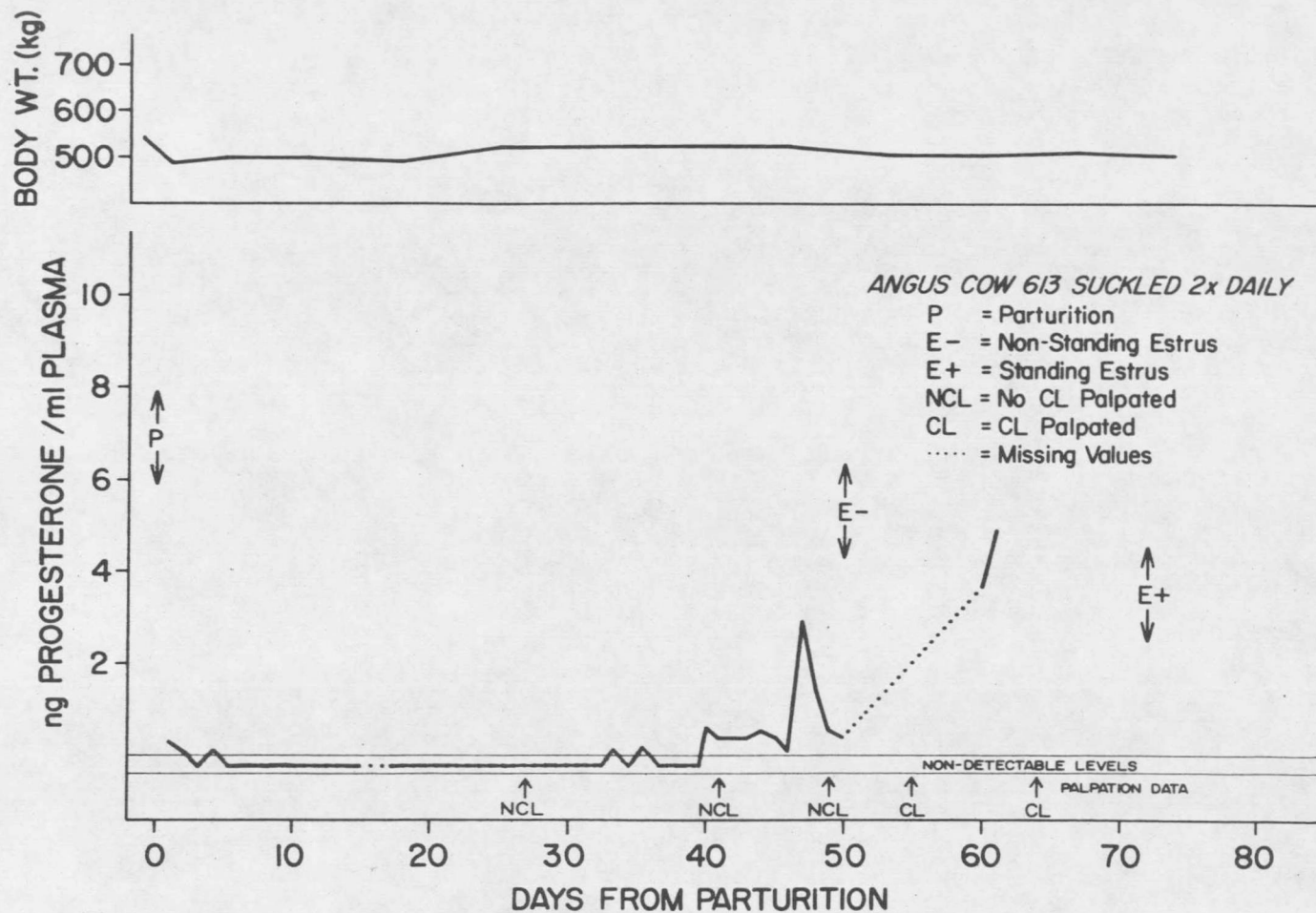
Appendix figure 9-6. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 615 (NON-LACTATING) during the postpartum period.



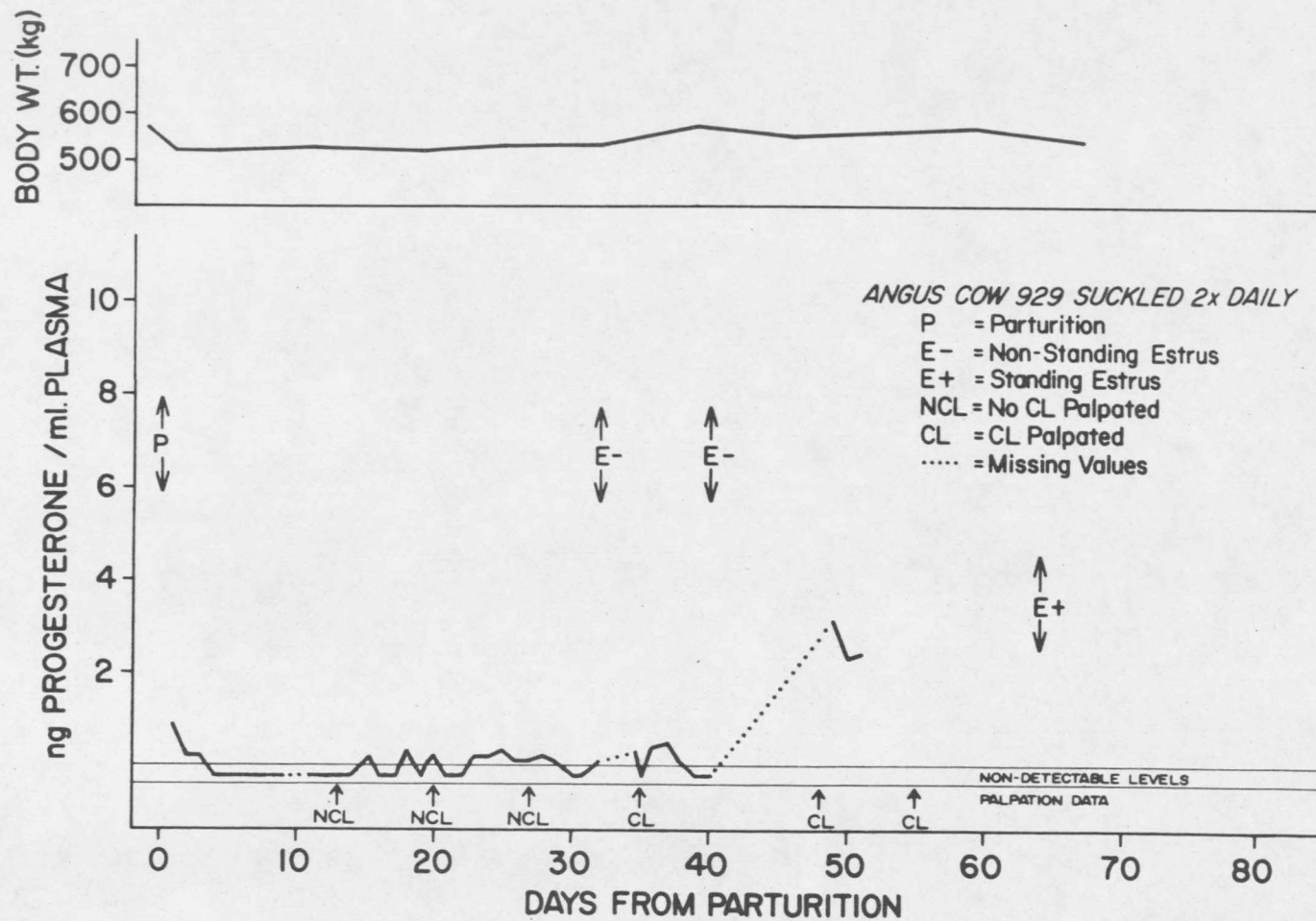
Appendix figure 9-7. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 314 (SUCKLED 2X) during the postpartum period.



