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EFFECT OF REDUCED ENERGY INTAKE ON PITUITARY RESPONSE TO GONADOTROPIN RELEASING HORMONE

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EFFECT OF REDUCED ENERGY INTAKE ON
PITUITARY RESPONSE TO
GONADOTROPIN RELEASING HORMONE

by
Joseph Augustine Shangosa Chipepa

A Dissertation Submitted to the Faculty of the
COMMITTEE OF ANIMAL PHYSIOLOGY (GRADUATE)
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1981
As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Joseph Augustine Shangosa Chipepa
entitled Effect of Reduced Energy Intake on Pituitary
Responsiveness to Gonadotropin-Releasing Hormone

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for the Degree of Doctor of Philosophy

Final approval and acceptance of this dissertation is contingent upon the
candidate's submission of the final copy of the dissertation to the Graduate
College.

I hereby certify that I have read this dissertation prepared under my
direction and recommend that it be accepted as fulfilling the dissertation
requirement.

Donald E. Ray
Dissertation Director

Date

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SIGNED: [Signature]
This is dedicated to my mom, Kayambila Chipepa,
my dad Noah Fears Chipepa, my wife
Christine and my three daughters,
Kayambila, Musenge and Maimbolwa
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ABSTRACT

An experiment was conducted with Brangus cows to evaluate the effect of loss of body weight and condition on pituitary responsiveness to gonadotropin releasing hormone (GnRH) stimulation during late lactation.

The treatment groups were lactating intact (LI), lactating ovariectomized (LO), nonlactating intact (NLI), and nonlactating ovariectomized (NLO). The study was carried out in two separate blocks, each one consisting of 3 periods. During period 1 the cows were fed a ration that supplied 90% or 88% of the NRC recommendations for TDN in lactating and nonlactating cows, respectively. This period lasted 170 in block 1 and 130 days in block 2. During period 2 the TDN was reduced to 55% or 52% for lactating and nonlactating cows, respectively. Period 2 lasted 100 days for cows in block 1 and 63 days for cows in block 2. At the beginning of period 3 TDN was further reduced to 25% or 27% for the lactating and nonlactating cows, respectively. Cows in block 1 were challenged with GnRH 40 days after the beginning of the 1st energy reduction, 30 days later and 7 days after the 2nd energy reduction. The cows in block 2 were challenged with GnRH 30 days after the 1st energy reduction, 30 days later and 25 days after the 2nd energy reduction.
reduction. At the end of the study body composition parameters and organ gland weights were determined.

No significant differences in the weights of the cows among the treatment groups were found. All cows were, however, losing weight through the course of this study. The nonlactating cows maintained higher body condition (P < .05) than lactating cows from 31 days after ovariectomies were performed until the end of the study.

The pituitary glands were significantly heavier in the lactating ovariectomized (2.3 g vs. 1.7 g, P < .05) than the nonlactating intact cows. The weight of the adrenals per unit of body weight of LO cows was significantly higher (.057 g/kg vs. .040g P < .05) than among NLO cows. The percent of carcass lipid was significantly higher (P < .05) in nonlactating as compared to lactating cows. Percent moisture and protein were higher (P < .05) in lactating cows.

Amount of LH released after GnRH stimulation tended to be higher in lactating than nonlactating cows. The magnitude of the LH peak did not differ significantly among the treatment groups at each of the dates GnRH was injected. Ovariectomized cows (LO and NLO) responded more rapidly (P < .05) to GnRH stimulation than intact cows (LI and NLI).

Time on reduced TDN did not affect cow's response pattern after GnRH injection.
Losses in body weight and condition in this study did not elicit changes in pituitary response to a GnRH challenge, although TDN was provided at only 53% of NRC-recommended quantities for approximately 80 days.
CHAPTER 1

INTRODUCTION

Reproductive performance in a beef herd is frequently the most important factor affecting the success of a beef production enterprise. Poor reproductive performance not only reduces the profit per cow or unit of land area, but may limit attempts for genetic improvement of the herd. For beef production to be lucrative, once-a-year calving is desirable; therefore, conception should ideally occur 60-80 days after calving. Several factors such as suckling (Wiltbank and Cook, 1958; Graves et al., 1968; Wetteman et al., 1978), amount of milk production (Morrow et al., 1966), level of nutrition (Wiltbank et al., 1964; Dunn et al., 1969; Topps, 1977), disease, climate and genetics influence the interval to first post-partum estrus and eventual conception.

Nutritional inadequacies undoubtedly are among the most important causes of increase in post-partum interval in many areas of the world. Some of the nutritional factors that have been shown to affect the interval from calving to first post-partum estrus are: level of energy intake before and after parturition (Wiltbank et al., 1962, 1964;
Dunn et al., 1969), level of protein intake (Topps, 1977), minerals in the diet (Peterson and Walden, 1977) and Vitamin A content (Lane, 1964).

The mechanism by which nutritional deficiencies affect reproduction have not been clearly elucidated. Initially, Oxeinreider and Wagner hypothesized that the effects of reproduction led to mild hypoglycemia which eventually led to depressed hypothalamic function. Radford et al. (1978) showed this was not the case as they found similar blood glucose levels in cows that had marked differences in length of the interval to first post-partum estrus.

Conflicting results have been reported on the blood levels of pituitary gonadotropins and ovarian steroids in cows that have been subjected to reduced energy intake.

A study was initiated to induce losses in body weight and condition of mature cycling Brangus cows by feeding them a low-energy diet. It was hoped that adequate losses in weight would be attained so as to eventually cause cessation of estrous activity. During the course of this loss in body weight, pituitary responsiveness to gonadotropin releasing hormone was assessed in lactating and nonlactating cows that were either ovariectomized or intact.
CHAPTER 2

LITERATURE REVIEW

Bovine Estrous Cycle

Physiological activities in the female reproductive system are cyclical in nature. The cyclic pattern, in behavioral, ovarian and uterine events, is controlled by hormones circulating in the female's blood (Sorenson, 1979). The estrous cycle may be broken into periods designated as proestrus, estrus, metestrus and diestrus. The estrous cycle is 20 days in heifers (S.D. 2-3 days) and 21 days in cows (S.D. 3-7 days) (Robinson, 1977; Swanson and Hafs, 1971). Estrus is the time during the cycle when the female is sexually receptive to the male. The duration of estrus is 12-24 hours with considerable variation (Robinson, 1977).

Hormones of the Estrous Cycle

The circulating hormones act on various target organs and induce the ovarian, uterine, behavioral and hormonal changes observed during the cycle.

Hypothalamic Hormone

Historical Background. Taleisnik and McCann (1961) showed that the hypothalamus exerted a regulatory influence
over the synthesis and the secretion of hypophyseal luteinizing hormone (LH). Ramirez and McCann (1963) also demonstrated LH releasing action of crude hypothalamic extracts on the pituitary. The hormone in the hypothalamus that was responsible for LH release from the pituitary was isolated (Schally et al., 1971), structurally elucidated (Baba et al., 1971) and synthesized (Matsuo et al., 1971). The elucidation of the amino acid sequence of LH-releasing hormone (Gonadotropin Releasing Hormone) isolated from porcine hypothalami has enabled a considerable number of related peptides to be synthesized and examined for biological activity.

The polypeptide responsible for both LH and FSH release from the pituitary was found to be a decapeptide with the amino acid sequence: PGLu-his-trp-ser-tyr-gly-leu-arg-pro-gly-NH₂. The synthetic decapeptide was found to behave in the same manner as the natural luteinizing hormone releasing hormone (Arimura et al., 1972; Kastin et al., 1974).

Gonadotropin releasing hormone (GnRH), was shown to control the synthesis and secretion of LH and follicle stimulating hormone (FSH) from the anterior pituitary (Arimura et al., 1972). Synthetic GnRH injected intramuscularly induced a significant increase in both serum LH and FSH in anestrous ewes (Reeves et al., 1972). White (1970) and Sandow et al., (1975) showed that there is probably only one releasing hormone for both FSH and LH.
In beef heifers levels of GnRH in serum were maximal 5 minutes after intravenous (IV) administration and decreased to baseline after 2 hours. However, maximum levels of bovine GnRH in serum were not observed until 15 and 30 minutes after IM injection of GnRH and did not return to baseline for 4 to 8 hours (Peterson and Nett, 1976). Redding et al., (1973) showed that the liver and kidney in man were the major sites for the degradation and excretion of GnRH. Gonadotropin Releasing Hormone rises during proestrus, peaks during estrus and decreases towards the end of metestrus. It stays low during diestrus before rising again in proestrus (Robinson, 1977).

The possibility that ovarian steroids may modify the responsiveness of the pituitary to exogenous GnRH has been considered by others. Reeves et al. (1971) demonstrated that LH release in ewes was greater during an 8-hour period on day 1 of the estrous cycle relative to any other time tested. These results provide indirect evidence that pituitary sensitivity to GnRH may be enhanced by removal of progesterone block or by estrogen stimulation. In addition, pretreatment of ewes with 250 to 500 μg of estradiol benzoate 20 hours before GnRH administration increased LH release relative to control ewes (Reeves et al., 1971). In Hostein heifers, Zolman et al., (1974) were able to demonstrate that endogenous serum estrogen levels influences
sensitivity of the pituitary to exogenous GnRH. In an earlier study, Zolman et al., (1973) observed an increase in magnitude of LH released with increasing doses of GnRH in heifers treated during the luteal phase of the estrous cycle.

**Pituitary Gonadotropins**

**Follicle Stimulating Hormone (FSH).** Follicle stimulating hormone is responsible for stimulation of growth and maturation of follicles and the stimulation of production and secretion of the steroid hormones which induce behavioral estrus (Rhaka and Robertson, 1965).

Follicle stimulating hormone is secreted by the adenohypophysis in response to GnRH stimulation (Sorenson, 1979). In anestrus ewes significant rises in FSH were observed after intramuscular injections of synthetic GnRH. In ewes that were normally cycling, Rhaka and Robertson (1965) showed that the release of FSH begins approximately 8 hours before the onset of estrus and continues for 14 hours, i.e., until 6 hours after the onset of estrus.

**Luteinizing Hormone (LH).** Together with FSH, LH functions synergistically in a number of roles: the initiation and maintenance of follicular growth and maturation, and ovulation. LH plays an important role in initiating ovulation and beginning the growth and maintenance of the corpus luteum. The luteotrophic action of LH in the cow, as
indicated by increasing progesterone has been demonstrated (Hansel and Snook, 1970). Snook et al. (1969) found that treatment with a specific LH antiserum resulted in a reduction in the size of the CL and progesterone content. Similarly, Karg et al. (1970) noticed a significant fall in the peripheral plasma concentration of progesterone after administration of LH antiserum on the 11th day of the cycle in one heifer. Hoffman et al. (1974) provided conclusive evidence that endogeneous LH acts as the main leuteotrophic factor in the cow.

Christensen et al., (1974) observed serum LH in cycling beef cows to peak at 7.38 ± 5.15 hours after onset of estrus and 24.1 ± 2.5 prior to ovulation. The elevation of LH level lasted 12.4 ± 1.6 hours. That the LH peak lasted 12 hours ± 7.4 hours after onset of estrus agrees with findings of other investigators (Henricks et al., 1970; Snook et al., 1971) who reported a peak of LH at the onset of estrus which lasted between 6-16 hours. The 24-hour interval between the LH peak and ovulation is in close agreement with Henricks et al. (1970) and indicates that considerable time is necessary for ovulation to occur after LH release. The interval from the LH peak to ovulation was 21 to 26 hours (Christensen et al., 1974).

During the remainder of the estrous cycle, mean values ranged from 0.6 ng/ml on day 15 to 1.8 ng/ml on days 3, 4,
10 and 12 in cycling beef cows. No LH values above 5 ng/ml were noted between the preovulatory peaks (Christensen et al., 1974). Echternkamp and Hansel (1973) found basal LH values to range from 0.5 to 3.0 ng/ml.

Prolactin. In rats (Evans et al., 1941), rabbits (Spies et al., 1968) and ewes (Denamur et al., 1973) prolactin is considered luteotrophic. However, in cattle it is not considered to be luteotrophic. Hoffman et al., (1974) observed no effect on corpus luteum function in the bovine despite the reduction in circulating prolactin caused by using a specific prolactin inhibitor, (B-154 (2 Br α - ergokryptin-methane-sulphonate). This was true even for the hysterectomized heifer with a persistent CL in which any long term effects of the prolactin inhibitor on CL function should have become obvious. These findings agree with earlier observations by Hoffman et al., (1973) who found no change in progesterone secretion after the administration of the inhibitor during the last 14 days of pregnancy. In order to demonstrate complete elimination of prolactin from peripheral plasma, the hysterectomized animal was treated with a combination of inhibitor and prolactin antiserum. This treatment did not significantly alter CL function (Hoffman et al., 1974).
Ovarian Steroids

**Progesterone.** When ovulation occurs, what is left of the graffian follicle rearranges to form the corpus luteum. The luteal cells are the main source of progesterone (Thibault and Levasseur, 1974). Christensen et al. (1974) observed that progestin levels rose and fell coincident with growth and regression of the corpus luteum in cycling beef cows. The serum levels were lowest on day 0 and remained low until day 4, when levels began to rise, reaching a peak on day 15. Thereafter, the levels declined until the next cycle. Smith et al., (1974) observed that circulating progesterone rapidly declined 4 days before estrus, remained low during and for 2 days after estrus, then increased to a maximum about day 10. The decrease in plasma progesterone which began 4-5 days before estrus was seen to coincide with an increase in plasma estradiol concentration. These findings are in agreement with earlier observations of Stabenfeldt et al. (1969) that the progesterone levels increased rapidly from day 3 to day 8 with a much slower rate of increase from day 8 to day 17, with rapid declines thereafter until ovulation. The decline in levels of progesterone are due to luteolysis which has been shown to be caused by prostaglandin F$_2$α (Caldwell and Moor 1971).
Estrogens

The major source of the plasma estrogens in non-pregnant cows are the ovarian follicles, and estradiol-17β, the major estrogenic steroid found in follicular fluid (Short, 1962) and in whole follicles (Lunaas, 1964). Smith et al., (1974) observed the occurrence in the normal cycling cow of two peaks of plasma estradiol, the greatest being the pre-estrous peak. Studies of the urinary excretion of total estrogen have shown two main peaks at days 7 and 20 after estrus (Mellin and Erb, 1966; Gaverick et al., 1971) corresponding closely with the peaks described in the plasma by Smith et al., (1974).

The pre-estrus peak has been shown to precede the preovulatory surge of LH, which occurs in the cow 15 to 22 hours before ovulation, or 3-6 hours after the onset of estrus (Schams and Karg, 1969). This is compatible with the view that a positive feedback of ovarian estrogen on the hypothalamic-pituitary axis is an essential part of the mechanism that triggers the release of LH in the cow.

Prostaglandin F$_2$α and Reproduction in the Cow

The interest in the effects of prostaglandin on reproductive processes was stimulated by a hypothesis implicating PGF$_2$α as the uterine factor responsible for termination of corpus luteum activity in several species (McCracken et al., 1970).
PGF$_2\alpha$ as a Luteolysin. Levels of PGF$_2\alpha$ vary with the stage of the estrous cycle in both the endometrium (Wilson et al., 1972) and uterine venous blood (McCracken, 1971; Thorburn et al., 1972), rising to peak levels by day 14 of the cycle which are fourfold those seen at earlier stages. Studies by Caldwell and Moor (1971) showed that dried uterine venous plasma collected on day 14 of the cycle in sheep caused luteal regression when infused into the ovarian artery in sheep at day 8, while infusion of similar material collected on day 8 had no effect. Pharriss et al., (1968) demonstrated that PGF$_2\alpha$ did not alter pituitary LH levels in the rat and concluded that the luteolytic effect of PGF$_2\alpha$ was not mediated through the hypothalamic-pituitary axis. The possibility that prostaglandin F$_2\alpha$ acts initially on the uterus to cause release of a specific uterine luteolytic substance has been negated by the work of Blatchley and Donovan (1969) who observed a luteolytic effect of PGF$_2\alpha$ in hysterectomized animals. Niswender et al., (1974) showed an increase in blood flow to the CL during the mid-luteal phase suggesting also an increase in the amounts of LH and prolactin as well as other substances such as oxygen, which supports the increased function of the CL. Results from the work by Pharriss et al., (1974) on rabbits and rats strongly support the concept that congestion of the ovary could follow administration of PGF$_2\alpha$ and in turn could
account for luteolysis, but also for the increased lysosomal fragility observed. The luteolytic effects of \( \text{PGF}_2\alpha \) are also manifest in tissue cultures of rat and rabbit ovarian tissue, where effects on the vascular system are not likely to be the causal factors (Behrman et al., 1971; O'Grady et al., 1972). \( \text{PGF}_2\alpha \) acts locally rather than as a systemic hormone (McCracken et al., 1972) as primary prostaglandins have very short half lives and are almost completely cleared from the blood after one passage through the lungs.