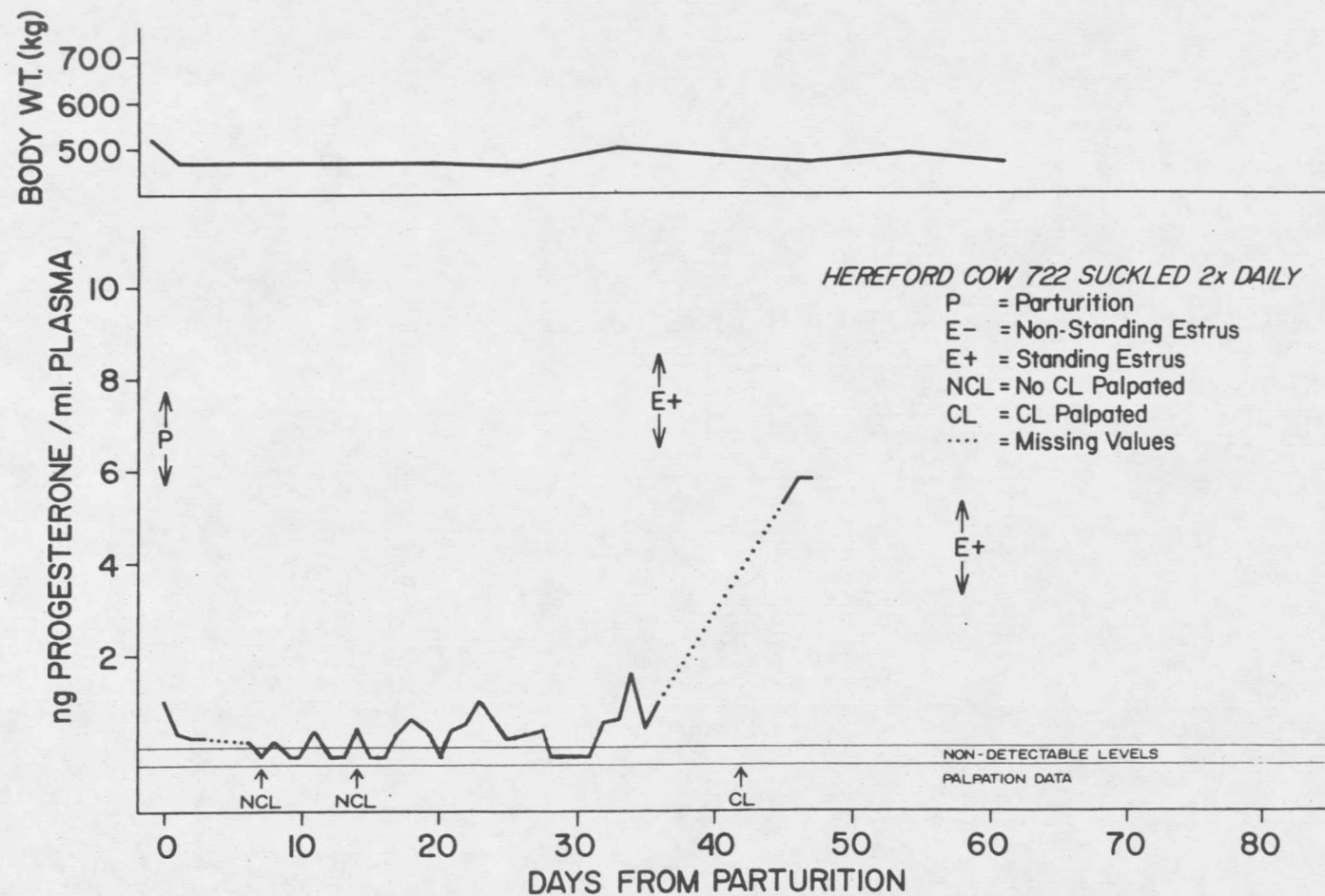
Appendix figure 9-8. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 601 (SUCKLED 2X) during the postpartum period.



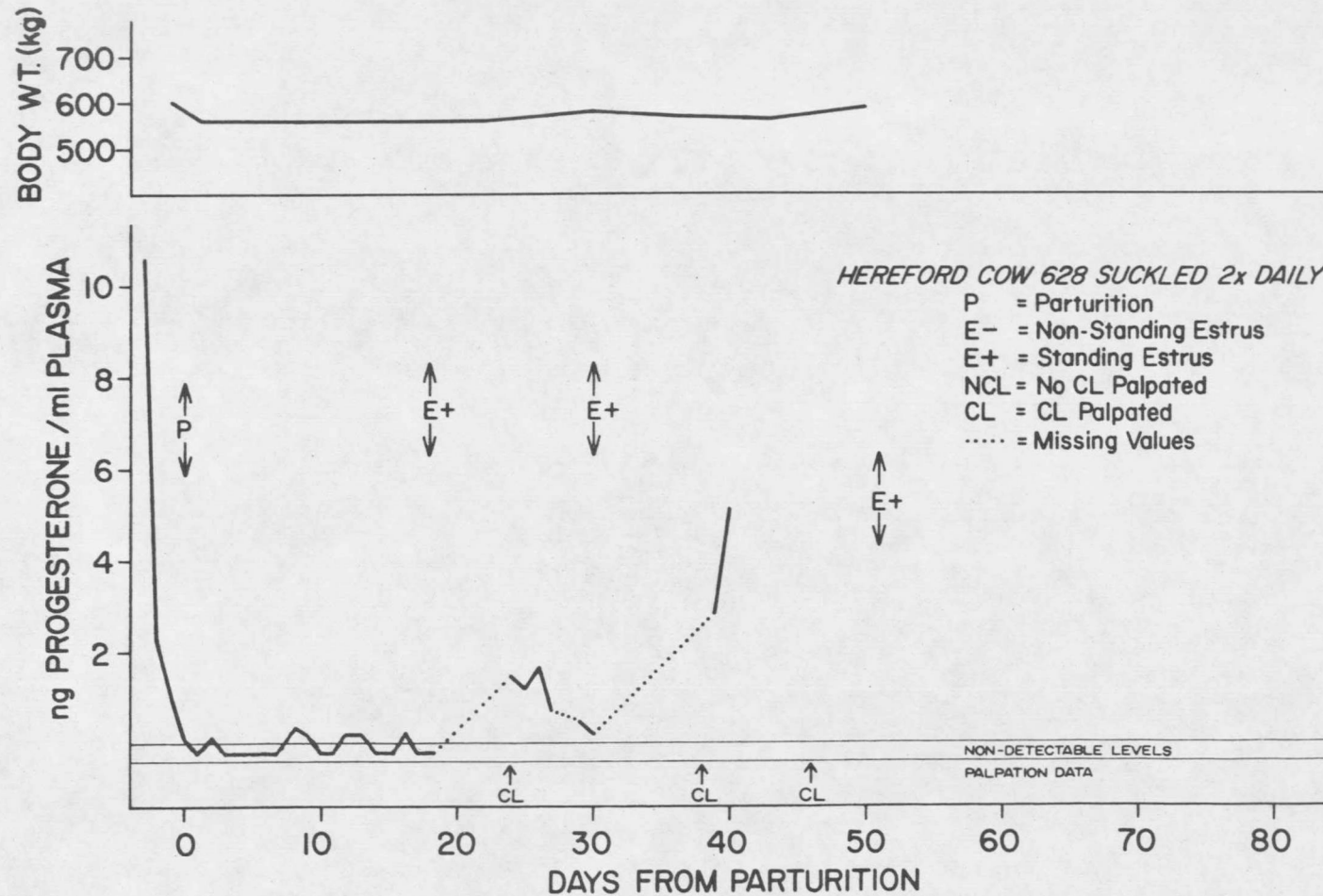
Appendix figure 9-9. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 613 (SUCKLED 2X) during the postpartum period.



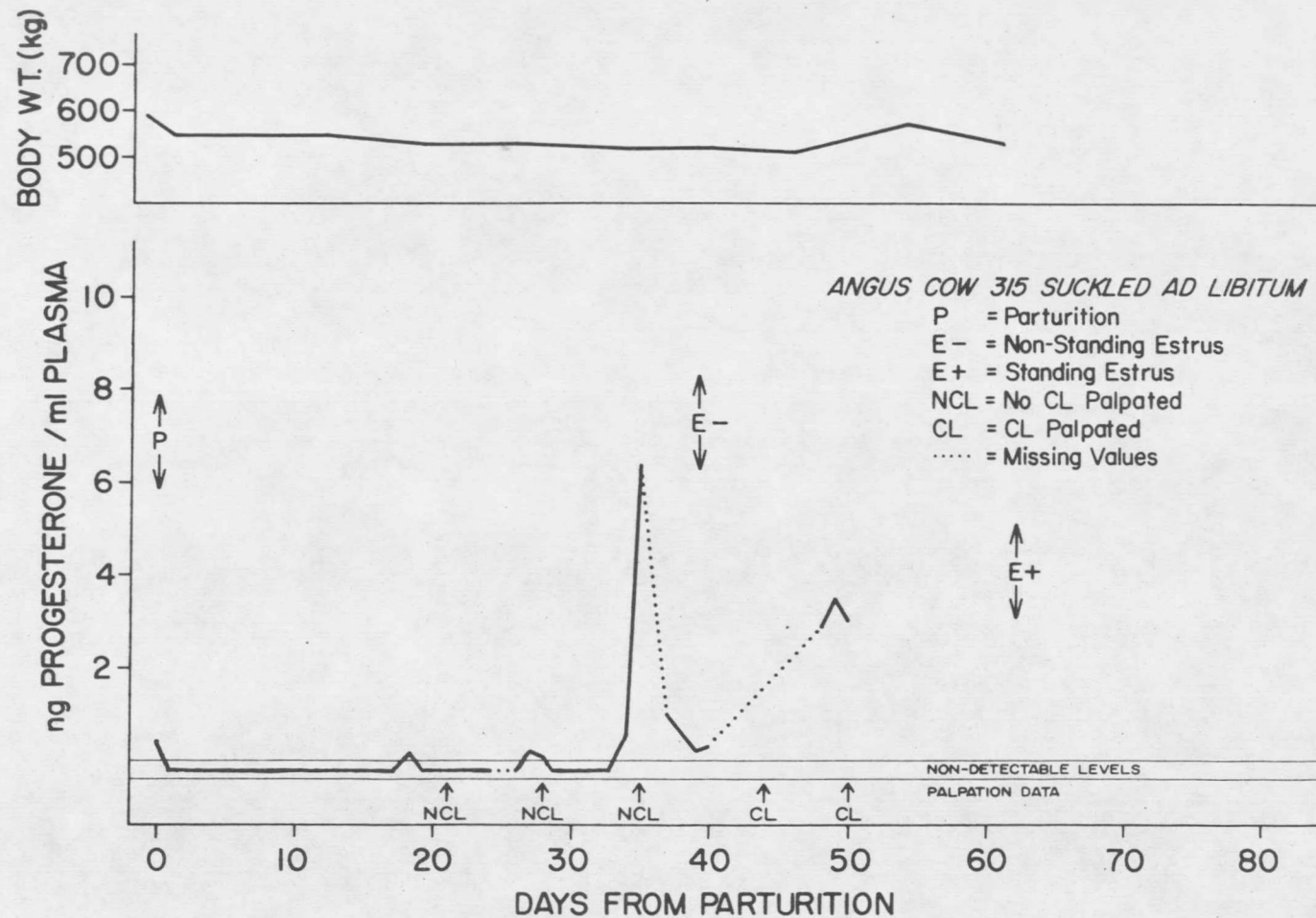
Appendix figure 9-10. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 929 (SUCKLED 2X) during the postpartum period.



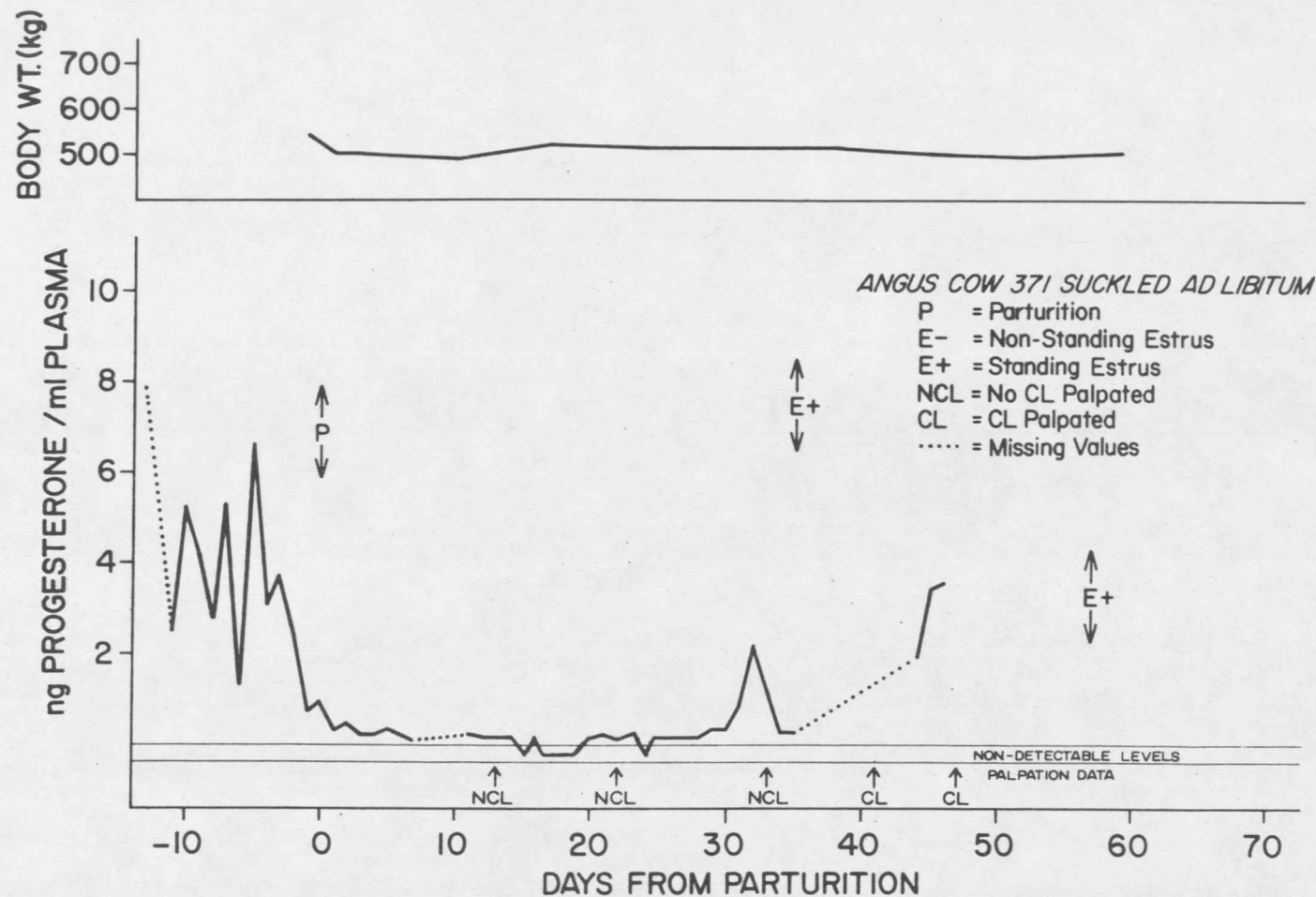
Appendix figure 9-11. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 722 (SUCKLED 2X) during the postpartum period.