Based on the short half-life and rapid systemic clearance of \( \text{PGF}_2\alpha \) a specialized mechanism providing the ovary with initial exposure to the hormone must be present. In sheep, McCracken et al. (1972) showed that when tritiated \( \text{PGF}_2\alpha \) was infused into the uterine vein it transversed into the ovarian artery by a counter-current exchange mechanism between the utero-ovarian vein and the ovarian artery. The data of Hixon and Hansel (1974) are also consistent with this hypothesis.

**Hormonal Interaction**

Hobson and Hansel (1972) were able to show the rise in estrogen levels which precede behavioral estrus in the beef cow was responsible for the initiation of LH release. Estradiol-17\( \beta \) may cause the release of LH by increasing
pituitary responsiveness to GnRH or by increasing the number of binding sites or the binding affinity of the releasing hormone to the receptor sites in the anterior pituitary (Reeves et al., 1971; Beck and Convey, 1977).

Echternkamp and Hansel (1971) observed that the same dose of estrogen that caused LH release had no effect on LH release when they were given to intact animals at mid-cycle, a time when progesterone were presumably high. This suggests that progesterone inhibits the stimulatory action of estrogen on LH. The negative correlation between serum LH and progestins during the estrous cycle was also observed by Garverick et al. (1971) in dairy cows and in beef cows by Tillson et al. (1970) and Christensen et al. (1974). The mechanisms by which progesterone influences secretion of LH have not been defined. Receptors to progesterone are present in the anterior pituitary and hypothalamus (Moguilewsky and Raynaud, 1977); therefore influence of progesterone on LH secretion may occur directly at either or both of these sites. This negative feedback could be exerted in several ways: (1) progesterone could decrease the number of receptors for GnRH, thereby increasing the concentration of GnRH required to elicit release of LH; (2) progesterone could inhibit synthesis of LH within the anterior pituitary, thereby limiting the amount of LH available for secretion;
and (3) progesterone could inhibit secretion of GnRH by the hypothalamus, and thus, indirectly inhibit secretion of LH (Nett et al., 1980). In ewes, Nett et al. (1980) postulated that the decreased secretion of LH they had observed after administration of progesterone was most likely due to depressed secretion of GnRH. They arrived at this conclusion from the observation that despite suppressed serum concentration of LH after administering progesterone in ovariectomized ewes, the concentrations of LH in the anterior pituitary gland were not affected. They also noted that the numbers of receptors did not change after ovariectomy, nor after administration of progesterone to the ovariectomized ewes.

The increasing levels of estrogen stimulate the myometrium which in turn causes release of PGF$_2\alpha$. The PGF$_2\alpha$, by a counter-current exchange mechanism, traverses from the uterine vein to the ovarian artery where it acts on the CL of the estrous cycle, causing it to regress (McCracken et al., 1972; Hixon and Hansel, 1974).

**Ovarian Activity**

Cyclic events occur in the ovaries in relation to the circulating hormones during estrous cycle. The changes involve growth, maturation and eventual ovulation or atresia for some follicles and/or the development of the corpus
luteum. Rajakowski (1960) reported the occurrence of two growth waves of follicles in bovine ovaries, the first being between 1 to 12 days and the second beginning 12 to 13 days and ending at ovulation.

After ovulation occurs the granulosa cells form into a corpus luteum. Weights of corpora lutea increased from days 1 to 17 then declined (Ireland et al., 1979). Christensen et al. (1974) observed that progestin levels rose and fell coincident with growth and regression of the corpus luteum.

Bovine Hormonal Profiles During Pregnancy and at Parturition

When fertilization occurs in the cow or heifer, changes occur that help in the maintenance of pregnancy. Changes in some hormonal profiles during this stage will be reviewed.

Progesterone

Progesterone produced by the corpus luteum (CL) plays an important role in the maintenance of pregnancy. In the bovine there seems to be no placental progesterone production (Gorski et al., 1958). This is in agreement with Estergreen et al. (1967) who showed that the corpus luteum (CL) of pregnancy was essential for maintaining pregnancy
in cows prior to days 165-180, as removal of the CL would lead to abortions or parturition before term, with complications in delivery and retained placentas.

Henricks et al. (1972) noted that progesterone concentrations in heifers that returned to estrus 18 to 20 days after mating were significantly lower than in pregnant heifers, as early as the ninth day after mating. During the first 15 days after mating, pregnant heifers had 1.7 times more progesterone present in peripheral plasma than those that returned to estrus. Progesterone concentration increased at the beginning of pregnancy with the development of the CL of pregnancy (Schams et al., 1972). Change from CL of normal cycle to CL of pregnancy was characterized by progesterone values higher than 5 ng/ml during days 18-24 (Schams et al., 1972). Robertson (1972) reported a decline in progesterone to relatively low concentrations from days 90 to 150 of pregnancy followed by variable rises. Allowing for individual variation, the concentration seemed to remain fairly constant after day 40 of pregnancy (Henricks et al., 1972).

Using intravascular catheters placed in the umbilical, uterine and maternal circulations of Jersey cows between 240 and 260 days of gestation, Comline et al. (1974) observed maternal peripheral plasma concentration to be
stable until a sudden fall 1-2 days before delivery. Edqvist et al. (1972) noted mean plasma levels of about 4 to 5 ng/ml during the last seven days before parturition. A significant drop in the peripheral plasma level of progesterone to an average of 1.8 ng/ml occurred about 24 hours before parturition. Plasma progesterone levels decreased gradually from 4.1 ± 0.3 ng/ml 2 days prior to calving to 1.6 ± 0.4 ng/ml on the day of calving (Garverick et al., 1974). There are, however, conflicting results since Erb et al. (1968) reported remarkable higher concentrations of progesterone during the last month and no decline prior to parturition.

O'Brien and Stott (1977) observed a decline in the concentration of progesterone until parturition in Holstein heifers that had normal delivery as well as those that had dystocia and were assisted at the time of parturition. Symons (1973), in dairy cows, also observed that progesterone levels began to fall between 15-20 days prepartum (7-8 ng/ml) and reached relatively low levels by 1 day prepartum (less than 2 ng/ml).

Serum 17-hydroxyprogesterone appeared to follow the same pattern in normal and assisted Holstein heifers, especially during the last 2 weeks of pregnancy.
Estrogens

Batson et al. (1973) observed higher estrogen concentration in bred cows than in nonbred cows on days 5, 6 and 10 post-estrus. This is in contrast to Henricks et al. (1972) who observed that estrogen levels were even lower during early pregnancy than during the cycle through the first 39 days of pregnancy. During the 14 days before parturition, estrogen increased from 500 pg/ml to 2660 pg/ml at parturition. For the last 5 days the estrogen concentration progressively increased at the rate of 248 pg/day (Henricks et al., 1972). Excretion of estrogen in urine increases dramatically at the end of gestation with a 30% increase seen the last 48 hours preceding parturition (Mellin et al., 1966).

Estrogen levels were observed to rise sharply about 15 days prepartum and reached maximum values 1-2 days before delivery in dairy cows (900-1700 pg/ml) (Symons, 1973).

Serum estrone concentrations in both the normal and dystocial heifers showed a 7-to-8-fold increase from day 23 prepartum until parturition. The highest average was before-parturition plasma levels of estrone in dairy cows of the Swedish and white breed. The levels recorded during the time period from the 20th to the 35th week of pregnancy were
below or about 0.1 ng per ml. After the 35th week of pregnancy the concentration increased gradually and maximum levels ranging from 0.5 to 2 ng/ml were found during the last week of gestation. Estrone levels ranged from about 0.7 to 0.9 ng/ml during the last eight days preceding the delivery (Edqvist et al., 1972).

The peripheral plasma levels of estradiol-17β followed the same pattern as for estrone. The concentration of estradiol was only 10 to 20 percent of the estrone level (Edqvist et al., 1972). During the last 3 weeks of gestation in both the unassisted and assisted holstein heifers, O'Brien and Stott (1977) observed that serum concentrations of estradiol-17β exhibited a continuous rise until parturition. Plasma estradiol levels observed at normal parturition, in a study by Garverick et al. (1974), followed a pattern similar to the changes previously observed in the urine samples with peak levels occurring on the day of parturition.