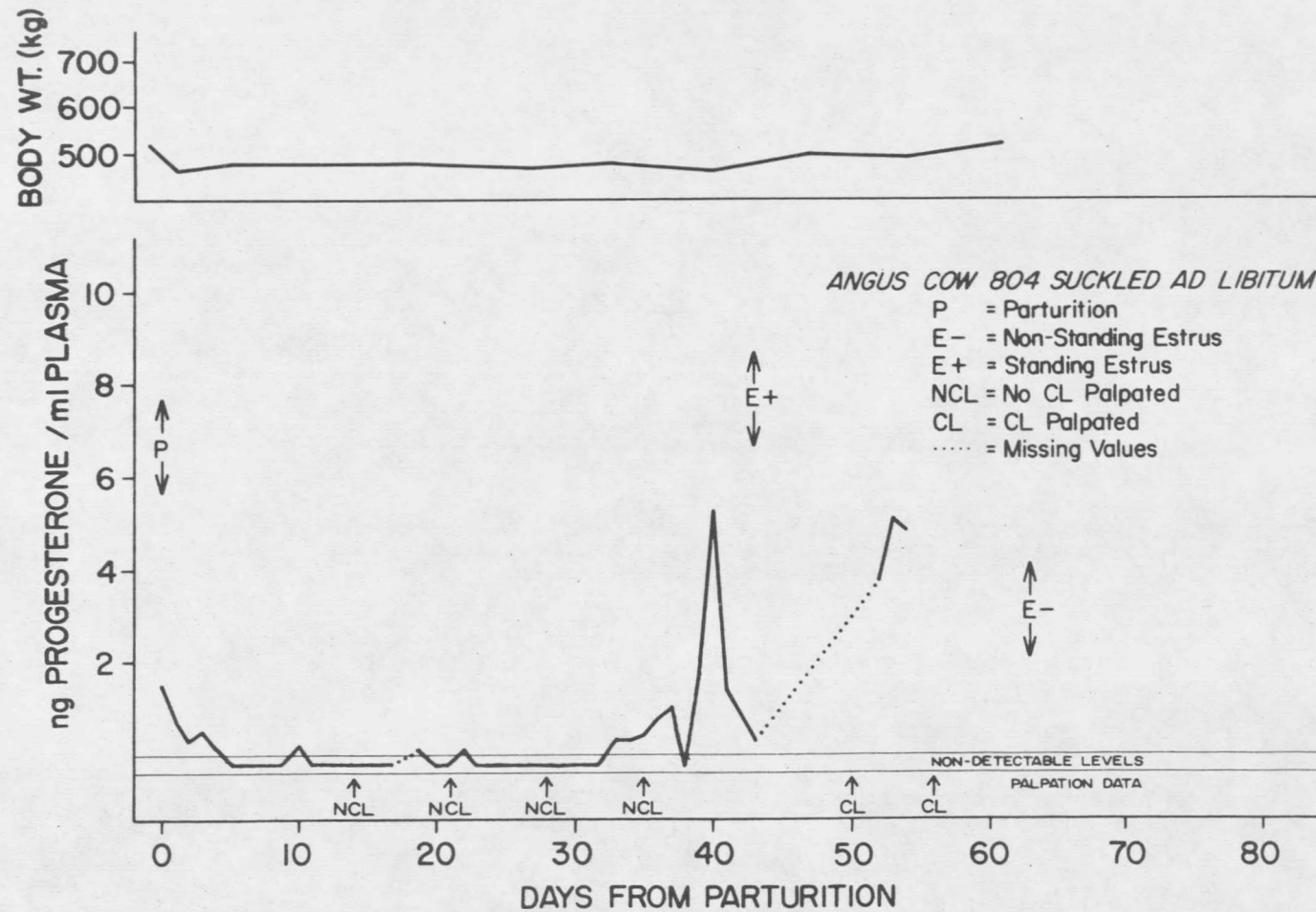
Appendix figure 9-12. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 628 (SUCKLED 2X) during the postpartum period.



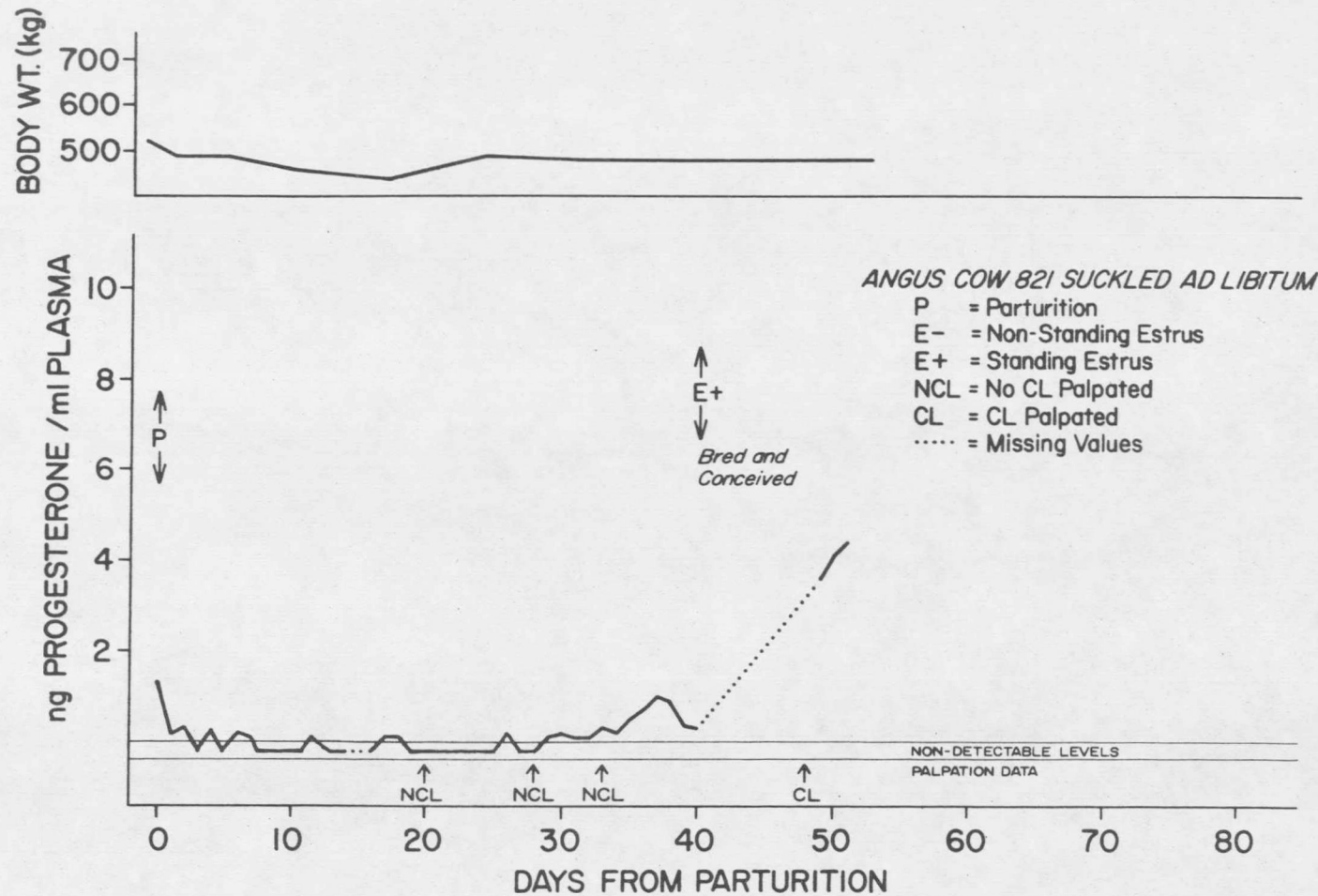
Appendix figure 9-13. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 315 (SUCKLED AD LIBITUM) during the postpartum period.



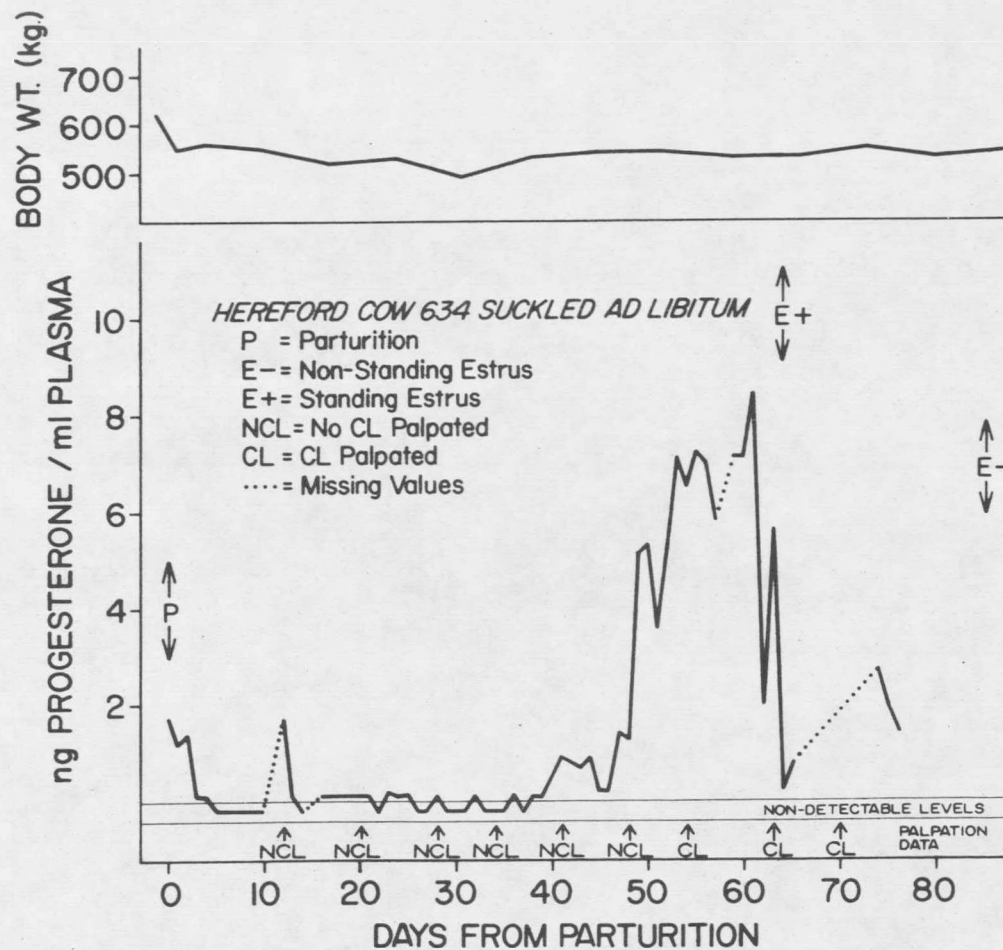
Appendix figure 9-14. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 371 (SUCKLED AD LIBITUM) during the postpartum period.