Corticosteroids

Hunter et al. (1977) monitored hormonal changes preceding calving by cannulation of the fetal posterior vena cava and maternal utero-ovarian and jugular veins in Jersey cows between days 240 and 260 of gestation. Fetal corticosteroids rose slowly from 5.0 ± 0.7 ng/ml at 20 days
to 9.3 ± 3.0 ng/ml at 10 days before term, then progressively to a mean of 74 ng/ml at calving. Maternal levels remained relatively constant at 5 to 15 ng/ml at calving. A similar pattern was observed earlier by Comline et al. (1974) who observed a gradual pre-partum rise in fetal plasma cortisol during the last week of gestation (from 10-20 ng/ml 7 days before parturition to 51 ± 5 ng/ml in the last 3 hours before delivery). The maternal plasma cortisol levels stayed fairly constant. In contrast to these findings, Hoffman et al. (1973) observed an increase in maternal plasma cortisol levels. Part of this increase was assumed to be an elevation due to experimental stress imposed on the animals. However, this did not wholly account for the levels observed. The concentration of maternal endogenous corticosteroids was observed to rise before parturition in Holstein heifers that had normal delivery (O'Brien and Stott, 1977). Adams and Wagner (1970) also found increased levels of endogenous glucocorticoids 1-4 days before parturition in maternal jugular vein plasma.

**Pituitary Hormones**

**LH and FSH**

In general, the LH levels ranged around 1 ng/ml throughout pregnancy (Schams et al., 1972; Echternkamp and
Hansel, 1973; Hoffman et al., 1973). These values agree with those of Arije et al. (1974) who reported prepartum LH concentrations of less than 1 ng/ml of plasma. Synthesis of LH is probably inhibited by high estrogen levels during late pregnancy through 3 days after calving (Erb et al., 1971). In contrast, pituitary FSH activity is higher at calving than during the first or second ovarian cycles (Saiddudin, 1968). The above studies show that the beginning of luteolysis of the corpus luteum of pregnancy is certainly not due to a deficiency of LH. The FSH levels are also unchanged before parturition.

Prolactin

Using 4-12 year old Brown Swiss and Simmental cows, Hoffman et al. (1973) looked at the role of prolactin in initiating parturition. In three of the cows treated with a specific protein inhibitor (2 BR-α-Ergokryptin, CB-154, Sandoz), no significant changes in the other hormone concentrations were found as compared to normal cows. The prapartum prolactin increase plays an important role in connection with the onset of lactation.
Prostaglandin F₂α (PGF₂α)

Fairclough et al. (1975) found that PGF₂α concentration in the utero-ovarian venous blood rose sharply 48 to 72 hours before parturition in the dairy cow and suggested that PGF₂α was the luteolytic factor responsible for the decrease in progesterone shortly before parturition. Prostaglandin F₂α levels increased gradually 36-48 hours before birth and then dramatically the last 24 hours, reaching peak levels (5.7 ± 0.6 ng/ml) during labor (Hunter et al., 1977). The control of prostaglandin F₂α production by the pregnant cow is unknown. In sheep there is good evidence that the controlling factor is the fetal secretion of cortisol, which determines the ratio of maternal production of estrogen and progesterone, which leads to the release of prostaglandin F₂α. Although the sheep and cow differ in the source of progesterone during the last third of pregnancy, the fact that the ratio of estrogen to progesterone increases over the last 10 days before term (Hunter et al., 1977) supports the suggestion that prostaglandin F₂α production in the cow may be stimulated by a rise in the estrogen-to-progesterone ratio.

Hormonal Relationships at Parturition

Successful parturition requires termination of the mechanisms for the maintenance of pregnancy and expulsion
of the fetus. The mechanisms controlling parturition differ slightly among the domestic species. In swine, goats and cattle, where progesterone is produced by the corpus luteum, luteolysis must occur and the progesterone block of uterine contraction be removed for induction of delivery (Jöchle et al., 1972). In sheep, placental production of progesterone is reduced preterm and is terminated after delivery (First, 1978). Evidence that the pituitary adrenal axis of the calf is involved in initiation of parturition came initially from observations that inherited defects in the fetal adrenal cortex or anterior pituitary resulted in prolonged gestation (Kennedy et al., 1957; Holm et al., 1961). Preterm delivery was introduced by administration to the bovine fetus of exogenous adrenocorticotropic hormone (Comline et al., 1974) or infusion of the fetus with dexamethasone at a low dose (3.3 mg/day) (Hunter, et al., 1974). The rise in maternal estrogens and fall in progesterone which precede normal parturition also occurs soon after administration of dexamethasone to the fetal calf (Comline et al., 1974; Fairclough et al., 1975).

However, exactly how fetal cortisol or exogenous glucocorticoids cause luteolysis and parturition is unknown. The mechanism responsible for controlling CL function
is poorly understood in the bovine (First, 1978). Denamur et al. (1973) identified the hormones responsible for the maintenance of the CL during the bovine estrus cycle to be prolactin and luteinizing hormone. In the bovine species during pregnancy maternal pituitary LH is more likely to cause maintenance of the CL than prolactin or prolactin-like substances (placental lactogen) because LH is detectable and stable at the end of pregnancy (Hoffman et al., 1973; Schamms et al., 1972). Hoffman et al. (1974) treated pregnant cows with a prolactin inhibitor or prolactin antisera, and the CL was maintained, whereas treatment with antisera to LH led to regression of corpora luteae.

The possible hormonal mechanisms controlling parturition in the bovine have not been clearly elucidated. Liggins et al. (1973) postulated the following sequence of events as initiating parturition in ewes. They believe the stimulus to parturition in the ewe originated in the fetal hypothalamus, though the factors influencing hypothalamic activity and determining the timing of the mechanism are still uncertain. As a result of hypothalamic and pituitary activity, the rate of secretion of cortisol increases rapidly due to combined effects of increased activation of the enzyme 11β-hydroxylase and of increased responsiveness of the adrenal cortex to ACTH. Cortisol would then act on
the placenta and reduce the secretion of progesterone and increase secretion of estrogen. Associated with rising concentration of estrogen is an equally sharp rise in PGF$_2$$\alpha$ in maternal cotyledons and the myometrium. The myometrium responds to PGF$_2$$\alpha$ with heightened sensitivity to oxytocin. The distension of the cervix and vagina by the descending fetus reflexly stimulates release of oxytocin from the posterior pituitary gland.

The uterine contractions that were started by PGF$_2$$\alpha$ are augmented by the added stimulation of oxytocin on the myometrium. The maternal abdominal musculature also becomes reflexly excited, leading to eventual completion of parturition.

A similar sequence of hormonal changes and events might control parturition in the bovine.

Endocrinology of the Postpartum Period

The interval from parturition to first estrus is variable in the bovine, being shorter in milked dairy cows and longer in beef cows. The postpartum anestrous period (interval from parturition to behavioral estrus) ranges from 30 to 72 days in dairy cows and from 46 to 104 days in beef cows. Similarly, the average interval from parturition until the first ovulation ranges from 14 to
45 days in dairy cows and from 36 to 71 days in beef cows (Casida, 1968).

Knowledge of the endocrinology of the postpartum period is essential for the understanding of the factors responsible for reinstating cyclic ovarian activity following parturition.

Gonadotropins

**Follicle Stimulating Hormone (FSH).** Pituitary follicle stimulating hormone (FSH) is greatest at parturition and decreases during the early postpartum period (Labhsetwar et al., 1964). Dobson (1978) observed that the mean FSH concentration was significantly greater between 0 and 20 days postpartum in 3 of 6 dairy cows and the values tended to be generally lower after the resumption of cyclic activity. Pituitary responsiveness to GnRH as measured by release of FSH was studied by Schallengerger et al., (1978) in Brown Swiss cows. They found that the response decreased significantly during the last 9 days antepartum. The FSH serum concentration during the period averaging 108 ± 34 ng/ml. The response was not changed until after 10 days postpartum when there was an obvious rise to 160 ± 61 ng/ml. The average duration of the interval between injection of GnRH and peak value was about 25 minutes shorter postpartum than antepartum.
Luteinizing Hormone (LH). At parturition pituitary LH content was low in Holstein cows (Labhsetwar et al., 1964). During the first 30 days after parturition Wagner et al., (1969) observed an increase in pituitary LH concentrations. The serum levels seem to follow the same pattern gradually increasing as the interval from parturition increases. Erb et al., (1971) observed an increase in plasma LH from 0.21 to 0.48 ng/ml from 0.5 to 8 days after calving in dairy cows. The same tendency was reported by Stevenson and Britt (1979), as the concentration of LH in blood samples collected on day 14 postpartum, from all the Holstein cows in the study, was higher than that in samples collected on day 7. In addition to this they also found that the number of episodic LH peaks and magnitude of the largest LH peak tended to increase from day 7 to day 14 postpartum. A similar pattern was observed in beef cows by Arije et al., (1974). The serum LH concentration from 3 weeks before until the day of parturition ranged between 0.4 and 1.4 ng with a mean of 1.1 ng/ml. The concentration after parturition varied between 0.5 and 2.0 ng/ml the first 21 days with a mean of 1.33 ng/ml.

Pituitary responsiveness to GnRH stimulation as measured by LH release is established as early as 7 days postpartum in dairy cows (Kesler et al., 1977) and day 10 in beef cows (Schallengerberger et al., 1978). Increased
concentrations of progesterone and estrogen during pregnancy may reduce responsiveness of the pituitary during late gestation and during the early postpartum period, or the lack of LH release could be caused by a reduction in pituitary LH content (Wetteman, 1980). Webb et al., (1977) also found that the responsiveness of the pituitary to GnRH in beef cows tended to increase postpartum up to day 20.

**Ovarian Steroids**

**Estrogens.** After parturition, estrogen levels ranged from non-detectable to 40 pg/ml in dairy cows (Henricks et al., 1972). The rate of excretion of estrogens in urine decreased 10-fold from 0.5 day as compared to 3 days after calving in dairy cows (1,599 versus 151 ng/ml creatinine) (Erb et al., 1971). Arije et al., (1974) measured estrogen levels in multiparous pre- and post-parturient beef cows. Values were high (972 to more than 1,300 pg/ml) during the last 2 weeks of gestation and dropped to 592 pg/ml the day after calving. Estrogen levels became markedly lower varying between 122 and 382 pg/ml with a mean of 269 10 days postpartum. Echternkamp and Hansel (1971) obtained values of 4,653 and 7,549 pg/100 ml of estrone and estradiol, respectively, in the plasma of dairy cows on the day of parturition. The concentrations are in agreement with those obtained by Henricks et al., (1972).
Estrogen concentration increased during the 5 days before estrus from 149 pg/ml to as much as 540 pg/ml on the day before estrus, indicating a rise in estrogens preceding the LH peak (Arije et al., 1974). Stevenson and Britt (1979) found that following return to basal concentrations, estradiol fluctuated between 7 and 15 pg/ml (estradiol peak in their assays averaging 20 pg/ml) in blood samples collected from Holstein cows between parturition and first ovulation, but in other cows concentration remained low until 2 to 3 days before estrus.

**Progesterone.** Mean progesterone concentration in Holstein cows fluctuated between 4 and 2 ng/ml from 14 days until 1 day before parturition (Henricks et al., 1972). They also found a downward trend in progesterone levels which began 3 days before parturition and on the day of parturition the concentration was below 1 ng/ml. In beef heifers progesterone levels, though low, were present in a cyclic pattern within 20 days after parturition. In this study Henricks et al., (1972), observed 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, indicating that the difference in postpartum interval appears to be related to the incidence of plasma progesterone. The CL of pregnancy is the major source of progesterone during pregnancy, however after calving ovarian
venous plasma (Erb et al., 1968) is very low and in the regressing CL (Labhsetwar et al., 1964). Erb et al., (1971) postulated that since they observed peripheral progesterone levels varying from 7 to 10 ng/ml, this was being produced by a nonluteal source, as they could not palpate any new CL. The adrenal gland was assumed to be the most probably nonluteal source of progesterone as levels in this gland had been found to be substantially high in earlier work, 7 to 30 days after calving by Wagner (1969). Castenson et al. (1976) examined ovaries of post-partum beef heifers in situ by laparoscopy, as well as by histological examination of the ovarian sections when peripheral progesterone became elevated for 2 days. Removal of ovary with the CL led to an immediate drop in peripheral progesterone concentration. These results demonstrated unequivocally that the CL was the source of the progesterone. Donaldson et al. (1970) also observed that in cattle the first normal cycle postpartum, whether accompanied by observed estrus or not, was usually preceded by a small increase in plasma progesterone three to five days before the beginning of the cycle, which was assumed to have a priming effect. LaVoie and Moody (1976), Humphrey et al., (1976) and Williams and Ray (1978) also reported the same phenomenon and prior to this "priming" effect by progesterone, estrogen and LH appeared to be out of phase with one another (Dickey et al.,
1975). Gonzalez-Padilla et al. (1975a, b and c) observed in changes in hormonal profiles at the attainment of puberty in bovine that were similar to the ones occurring before first postpartum estrus.

Factors Causing Increase in Postpartum Anestrous Period

Once-a-year calving is dependent upon early establishment of reproductive activity after parturition. Conception should ideally take place between two and three months after calving. Duration and variability of intervals from calving to standing estrus are known to be influenced by suckling (Graves et al., 1968; Wetteman et al., 1978), amount of milk produced (Morrow et al., 1966) and level of nutrition (Dunn et al., 1969). Another cause of delayed fertility after calving in some cows may be dystocia. Calving difficulty increases the interval to first estrus and decreases fertility to subsequent breeding. The effect may be due to trauma, physical damage or resulting infection (Foote, 1974). Breed and age of the beef cows have also been established as having an effect on postpartum interval to estrus. Mechanisms by which these factors alter the postpartum anestrous interval are however, unclear.

Effect of Suckling and Lactation

Among suckled beef cows, the interval from parturition to first estrus is variable, but means generally range
from 60 to 100 days (Wiltbank and Cook, 1958; Short et al., 1972). Suckling prolongs the postpartum interval to first estrus (Oxenreider, 1968; Short et al., 1972). Wiltbank and Cook (1958) demonstrated that the postpartum anestrous period was longer for cows suckled or cows milked four times daily than for cows milked twice daily. The mechanism by which suckling causes this effect, however, is not known. Saiduddin et al., (1968) reported that the concentration of LH was similar in pituitaries from suckled and non-suckled cows, whereas Short et al., (1972) and Carruthers et al., (1978) observed that suckling suppressed plasma LH concentration in cows postpartum. Troxel et al., (1980) found that concentrations of pituitary LH were similar for suckled and non-suckled cows, but suckling appeared to decrease the concentration of LH in plasma. Furthermore, pituitary responsiveness appears to be regained about 8 to 9 days earlier in milked cows (Kesler et al., 1977) than suckled cows (Irvin et al., 1977). Troxel et al., (1980) observed an increase in the concentrations of LH in plasma and the GnRH-induced release of pituitary LH after short-term calf removal. Therefore, suckling appears to suppress release of LH in response to GnRH even though hypothalamic GnRH (Carruthers et al., 1978) and pituitary content of LH (Saiduddin et al., 1968) do not appear to be affected.
In Angus cows, Short et al., (1972) showed that mastectomy decreased the length of the postpartum interval. The mechanism by which it did is not understood. They postulated however, that mastectomy might have been magnifying the effect of nonsuckling by completely removing any inhibitory effect of lactation or nursing. Radford et al., (1978) in crossbred beef cows found that non-suckled cows experienced regular ovarian cycles from 10 to 33 days postpartum, while suckling cows did not do so until at least 14 weeks postpartum. Suckled cows also had lower plasma LH concentrations before 30 days postpartum but were similar thereafter.

Episodic releases of LH may be associated with events leading to first ovulation postpartum. Carruthers and Hafs (1980) observed that suckling, which prolongs the interval to first ovulation, also reduces the frequency and amplitude of episodic LH releases in dairy cows. They observed that the frequency and amplitude of LH release in Holstein cows suckling one calf were 50% less than those in nonsuckled controls and that the interval from parturition to first ovulation was doubled by suckling (Carruthers et al., 1980). Episodic release of LH is probably caused by LHRH released from the hypothalamus. The decrease in LH secretion caused by suckling may be due to a decrease in hypothalamic GnRH content. Minaguchi and Meites
(1967) showed that bioassayable GnRH content was lower postpartum in lactating rats, suggesting that availability of GnRH may have been the limiting factor to resumption of estrous cycles. In contrast to this, Carruthers et al., (1980) found no difference in total content or gross distribution of radioimmunoassayable GnRH in hypothalami from suckled and nonsuckled cows. They concluded therefore, the amount of GnRH available in the hypothalamus did not limit resumption of LH secretion in postpartum cows. However, measurement of total gland content does not provide information regarding GnRH release and differences in biologically active LHRH might not be detected by radioimmunoassays. Carruthers et al., (1980) detected no differences in the LH content between the pituitaries from suckled and nonsuckled cows. The decreased release of LH they observed in the suckled cows must have been due to lower quantities of "releasable" LH. Foster (1978) reported that a "priming" injection of GnRH increased the LH response to a second injection of LHRH in dairy cows. This same effect was demonstrated in vitro by Padmangbhan et al., (1980). Thus, quantity of LH released from bovine anterior pituitary cells is increased by previous exposure of cells to GnRH. Assuming that the frequency of episodic LH releases observed in nonsuckled cows results from more frequent releases of LHRH, pituitaries of nonsuckled cows receive more GnRH "priming"
than do those of suckled cows and have greater releasable pools of LH as a consequence. Frequency and amplitude of episodic LH secretion and amount of releasable LH in the pituitary are reduced by suckling. On the basis of the above observations, Carruthers et al. (1980) hypothesize that suckling prolongs the interval of postpartum anestrus and anovulation by reducing frequency and perhaps amplitude of LHRH secretion. As a consequence of reduced GnRH secretion, LHRH "self priming" is reduced, which decreases the releasable pool of LH in the pituitary and frequency and magnitude of episodic LH release, leading to delayed follicular maturation.