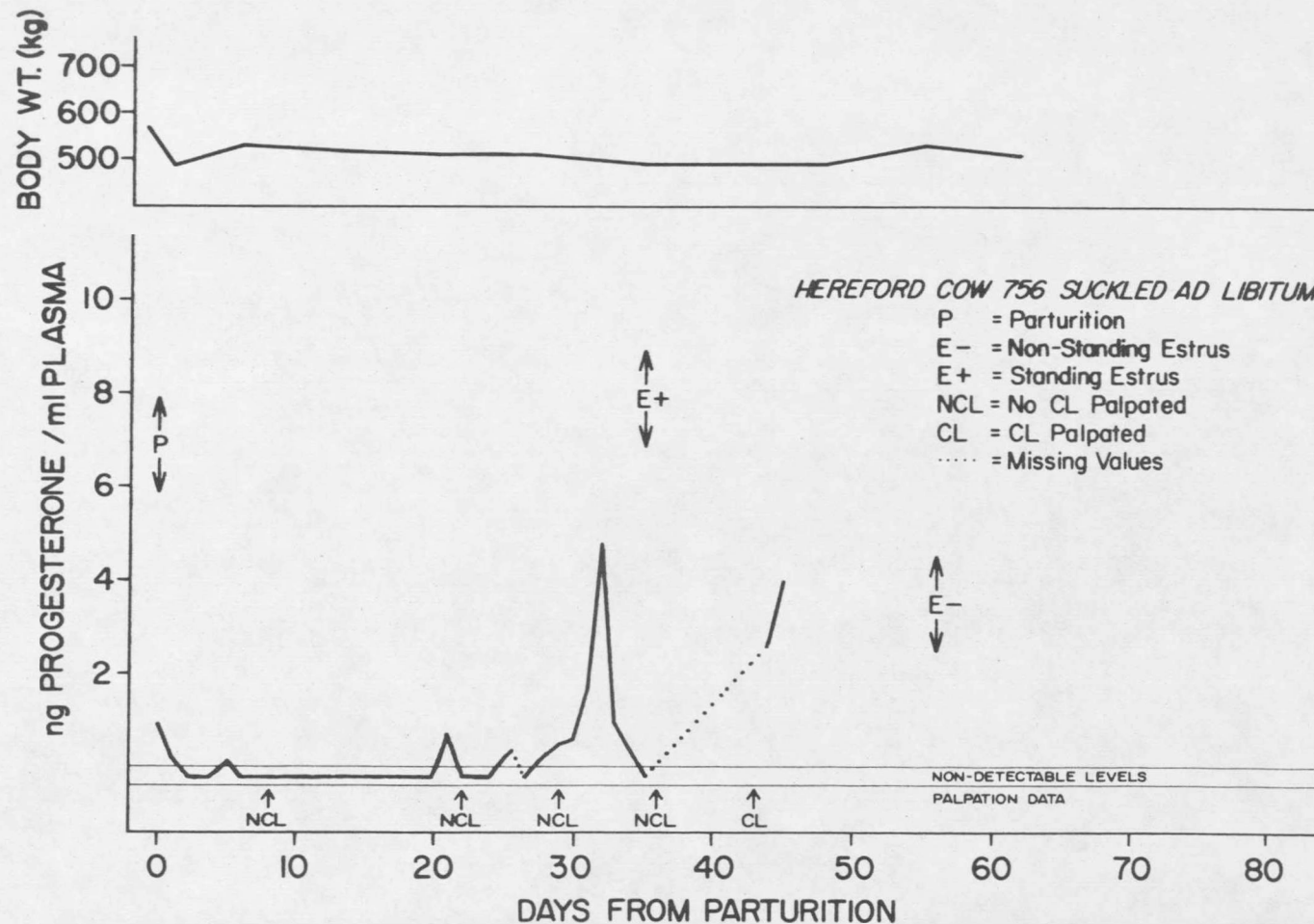
Appendix figure 9-15. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 804 (SUCKLED AD LIBITUM) during the postpartum period.



Appendix figure 9-16. Blood plasma progesterone levels, palpation and estrous information and body weights for Angus cow 821 (SUCKLED AD LIBITUM) during the postpartum period.



Appendix figure 9-17. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 634 (SUCKLED AD LIBITUM) during the postpartum period.



Appendix figure 9-18. Blood plasma progesterone levels, palpation and estrous information and body weights for Hereford cow 756 (SUCKLED AD LIBITUM) during the postpartum period.

CHAPTER 10

LITERATURE CITED

- Adams, W. M. 1968. The elective induction of labour and parturition in cattle. *J. Amer. Vet. Med. Ass.* 154:261.
- Adams, W. M. and W. C. Wagner. 1970. The role of corticoids in parturition. *Biol. Reprod.* 3:223.
- Akbar, A. M., L. E. Reichert, Jr., T. G. Dunn, C. C. Kaltenbach and G. D. Niswender. 1973. Bovine FSH in serum measured by radio-immunoassay. *J. Anim. Sci.* 37:299.
- Albrechtsen, J. 1917. Sterility of cattle and methods of treatment. *Cornell Vet.* 57.
- Arije, G. R., J. N. Wiltbank and M. L. Hopwood. 1974. Hormone levels in pre- and postparturient beef cows. *J. Anim. Sci.* 38:338.
- Arimura, Akira and Alan Findlay. 1974. Hypothalamic map for the regulation of gonadotrophin release. *Research in reproduction* 3:1 (Jan. 1974 revision).
- Asdell, S. A., J. DeAlba and S. J. Roberts. 1948. Studies on the estrous cycle of dairy cattle: Cycle length, size of corpus luteum and endometrial changes. *Cornell Vet.* 39:389.
- Bailey, L. F., M. W. McLennan, D. M. McLean, P. R. Hartford and G. L. Munro. 1973. The use of dexamethasone trimethylacetate to advance parturition in dairy cows. *Australian Vet. J.* 48:567.
- Balfour, W. E., R. S. Comline and R. V. Short. 1957. Secretion of progesterone by the adrenal gland. *Nature* 180:1480.
- Banerjee, A. K. 1963. A study of the action of terramycin on the bacterial flora of the uterus in cattle following retained placenta. Thesis, Utrecht.
- Bellows, R. A., R. E. Short, J. J. Urlick and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. *J. Anim. Sci.* 38:589.
- Benesch, F. and J. G. Wright. 1971. *Veterinary Obstetrics.* Williams and Wilkins, Baltimore.
- Beery, R. O., A. M. Sorensen and G. T. King. 1958. Physiological effects of testosterone propionate on the development of sexually immature heifers. *J. Anim. Sci.* 17:1235 (Abstr.).

- Bosc, M. J. 1971. Étude de l'induction de la parturition chez la vache par un corticostéroïde de synthèse. Am. Biol. Anim. Bioch. Biophys. 11:581.
- Boyd, W. L. 1925. A study of the physiological and pathological changes occurring in the reproductive organs of the cow following parturition. Minn. Agr. Exp. Sta. Bull. 23:1.
- Britt, Jack H., Roger J. Kittok and David S. Harrison. 1974. Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J. Anim. Sci. 39:915.
- Brown, J. G., D. W. Peterson and W. D. Foote. 1972. Reproductive response of beef cows to exogenous progestogen, estrogen and gonadotrophins at various stages postpartum. J. Anim. Sci. 35:362.
- Buch, N. C., W. J. Tyler and L. E. Casida. 1955. Postpartum estrus and involution of the uterus in an experimental herd of Holstein-Friesian cows. J. Dairy Sci. 38:73.
- Callahan, C. J., R. E. Erb, A. H. Surve and R. D. Randal. 1971. Variables influencing ovarian cycles in postpartum dairy cows. J. Anim. Sci. 33:1053.
- Cameron, N. W. and O. T. Fosgate. 1964. Effects of oxytocin upon the reproductive tract of the lactating bovine. J. Dairy Sci. 47:79.
- Carman, G. M. 1955. Interrelationships of milk production and breeding efficiency in dairy cows. J. Anim. Sci. 14:753.
- Carroll, E. J. 1974. Induction of parturition in farm animals. J. Anim. Sci. 38:1 (Suppl. I).
- Casida, L. E. 1971. Postpartum interval and its relation to fertility in the cow, sow and ewe. J. Anim. Sci. 32:66 (Suppl. I).
- Casida, L. E., and A. B. Chapman. 1951. Factors affecting the incidence of cystic ovaries in a herd of Holstein cows. J. Dairy Sci. 34:1200.
- Casida, L. E., E. R. Hauser, W. J. Tyler, W. E. Graves, J. W. Lauderdale, J. W. Riesen and Syed Saiduddin. 1968. Studies on the postpartum cow. Wis. Agr. Exp. Sta. Bull. 270:1.

- Casida, L. E., R. K. Meyer, W. H. McShan and W. Wisnicky. 1943. Effects of pituitary gonadotrophins on the ovaries and induction of superfecundity in cattle. *Amer. J. Vet. Res.* 4:76.
- Casida, L. E. and W. G. Venzke. 1936. Observations on reproductive processes in dairy cattle and their relation to breeding efficiency. *Proc. Amer. Soc. Ania. Prod.* 29:221.
- Casida, L. E. and W. Wisnicky. 1950. Effects of diethylstilbestrol dipropionate upon postpartum changes in the cow. *J. Anim. Sci.* 9:238.
- Chapman, A. B. and L. E. Casida. 1934. Factors associated with breeding efficiency in dairy cattle. *Proc. Amer. Soc. Anim. Prod.* 27:57.
- Chapman, A. B. and L. E. Casida. 1937. Analysis of variation in the sexual cycle and some of its component phases with special reference to cattle. *J. Agr. Res.* 54:417.
- Christensen, D. S., J. N. Wiltbank and M. L. Hopwood. 1971. Blood hormone levels during the bovine estrus cycle. *J. Anim. Sci.* 33:251.
- Clapp, H. 1937. A factor in the breeding efficiency of cattle. *Proc. Amer. Soc. Anim. Prod.* 30:259.
- Cole, H. H. and R. F. Miller. 1933. Artificial induction of ovulation and oestrus in the ewe during anoestrus. *Amer. J. Physiol.* 104:165.
- Connor, J. S., R. L. Tribble, T. L. Woodward, J. L. Fleeger, J. R. Beverly and A. M. Sorensen, Jr. 1974. Postpartum ovarian activity in first calf Hereford heifers. *Tex. Agr. Exp. Sta. Progrs. Report* 3216:24.
- Corah, L. R. Alice P. Quealy, T. G. Dunn and C. C. Kaltenbach. 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. *J. Anim. Sci.* 39:380.
- Cupps, P. T. 1969. Steroid biosynthesis by the bovine ovary. *J. Anim. Sci.* 29:187 (Abstr.).
- Cross, B. A. 1973. Unit responses in the hypothalamus. In L. Martini and W. F. Ganong (Eds.) *Frontiers in Neuroendocrinology*. Oxford University Press, New York.

- Davis, S. L., L. E. Reichert, Jr. and G. D. Niswender. 1971. Serum levels of prolactin in sheep as measured by radioimmunoassay. *Biol. Reprod.* 4:145.
- Desjardins, C. and H. D. Hafs. 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. *J. Anim. Sci.* 27:472.
- Donaldson, L. E., J. M. Bassett and G. D. Thornburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrus cycles, pregnancy and lactation, and the effects of undernutrition and exogenous oxytocin on progesterone concentration. *J. Endocrinol.* 48:599.
- Donaldson, L. E. and W. Hansel. 1968. Cystic corpora lutea and normal and cystic Graafian follicles in the cow. *Australian Vet. J.* 44:304.
- 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.
- Echternkamp, S. E. and William Hansel. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. *J. Anim. Sci.* 37:1362.
- Edwards, J. 1950. Fertility in cattle: effect of postpartum interval. *Vet. Rec.* 62:310.
- Elliott, Lloyd, K. J. McMahon, H. T. Gier and G. B. Marion. 1968. Uterus of the cow after parturition: Bacterial Content. *Amer. J. Vet. Res.* 29:77.
- Erb, R. E., R. D. Randel and C. J. Callahan. 1971a. Female sex steroid changes during the reproductive cycle. *J. Anim. Sci.* 32:80 (Suppl. I).
- Erb, R. E., A. H. Surve, C. J. Callahan, R. D. Randel and H. A. Garverick. 1971b. Reproductive steroids in the bovine. VII. Changes postpartum. *J. Anim. Sci.* 33:1060.
- Flerko, B. 1970. Control of follicle-stimulating hormone and luteinizing hormone secretion. In L. Martini, M. Motta and F. Fraschini (Eds.) *The Hypothalamus*. Academic Press, New York.

- Foote, W. Darrell. 1971. Endocrine changes in the bovine during the postpartum period. J. Anim. Sci. 32:73 (Suppl. I).
- Foote, W. D., E. R. Hauser and L. E. Casida. 1960a. Some causes of variation in postpartum reproductive activity in Hereford cows. J. Anim. Sci. 19:238.
- Foote, W. D., E. R. Hauser and L. E. Casida. 1960b. Influence of progesterone treatment on postpartum reproductive activity in beef cattle. J. Anim. Sci. 19:674.
- 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. and S. Saiduddin. 1964. Hormone treatment of postpartum beef cows. J. Anim. Sci. 23:592 (Abstr.).
- Foote, W. D., D. C. Sawhney, M. M. Quevedo and D. W. Peterson. 1966. Chorionic gonadotrophin treatment of beef cows. J. Anim. Sci. 25:585 (Abstr.).
- Gay, Vernon L. 1972. The hypothalamus: Physiology and clinical use of releasing factors. Fertil. and Steril. 23:50.
- Geschwind, I. I. 1972. Dynamics of pituitary gonadotrophin secretion. J. Anim. Sci. 34:19 (Suppl. I).
- Gier, H. T. and G. B. Marion. 1968. Uterus of the cow after parturition: Involutional changes. Amer. J. Vet. Res. 29:83.
- Gier, H. T., N. P. Singh and G. B. Marion. 1962. Histopathology of the postpartum bovine uterus. J. Anim. Sci. 21:1023.
- Gomes, W. R. and E. R. Erb. 1965. Progesterone in bovine reproduction: A review. J. Dairy Sci. 48:314.
- Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham and J. N. Wiltbank. 1975a. Puberty in beef heifers. III. Induction of fertile estrus. J. Anim. Sci. (in preparation).
- Gonzalez-Padilla, E., J. N. Wiltbank and G. D. Niswender. 1975b. Puberty in beef heifers: I. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. (in preparation).

- Gorski, Marianne, E. and Irene E. Lawton. 1973. Adrenal involvement in determining the time of onset of puberty in the rat. *Endocrinol.* 93:1232.
- Graves, W. E., J. W. Lauderdale, E. R. Hauser and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotrophins, ovarian follicular development and fertility in beef cows (Effect of suckling and interval to breeding). *Wis. Agr. Exp. Sta. Bull.* 270:23.
- Grosvenor, Clark E. and Flavio Mena. 1971. Effect of suckling upon the secretion and release of prolactin from the pituitary of the lactating rat. *J. Anim. Sci.* 32:115 (Suppl. I).
- Halasz, B. 1969. In W. F. Ganong and L. Martini (Eds.) *Frontiers in Neuroendocrinology 1969*. Oxford University Press, New York.
- Halasz, B. and L. Pupp. 1965. Hormone secretions of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. *Endocrinol.* 77:553.
- Hammond, J. and H. G. Sanders. 1923. Some factors affecting milk yield. *J. Agr. Sci.* 13:74.
- Han, D. K. 1973. LH and prolactin levels in postpartum beef cows and prolactin levels in cyclic beef cows subjected to various mating stimuli. M. S. Thesis. Montana State University, Bozeman.
- Han, D. K. and E. L. Moody. 1974. LH, prolactin and progesterone in postpartum beef cows. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 25:234.
- Harvey, W. R. 1960. Least squares analysis of data with unequal subclass numbers. *ARS* 20:8.
- Hendricks, D. M., J. F. Dickey, J. R. Hill and W. E. Johnson. 1972. Plasma estrogen and progesterone levels after mating and during late pregnancy and postpartum in cows. *Endocrinol.* 90:1336.
- Herman, H. A. and J. H. Edmondson. 1950. Factors affecting interval between parturition and first estrus in dairy cattle. *Mo. Agr. Exp. Sta. Bull.* 462:1.