Follicle-stimulating hormone appears to be present in high concentration during the postpartum period (Schamms et al., 1978). Secretion of FSH is not limited by suckling and therefore is not a limiting factor in the resumption of cyclicity in postpartum cattle. The FSH concentration reported by Carruthers et al. (1980) agrees with the levels found by Dobson (1978) for milked Holstein cows during the postpartum period and during the luteal phase of the estrous cycle.

Hyperprolactinemia has been implicated as a cause of anovulation postpartum in other species, but not in cows. Decreasing prolactin concentration in the blood by various
procedures has not been effective in shortening the interval to first postpartum ovulation in cattle (Clemente et al., 1978; Williams and Ray, 1978; Carruthers and Hafs, 1980; Carruthers et al., 1980).

Effect of Nutrition

Nutrition plays a highly significant role in the reproductive performance of farm animals.

The length of postpartum interval in the bovine has been shown to be affected by various nutritional factors. Several workers have shown the level of energy before and after parturition influences reproductive performance (Wiltbank et al., 1962, 1964; Dunn et al., 1969). The level of protein intake too has been shown to play a significant role on the postpartum reproductive performance (Topps, 1977; Williams et al., 1980). Among minerals, phosphorus (Bosticco and Corrias, 1961) and vitamin A among vitamins (Lane, 1964) have been shown to affect reproductive performance during the postpartum interval.

The intensity of the effects of the above factors on reproduction are influenced by the condition of the heifers or cows before and/or after parturition (Whitman et al., 1975).
Effect of Energy Level

Wiltbank et al. (1962) studied the effect of level of energy on reproductive performance in mature Hereford cows. Two levels of energy, 9 lb. of TDN per head daily (high) and 4.5 lb. of TDN per head daily (low) were fed prior to calving. Following calving, one-half of the low ratio received 16.0 lb. of TDN per head daily (high-high and low-high). The remainder of the cows received 8.0 lb. of TDN per head daily (high-low and low-low). The conception rates during the experiment were 95%, 77%, 95% and 20% for cows fed the high-high ration, high-low ration, low-high ration and low-low ration, respectively, showing that level of energy can markedly influence reproduction in the mature beef cow suckling a calf. Wiltbank et al. (1964) found that TDN levels 25% below or 50% above recommended levels lengthened the interval from calving to estrus and that low levels of TDN after calving reduced calving percent. Dunn et al. (1969) studied the influence of pre- and post-calving energy intake in 2-year-old Hereford and Angus heifers nursing their first calves. They fed two levels of digestible energy (DE) before calving (8.7 M cal low and 17.3 M cal). The precalving low group was divided into two
groups, one to be fed a moderate level of energy (27.3 M cal) and one a high level of energy (48.2 M cal) after calving. The high precalving group was divided into three groups at calving time and fed three levels of energy daily: high-low (HL), 14.2 megacalories; high-moderate, 27.3 megacalories; and high-high (H-H), 48.2 megacalories. The heifers were assigned to the treatment until 120 days after calving. The 60-day breeding started after calving. The pregnancy rate 120 days after calving was directly related to the postcalving level. Eighty-seven percent of the cows fed the high-energy level after calving were pregnant and compared with 75% of those fed moderate level and 64% of those on the low energy those fed moderate level and 64% of those on the low energy level. The pregnancy rate 120 days after calving was directly related to the low level of energy before calving. Clemente et al. (1978), using fall-calving cows, showed that precalving nutrition affected length of the postpartum interval.

Diet was shown to have no effect on number of follicles or follicular or luteal volume. There was, however, a trend for the corpus luteum of heifers fed the low-level diet to be smaller. In the non-ovulatory ovary, the level of nutrition did not appear to have any significant effect
on ovarian size or follicular volume (Spitzer et al., 1978). Lishman et al. (1979) also showed that level of feeding had no effect on size of the largest follicle or the proportion of beef cows that had a follicle 12 mm in diameter. Eighty-eight percent of ova recovered from heifers on the low level of energy were fertilized compared to 79% fertilization rate for ova recovered from heifers on the high level of energy (Spitzer et al., 1978). These differences were, however, not significant, showing that level of nutrition did not appear to affect the ability of ova to be fertilized.

Effect of Protein

Foster et al. (1945) showed calf crops of cows grazing on the southeastern coastal plains in the United States varied according to the level of winter supplement received. Cows receiving 2 lbs. of protein supplement per day had an average calf crop of 48% compared to 63% for those that received 4 lbs. of protein supplement per day and 68% for those that had received 6 lbs. of protein supplement. Williams et al. (1980), using primiparous Hereford heifers, studied the effect of pre- and postpartum protein restriction on postpartum reproductive performance. The heifers were fed one of the two isocaloric diets from 150 days prepartum to 110 days postpartum. Ration I (adequate) was
adjusted to supply 0.96 kg and ration II (deficient) supplied 0.32 kg crude protein per day. Intake of the respective rations was increased by 33%. Heifers consuming ration I had a shorter interval from parturition to first estrus than those consuming ration II (75 versus 86 days), to first service (76 versus 87 days) and conception (81 versus 92 days). Eighty-nine percent of animals receiving ration I exhibited estrus within 110 days postpartum compared to only 63% of those deficient in protein. First service conception rate (71 versus 33%) overall conception rate (74 versus 32%) were adversely affected by reduced crude protein intake.

Effect of Minerals and Vitamin A

Butcher et al. (1979) individually fed 7-month-old Hereford heifers a basal ration containing either 0.14% phosphorus or approximately 66% of the NRC requirement over a 4-year period and the other half of the cows were supplemented with monosodium phosphate to have an average phosphorus intake of 0.36% or 174% of NRC requirements. No significant differences in growth rate, appetite or reproductive rate were found during the four-year period. From the results of breeding performance in this study, they recommended that phosphorus levels below 50% NRC are
still adequate for maintaining fertility. Simmons et al. (1980) supported the above findings, as phosphorus-deficient diets did not have any significant effect on the estrous cycles and the breeding capabilities of lactating dairy cows. The above findings are in agreement with earlier studies by Palmer et al. (1941) who did not observe any interference with the normal regularity of ovulation or the ease of conception in dairy cows on a phosphorus-deficient diet.

Differences in mineral nutrition between herds were shown to be associated with reproductive performance in a study by Peterson and Walden (1977) using 261 dairy herds in the Lower Fraser Valley of British Columbia. The copper/molybdenum ratio in silage was the most important factor, with increases in Cu relative to Mo being associated with low herd non-return rates. Other minerals found to be important included Cu/Mo ration in grain and hay, Cu in hay, Mo and Se in grain, Ca and P in silage and the variability of Mg levels in soil.

Among vitamins, deficiencies in vitamin A have been shown to result in poor production and distocia at calving in cows. Bradfield and Behrens (1968), in a four-year study involving nine Southern Nevada beef ranches, obtained an increase of 11.8% and 14.0% in fall
pregnancy rate for aqueous and paste suspension, respectively, of vitamin A, D and E injected prior to spring breeding.

Lane (1964), using 2,200 cows in Arizona, injected 1,000,000 IU of vitamin A plus 100,000 units of vitamin D per cow. They observed that vitamin A injections on yearling heifers as two-year-olds when treatment was given prior to the breeding season, led to an increase in the percent calf crop.

Body Condition

The expression of the effects of nutritional deficiencies on the cow depend largely on the condition the animal is in before or after calving. Body condition is a subjective concept intended to summarize the degree of fat cover of an animal in relation to its size. Seasonal variation in availability and quality of forage usually causes cyclic loss and gain in live weight of beef cows. Changes in cow weights and condition score have both been shown to affect reproductive performance. Richardson (1975) observed that the percentage of cows that calved tended to increase with decreases in the percentage loss of body weight from the peak in autumn to either postpartum or midmating season. The estimated percentage body weight changes from autumn peak to mean body weight at the
beginning and end of the mating season, which would be followed by successful conception rates of 50%, 75% and 90% were -14%, -6% and +1% of autumn body weight, respectively (Richardson, 1975). The above findings are in agreement with earlier findings by Wiltbank et al. (1964) that cows that lost weight severely postpartum would be unlikely to conceive in the next mating period. A significant correlation was observed between the duration of postpartum anoestrus and post-partum live weight by Holness et al. (1978) in Africander and Mashona cows. Their observations were in agreement with Wiltbank et al. (1962) who found that pre-partum plane of nutrition and thus post-partum live weight have a major influence on subsequent conception. During the post-partum period, Holness et al. (1978) hypothesized that cows that lost live weight were able to mobilize tissue reserves more rapidly and were thus better equipped to provide for normal metabolic function during this time of heavy demand for available nutrients.

Joubert (1954), using Shorthorn, and Zebu, Africander, Fresian and Jersey breeds with an equal number of animals assigned to two nutritional regimes (high-plane and low-plane), observed 1.42 and 1.00 services per conception, respectively. Degree of fatness was in this case shown to affect the ease with which an animal could conceive. This
is in agreement with Wiltbank et al. (1964), who observed a delay of the onset of oestrus during the post-partum period by feeding Hereford cows 150% of their estimated NRC energy requirements.

Butler (1980) assigned 40 primiparous Brangus heifers to two treatment groups to evaluate effects of body composition on reproductive performance. One group of heifers was fed 100% of NRC energy requirements to maintain normal (N) body condition; the other group was fed 85% of the NRC requirements to reduce body condition (BN). Forty-five percent of the BN group and 84% of the N group conceived within a 120-day breeding season.

Guimaraes (1981) used 71 mature Brangus cows (33 in late stage of lactation and 38 non-lactating) and randomly assigned them to two treatment groups, a high- and low-energy group and looked at the effect of lactation status and body composition on reproductive performance. The high-energy group was fed about 120% of the recommended NRC requirements for TDN while the low-energy group received about 50% of the NRC requirements. The treatment groups did not differ (P < 0.05) in conception rate, number of days from beginning of breeding season to mating and conception, percent of cows conceiving at first service, and number of services required per conception. Conception
rates were 68, 82, 68 and 68% for non-lactating/high energy (NL-H), lactating/high-energy (L-H), non-lactating/low energy (NL-LH), and lactating/low energy (L-L), respectively. Rapid body weight and condition losses during the experimental period did not influence fertility substantially, although a five-fold difference in body fat was observed (NL-H versus L-L). Feeding high energy levels significantly increased percent of carcass lipid, udder weights (P < .01) and fat thickness (P < .05). Adrenals, pituitary, reproductive tract weights and amount of follicular fluid were not affected by the high-energy diet. From these results it was concluded that feeding cows above recommended levels of energy during a restricted breeding season did not improve conception rates as compared to cows fed below recommended levels. From these studies it also appeared that changes in condition and liveweight had little effect on conception ability. Note, however, that the lack of effect of lactation in this study was most probably due to the fact that the cows were in late lactation.

Possible Mechanisms by Which Nutrition Affects Reproduction During the Postpartum Period

The precise mechanisms by which deficiencies and/or excesses in nutritional factors affect reproduction during the postpartum period in the bovine have not yet been clearly...
elucidated. However, various possible mechanisms have been postulated.

In a study by Oxenreider and Wagner (1971), the nutritional treatments used caused significant alterations in blood glucose levels that were related to a variation in ovarian activity in Holstein cows. McClure (1968) previously related hypoglycemia to infertility in dairy cows; however, it has not been shown where the hypoglycemia was exerting its influence. Oxenreider and Wagner (1971) hypothesized that since brain tissue function depends on glucose for its energy source, even mild hypoglycemia may depress hypothalamic function, thus resulting in loss of ovarian function. Radford et al. (1978), using crossbred beef cows, did not agree with these findings, as they observed similar blood glucose levels in both the lactating and non-lactating cows, in spite of the fact that the interval from parturition to first estrus was 76 days and 22 days, respectively. Topps (1977) postulated that protein deficiency effects on reproduction may be brought about by the animal's reduced feed intake. This is in agreement with an earlier study by Bond et al. (1962) where a low level of protein in the diet appeared to reduce the voluntary intake of feed and thus had the effect of lowering the total TDN intake. They went on to recommend consumption of a minimum amount
of protein to insure that cattle consume an adequate amount of total feed. Joubert (1954) felt that with the approach of favorable nutritional conditions, animals in low body condition first restored depleted body tissues before the sexual cycle could return to normal activity.

Ovarian Activity. Ovarian activity was greater in the full-fed cows than in the other groups. Full-fed cows had larger follicles and greater ovarian volume during the 5-week period before the onset of estrus. Large follicles were observed continuously on the ovaries of the cows that were fed higher than the recommended level of TDN at least 3 weeks prior to estrus. These large follicles may have conditioned the reproductive tract or ova in some manner and improved fertility (Wiltbank et al., 1962). Gombe and Hansel (1973) noted significant differences in CL removed on the 10th day of the third estrous cycle. The heifers fed low-energy rations had decidedly smaller corpora lutea than the controls, both in terms of wet weight and lyophilized weights. The ovulatory ovary in heifers on high-energy intake was 57% larger than in heifers on restricted energy intake (Spitzer et al., 1978). Unlike earlier findings by Wiltbank et al. (1962), they observed no significant difference among heifers on different diets for either the number of follicles or follicular or luteal volume. There
was, however, a trend for the corpus luteum of heifers fed the low-level diet to be smaller. In the non-ovulatory ovary, the level of nutrition did not appear to have any significance on ovarian size or follicular volume (Spitzer et al., 1978). Lishman et al. (1979) also showed that level of energy intake had no effect on size of the largest follicle or the proportion of beef cows that had a follicle 12 mm in diameter. Spitzer et al. (1978) showed that fertilization rate of ova recovered from heifers on two levels of energy did not appear to be affected by level of energy intake.

**Pituitary Gonadotropins.** Gombe and Hansel (1973) observed that significant changes in plasma LH occurred during the second and third cycles when heifers are maintained on low energy intakes. This is in contrast with the observation by Hill et al. (1970) who reported no change in basal plasma LH levels in beef heifers receiving 85% of NRC requirements for energy and protein during one estrous cycle. Progesterone secreted by the corpus luteum appears to exert a negative feedback on LH secretion in the cow (Hansel and Echternakamp, 1972). The elevated LH levels observed by Gombe and Hansel (1973) may have been caused by lowered progesterone levels. Beal et al. (1978) observed in beef heifers and beef cows spayed and intact that the
release of LH by GnRH or after a synchronized estrus was as high or higher in cows fed the low-energy ration as compared to cows fed the high-energy ration. They postulated that restricted energy intake presumably acted directly on the pituitary to increase the responsiveness to GnRH. In contrast to earlier findings, Spitzer et al. (1978) found that level of nutrition had no significant effect on systemic blood levels of LH over two and one-half estrous cycles. Concentration of LH remained at baseline levels of less than 1 ng/ml through the estrous cycle with an elevation of 4 to 7 ng/ml on the day of estrus. The lack of significant differences in blood levels of LH between heifers on the low levels of energy is in agreement with reports by Hill et al. (1970) and Dunn et al. (1974). However, the findings by Spitzer et al. (1978) are in conflict with those of Gombe and Hansel (1973) who reported progressive increases in mean systemic levels of LH during the first, second and third cycles for heifers restricted in energy intake.

Ovarian Steroids. In beef heifers, Gombe and Hansel (1973), during the first cycle postpartum, observed no significant differences in the levels of plasma progesterone between beef heifers on restricted energy intake and controls. However, during the second and third cycles the progesterone values of the underfed animals fell below the
control values by 1-2 ng/ml. Heifers on restricted energy diet had significantly smaller corpora lutea and lower total progesterone content than control heifers. Gombe and Hansel (1973) hypothesized that the first effects of restricted energy intake were at some step during steroidogenesis within the corpus luteum, causing the observed reduction in plasma progesterone.

Plasma progesterone immediately prepartum and postpartum was not significantly affected by 100-day prepartum energy restriction. Heifers fed high energy tended to have slightly higher progesterone levels on days 3 and 5 prepartum as well as the first 5 days postpartum, but the differences were inconsistent and non-significant (Corah et al., 1974).