- Higaki, S., H. Ika, Y. Awai, T. Kirisawa and N. Yoshida. 1959. Postparturient uterine involution in dairy cows. I. Relationship in uterine involution to the breeding efficiency in normal dairy cows. *Bul. Natl. Instit. Agr. Sci. (Japan)*, series G. No. 18:65.
- Hill, D. D., J. N. Wiltbank, D. G. LeFever. 1972. Induction of postpartum estrus in beef cows. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 23:543.
- Hofstad, M. S. 1941. A study of breeding records of one large herd of dairy cattle. *Cornell Vet.* 31:379.
- Hubenov, M., N. Naidenov and G. Atesv. 1964. Stimulation of postpartum involution of the cow's genital organs. I. Influence of ergotamine on postpartum involution and sexual activity. *Vet. Med. Nauk.* 1:79.
- Ingalls, W., H. D. Hafs and W. D. Oxender. 1971. Growth hormone in heifers before and after parturition. *J. Dairy Sci.* 54:768.
- Inglebrecht, P. 1935. Influence du systeme nerveux central sur la mamelle lactante chez le rat blanc. *C. R. Seanc. Soc. Biol.* 120:1369.
- Jöchle, W. 1971. Corticoid induced parturition in domestic animals: Mechanism of action and economic importance. *Folia Vet. Latina. Roma* 1:229.
- Jöchle, W., H. Esparza, T. Gimenez and M. A. Hidalgo. 1972. Inhibition of corticoid-induced parturition by progesterone in cattle: Effect on delivery and calf viability. *J. Reprod. Fert.* 28:407.
- Johnson, E. F., Frank Hudson, Ralph Bogart, A. W. Oliver and F. F. McKenzie. 1956. The effects of injected testosterone on adult ewes. *J. Anim. Sci.* 15:177.
- Jubb, K. V. F. and P. C. Kennedy. 1963. *Pathology of Domestic Animals*. Academic Press, New York.
- Kato, J. and C. A. Villee. 1967. Preferential uptake of estradiol by the anterior hypothalamus of the rat. *Endocrinol.* 80:567.
- Kidder, H. E., G. R. Barrett and L. E. Casida. 1952. A study of ovulations in six families of Holstein-Friesians. *J. Dairy Sci.* 35:436.

- Labhsetwar, A. P., W. E. Collins, W. J. Tyler and L. E. Casida. 1964. Some pituitary-ovarian relationships in the periparturient cows. J. Reprod. Fertil. 8:85.
- Labhsetwar, A. P., W. J. Tyler and L. E. Casida. 1963. Genetic and environmental factors affecting quiet ovulations in Holstein cattle. J. Dairy Sci. 46:843.
- Lammond, D. R. 1964. Synchronization of ovarian cycles in sheep and cattle. Anim. Breed. Abstr. 32:269.
- Laster, D. B., H. A. Glimp, L. V. Cundiff and K. E. Gregory. 1973a. Factors affecting dystocia and the effects of dystocia on subsequent reproduction in beef cattle. J. Anim. Sci. 36:695.
- Laster, D. B., H. A. Glimp and K. E. Gregory. 1973b. Effects of early weaning on postpartum reproduction of cows. J. Anim. Sci. 36:734.
- Lauderdale, James W. 1972. Effect of corticoid administration on bovine pregnancy. J. Amer. Vet. Med. Ass. 160:867.
- Lauderdale, J. W., W. E. Graves, E. R. Hauser and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in beef cows. Wis. Agr. Exp. Sta. Bull. 270:42.
- Lauderdale, J. W., R. L. Kirkpatrick, N. L. First, E. R. Hauser and L. E. Casida. 1965. Ovarian and pituitary gland changes in periparturient sows. J. Anim. Sci. 24:1100.
- LaVoie, V. A., C. H. Clark, D. K. Han and E. L. Moody. 1974. Reproductive performance of mastectomized cows. Proc. West. Sec. Amer. Soc. Anim. Sci. Vol. 25.
- LaVoie, V. A., M. W. Hull and E. L. Moody. 1971. Progesterone ratios in heifers at estrus. J. Anim. Sci. 33:258 (Abstr.).
- LaVoie, V. A. and E. L. Moody. 1973a. Estrogen pre-treatment of corticoid induced parturition in cattle. J. Anim. Sci. 37:770.
- LaVoie, V. A. and E. L. Moody. 1973b. Cow and calf performance after dexamethasone induced calving. Proc. West. Sec. Amer. Soc. Anim. Sci. 24:285.

- Lobel, B. L. and E. Levy. 1968. Enzymic correlates of development, secretory function and regression of follicles and corpora lutea in the bovine ovary. *Acta Endocrinol.* 132:7 (Suppl.).
- Lovis, T. M., H. D. Hafs and B. E. Seguin. 1973. Progesterone, LH, estrus and ovulation after prostaglandin $F_{2\alpha}$ in heifers. *Proc. Soc. Exp. Biol. Med.* 143:152.
- Marion, G. B., J. S. Norwood and H. T. Gier. 1968. Uterus of the cow after parturition: Factors affecting regression. *Amer. J. Vet. Res.* 29:71.
- McClure, T. J. 1968. Hypoglycemia, and apparent cause of infertility of lactating cows. *Brit. Vet. J.* 124:3.
- Menge, A. C., S. E. Mares, W. J. Tyler and L. E. Casida. 1962. Variation and association among postpartum reproductive and productive and production characteristics in Holstein-Friesian cattle. *J. Dairy Sci.* 45:233.
- Moller, Knud. 1970. A review of uterine involution and ovarian activity during the postparturient period in the cow. *New Zealand Vet. Jour.* 18:83.
- Morrow, D. A. 1971. Effects of periparturient disease on postpartum reproduction in dairy cattle. *J. Anim. Sci.* 32:17 (Suppl. I).
- Morrow, D. A., S. J. Roberts and K. McEntee. 1969. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. *Cornell Vet.* 59:134.
- Morrow, D. A., S. J. Roberts, K. McEntee and H. G. Grey. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. *J. Am. Vet. Med. Ass.* 149:1596.
- Motta, M., F. Piva and L. Martini. 1970. The hypothalamus as the center of endocrine feedback mechanisms. In L. Martini, M. Motta and F. Fraschini (Eds.) *The Hypothalamus*. Academic Press, New York.
- Naess, Oddvar and Arne Attramadal. 1974. Uptake and binding of androgens by the anterior pituitary gland, hypothalamus, preoptic area and brain cortex of rats. *Acta Endocrin.* 76:417.
- Nalbandov, A. and L. E. Casida. 1940. Gonadotropic action of pituitaries from pregnant cows. *Endocrinol.* 27:559.

- Niswender, Gordon D. 1973. Influence of the site of conjugation on the specificity of antibodies to progesterone. *Steroids* 22:413.
- Niswinder, G. D. 1974. Influence of 2-Br- α -ergocryptine on serum levels of prolactin and the estrous cycle in sheep. *Endocrinol.* 94:612.
- Olds D. and D. Cooper. 1970. Effect of postpartum rest periods in dairy cattle on the occurrence of breeding abnormalities and on calving intervals. *J. Amer. Vet. Med. Ass.* 157:92.
- Olds, D., H. B. Morrison and D. M. Seath. 1949. Efficiency of natural breeding in dairy cattle. *Ky. Agr. Exp. Sta. Bull.* 539:1.
- Olds, D. and D. M. Seath. 1953. Repeatability, heritability, and effect of level of milk production on the occurrence of first estrus after calving in dairy cattle. *J. Anim. Sci.* 12:10.
- Oxenreider, S. L. 1968. Effects of suckling and ovarian function on postpartum reproductive activity in beef cows. *Amer. J. Vet. Res.* 29:2099.
- Oxenreider, S. L. and W. C. Wagner. 1971. Effect of lactation and energy intake on postpartum ovarian activity in the cow. *J. Anim. Sci.* 33:1026.
- Perkins, J. L. and H. E. Kidder. 1963. Relation of uterine involution and postpartum interval to reproductive efficiency in beef cattle. *J. Anim. Sci.* 22:313.
- Peters, J. B., N. L. First and L. E. Casida. 1969. Effect of pig removal and oxytocin injections on ovarian and pituitary changes in mammillectomized postpartum sows. *J. Anim. Sci.* 28:537
- Poncelet, G. R., J. L. Winter, V. A. LaVoie and E. L. Moody. 1974. Subsequent fertility after induced parturition. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 25:279.
- Pope, G. S., S. K. Gupta and I. B. Munro. 1969. Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. *J. Reprod. Fertil.* 20:369.
- Rasbech, N. O. 1950. The normal involution of the uterus in the cow. *Nord. Vet. Med.* 2:655.

- Rice, B. F. and K. Savard. 1966. Steroid hormone formation in the human ovary: IV Ovarian stromal compartments; formation of radioactive steroid from acetate-1 ^{14}C and action of gonadotrophins. J. Clin. Endocrinol. Metab. 26:593.
- Riesen, J. W., Syed 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. Wis. Agr. Exp. Sta. Bull. 270:27.
- Roberts, S. J. 1971. Veterinary Obstetrics and Genital Diseases (Theriogenology). Published by author. Ithaca, New York.
- Rondell, Paul. 1970. Biophysical aspects of ovulation. Biol. Reprod. Suppl. 2:64.
- Saiduddin, Syed and W. D. Foote. 1964. Pituitary lutenizing hormone activity of postpartum bovine. J. Anim. Sci. 23:592 (Abstr.).
- Saiduddin, S., M. M. Quevedo and W. D. Foote. 1968a. Response of beef cows to exogenous progesterone and estradiol at various stages postpartum. J. Anim. Sci. 27:1015.
- Saiduddin, S., J. W. Riesen, W. E. Graves, W. J. Tyler and L. E. Casida. 1966. Pituitary luteinizing hormone activity in the postpartum cow. J. Anim. Sci. 25:930.
- Saiduddin, S., J. W. Riesen, W. E. Graves, W. J. Tyler and L. E. Casida. 1967a. Effect of suckling on the interval from parturition to first estrus in dairy cows. J. Anim. Sci. 26:950.
- Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1967b. Some carry over effects of pregnancy on postpartum ovarian function in the cow. J. Dairy Sci. 50:1846.
- Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1968b. Relation of postpartum interval to pituitary gonadotrophins, ovarian follicular development and fertility in dairy cows. Wis. Agr. Exp. Sta. Bull. 270:27.
- Savard, K. and G. Telegdy. 1965. Steroid formation in the bovine corpus luteum. Steroids, Suppl. 11:205.

- 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. 1971. Gonadotrophin-releasing hormone: One polypeptide regulates secretion of luteinizing and follicle stimulating hormone. *Science* 173:1036.
- Schams, D., B. Hoffman, S. Fischer, E. Mary and H. Karg. 1972. Simultaneous determination of LH and progesterone in peripheral bovine blood during pregnancy, normal and corticoid induced parturition and the postpartum period. *J. Reprod. Fert.* 29:37.
- Schmidt, G. H. and L. D. Van Vleck. 1974. Principals of Dairy Science. W. H. Freeman and Co., San Francisco.
- Shannon, F. P., G. W. Salisbury and N. L. VanDemark. 1952. The fertility of cows inseminated at various intervals after calving. *J. Anim. Sci.* 11:355.
- Shemesh, Mordechai and William Hansel. 1974. Measurement of bovine plasma testosterone by radioimmunoassay (RIA) and by a rapid competitive protein binding (CPB) assay, *J. Anim. Sci.* 39:720.
- 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.
- Short, R. E., P. J. Burfening, R. A. Bellows and B. E. Howland. 1973. Effects of suckling in fall lambing ewes. *J. Anim. Sci.* 37:329 (Abstr.).
- Short, R. E. 1962. Steroid concentration in normal follicular fluid and ovarian cyst fluid from cows. *J. Reprod. Fertil.* 4:27.
- Smith, L. E., Jr. and C. K. Vincent. 1972. Effects of early weaning and exogenous hormone treatment on bovine postpartum reproduction. *J. Anim. Sci.* 35:1228.
- Snedecor, G. W. and W. G. Cockran. 1967. Statistical Methods (6th Ed.). Iowa State University Press, Ames.
- Stabenfeldt, G. H. 1969. Progesterone studies in domestic animals. *J. Amer. Vet. Med. Ass.* 154:11 (Abstr.).
- Swanson, L. V. and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. *J. Anim. Sci.* 33:1038.

- Tennant, B., J. W. Kendrick and R. G. Peddicord. 1967. Uterine involution and ovarian function in the postpartum cow. A retrospective analysis of 2,338 genital organ examinations. Cornell Vet. 57:543.
- Tennant, Bud and R. G. Peddicord. 1968. The influence of delayed uterine involution and endometritis on bovine fertility. Cornell Vet. 58:185.
- Tribble, R. L., T. L. Woodward, J. S. Connor, A. M. Sorensen, Jr., J. R. Beverly and J. L. Fleeger. 1974. Serum hormone levels in suckled and non-suckled first-calf Hereford heifers. Tex. Agr. Exp. Sta. Progs. Report 3215:21.
- Trimberger, G. W. 1954. Conception rates in dairy cattle from services at various intervals after parturition. J. Dairy Sci. 37:1042.
- Trimberger, G. W. and M. G. Fincher. 1956. Regularity of estrus, ovarian function, and conception rates in dairy cattle. Cornell Univ. Agr. Exp. Sta. Bull. No. 911.
- Turner, D. C. and J. T. Bagnara. 1971. General Endocrinology. (5th Ed.). W. B. Saunders Company, Philadelphia.
- Ulberg, L. C. and C. E. Lindley. 1960. Use of progesterone and estrogen in the control of reproductive activities in beef cattle. J. Anim. Sci. 19:1132.
- Ulberg, L. C., Lucy Brennen and P. L. Craven. 1974. Programs for improving reproduction in dairy herds through management. J. Anim. Sci. 38:31 (Supple. I).
- VanDemark, N. L. and G. W. Salisbury. 1950. The relation of the postpartum breeding interval to reproductive efficiency in the dairy cow. J. Anim. Sci. 9:307.
- Warnick, A. C. 1955. Factors associated with the interval from parturition to first estrus in beef cattle. J. Anim. Sci. 14:1003.
- Wagner, William Charles. 1968. Reproductive physiology of the postpartum cow. Ph.D. Thesis. Cornell University, Ithaca, New York.
- Wagner, W. C. and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological findings. J. Reprod. Fertil. 18:493.

- Wagner, W. C. and S. L. Oxenreider. 1971. Endocrine physiology following parturition. J. Anim. Sci. 32:1 (Supple. I).
- Wagner, W. C., Raymond Saatman and William Hansel. 1969. Reproductive physiology of the postpartum cow. II. Pituitary, adrenal and thyroid function. J. Reprod. Fert. 18:501.
- Wagner, W. C., R. L. Willham and L. E. Evans. 1974. Controlled parturition in cattle. J. Anim. Sci. 38:485.
- Welch, R. A. S., P. Newling and D. Anderson. 1973. Induction of parturition in cattle with corticosteroids: An analysis of field trials. New Zealand Vet. J. 21:103.
- Wetherill, G. D. 1965. Retained placenta in the bovine. A brief review. Canadian Vet. J. 6:290.
- Whitmore, H. L., W. J. Tyler and L. E. Casida. 1974. Effects of early postpartum breeding in dairy cattle. J. Anim. Sci. 38:339.
- Williams, W. L. 1943. The Diseases of the Genital Organs of Domestic Animals. Published by W. L. Williams, Ithaca, New York.
- Wiltbank, J. N. 1972. Management of heifer replacements and the brood cow herd through the calving and breeding period. In C. C. O'Mary and Irwin A. Dyer (Ed.). Commercial Beef Cattle Production. Lea and Febiger, Philadelphia.
- Wiltbank, J. N. and A. C. Cook. 1958. The comparative reproductive performance of nursed and milked cows. J. Anim. Sci. 17:640.
- 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 levels on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23:1049.
- Wiltbank, J. N. 1970. Research needs in beef cattle reproduction. J. Anim. Sci. 29:755.

Winter, J. L., G. R. Poncelet, V. A. LaVoie and E. L. Moody. 1974.
Induced calving in beef production. Proc. West. Sec. Amer. Soc.
Anim. Sci. 25:289.

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

MONTANA STATE UNIVERSITY LIBRARIES

3 1762 10014470 6

L395

cop.2

LaVoie, Verne A

The bovine postpartum interval

[illegible]

N378
L395
Cap 2