Other Factors Affecting Postpartum Interval

Effect of Season

Seasonal variations in fertility occur in beef cattle. Gangwar et al. (1965) demonstrated that the length of the estrous cycle in heifers was longer under hot climatic conditions, and that duration and intensity of estrus was decreased. Prolonged exposure of winter-conditioned heifers to 90°F (32°C) and 60% relative humidity has been demonstrated to cause anestrous (Bond et al., 1960). Cows
with elevated body temperatures at breeding have also been shown to have lower conception rates than cows with normal body temperature (Fallon, 1962). In a study by Dunlap and Vicent (1971), none of 23 heifers exposed to 90°F (32°C) for 72 hours immediately after breeding maintained pregnancy whereas 12 of 25 control heifers did maintain pregnancy. These detrimental effects of thermal stress on reproduction have been well-documented in domestic animals (Hafez, 1965; Thatcher, 1974). Loyacano et al. (1974) concluded that the decrease in calving percent for spring-bred cows versus winter-bred beef cows was due to increased environmental temperature during the breeding season rather than differences in the nutritive intake of the cows. They had observed all the animals were in estrus at some time during the breeding season; therefore the differences in calving percent were due to differences in conception rate rather than failure to come into estrus.

Heat stress may affect reproductive efficiency via four possible mechanisms: (1) a direct effect on spermatozoa; (2) a direct effect on the embryo; (3) a disturbance in the endocrine balance; and (4) a disturbance in the uterine status.

Phillips and McKenzie (1934) described a thermo-regulatory mechanism in the scrotum that allowed the testes
to be maintained at a lower temperature than the rest of the body. Skinner and Louw (1966) reported reduced spermatogenesis and increase in percent of abnormal sperms in bulls subjected to 40°C environment for as short a period as 12 hours. All of the above studies point to the fact that high temperatures will adversely affect fertility in bulls.

A decrease in blood flow to the reproductive tract during the early stages of zygote development is one possible way by which thermal stress might affect fertility in the domestic animal (Dutt, 1963). Uterine blood flow (UBF) is responsible for transporting nutrients, oxygen and water to the developing embryo (Bazer et al., 1969) and dissipation of heat and removal of metabolic waste products. Oakes et al. (1976) detected a decrease in UBF (25 to 48%) in response to induced hyperthermia in pregnant ewes. Romance-Ponce (1978), using ovariectomized dairy cows, observed a greater increase in rate of uterine blood flow in cows subjected to shade than those in no shade. From their studies, it appeared that uterine blood flow was significantly affected by thermal stress when tested under practical farm conditions under which shade is known to improve conception rates.

Thermal stress might therefore affect conception rates by reducing blood flow and this would, in turn, affect
the fetus due to reduced nutrient inflow and reduced outflow of metabolic end-products and heat dissipation.

During the onset of pregnancy, changes occur in hormonal profiles which aid in its maintenance. A disturbance in this pattern may be the route by which thermal stress affects reproduction. When Holstein cows were put under stress, an elevation in circulating catecholamines was observed (Alvarez and Johnson, 1973), as well as transient elevations in progesterone and glucocorticoids (Stott and Robinson, 1970; Madan and Johnson, 1972). The transient elevation in glucocorticoids and progesterone were followed by an eventual decrease even below normal levels in these hormones (Madan and Johnson, 1972), if the heat stress was continued for a longer period of time. From the above studies, the possible route of hormonally induced early embryo mortality might be:

1. The increase in heat stress leads to an elevation in LH serum levels.
2. The LH then acts on the CL and increases progesterone output.
3. The ACTH levels from the pituitary are elevated too, and these not only increase the glucocorticoid output but also that of progesterone.
4. The increase in circulating levels of progesterone then act on the luteotrophic cells in the anterior
pituitary and lead to a negative inhibition on LH production and secretion.

5. The depressed levels of LH fail to maintain the CL, leading to a depression of circulating progesterone; eventually the CL will regress.

6. Due to depressed progesterone, there is a decrease in the secretion by the endometrial glands.

7. The lack or depression in the uterine milk denies blastocyst of its nourishment, leading eventually to embryo mortality.

Effects of Breed and Age

Both breed and age have been shown to be associated with postpartum anestrus in beef cows. Data on the occurrence of estrus or a palpable CL on a single day in 1164 cows that were 30 to 99 days postpartum in 24 herds in West Virginia were obtained. The pattern of return to a functional reproductive status after parturition was linear, and varied with breed, age, year, herd and the interaction of age and breed (Inskeep and Lishman, 1978). At two years of age, while rearing the first calf, Angus cows and crossbreds (crosses of Angus or Hereford with Charolais, Simmental, Brown Swiss or Holstein) were more likely to have a CL at a given stage postpartum than Herefords or various crosses of the British breeds. Mature cows, four
years of age and older, were reproductively active earlier than the two- and three-year-olds (Inskeep and Lishman, 1978). These findings are in agreement with earlier findings by Laster et al. (1973), who obtained conception rates of 25.9% in 2-year-old cows, 15.6% in 3-year-old cows, and 7.9% in cows 4 years old and older, using Hereford, Angus and Charolais crosses.
CHAPTER 3

MATERIALS AND METHODS

General

A study was carried out at the University of Arizona River Road Farm using lactating and nonlactating 4-year-old Brangus cows. The lactating cows averaged 70.6 days post-partum (range 24 to 114 days). The nonlactating cows had been dry for approximately 6 months. They were kept in individual pens 2.46 by 4.92 meters in size. Each pen had a mobile partition that helped to restrain the cow in a section of the pen at the time blood samples were collected. The cows were turned out twice a day with a bull that had a surgically deviated penis and observed for estrous activity. They were palpated per rectum and weighed every two weeks to monitor changes in ovarian activity and body weight.

The cows were on diets that met or exceeded the National Research Council (1976) (NRC) recommendations for digestible protein, calcium, phosphorus and other minerals. The calves were removed from the pens at feeding time and had access to creep feed consisting of 10.0% ground alfalfa hay, 39.2% cottonseed hulls, 42.0% steam-processed milo, 3.0% cottonseed meal, 1.0% urea, 0.55% dicalcium phosphate,
0.20% salt, 0.05% trace mineral premix, 4.0% molasses, and 0.33g vitamin-A-10-P per kg of diet.

Due to the number of cows involved, it was necessary to carry out the study in two separate blocks. At the beginning of each block the cows were injected with 25 mg PGF$_{2\alpha}$ and 10 days later a second PGF$_{2\alpha}$ injection was administered for estrous synchronization. Approximately 10 days after estrous synchronization 4 nonlactating and 4 lactating cows were ovariectomized from the first block. During the second block 3 nonlactating and 3 lactating cows were ovariectomized. This was done to remove ovarian steroid influence on pituitary secretion of LH.

**Experimental Design**

A 2-by-2 factorial design was used in the study with lactating status being one factor and ovariectomized or intact being the other factor. The number of cows in each group is shown in Table 1.

Two cows from the lactating and 2 from the nonlactating groups were not put on reduced energy intake. These animals were not originally part of the study; but as they were available, data on condition scores, weights and parameters at slaughter were collected from them and compared with the cows that were on reduced energy intake. The study was carried out in three periods.
Table 1. Number of animals per treatment group in the study.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Lactating)</th>
<th>Group 2 (Non-lactating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact Cows</td>
<td>6</td>
<td>5*</td>
</tr>
<tr>
<td>Ovariectomized Cows</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

* One cow died.
Period 1 (Period of Adaptation)

During this period the cows were conditioned to the experimental facilities and procedures, and were fed 4.21 kg of TDN per cow per day for the lactating cows and 3.16 kg for the nonlactating cows. These rations supplied 90% and 88% for lactating and nonlactating cows, respectively, of the NRC requirements for TDN. This period lasted 130 days before the start of the first block and 170 days before the second block was started. This period was longer than planned as modifications had to be made to the experimental facilities. See Table 2 for diet composition.

Period 2 (Period of First Energy Reduction)

Within a week after ovariectomy the cows were placed on a ration that furnished 55% and 52% of the NRC requirements for TDN for lactating and nonlactating cows, respectively. With these energy reductions it was hoped that the intake cows would eventually stop cycling. Forty days after the beginning of this period, cows in block 1 of the study were fitted with indwelling jugular cannulas for blood collection. The procedure for cannulation and blood sample collection is described in detail in Appendix . Six blood samples were collected at ten-minute intervals and then each cow was challenged with 200 mcg of GnRH intramuscularly.
Table 2. Diet composition (kg/cow/day).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Period</th>
<th>Cow's Average Wt. (kg)</th>
<th>60% Alfalfa Plus 40% Cottonseed Hulls</th>
<th>Grain</th>
<th>Supplementa</th>
<th>TDN Intake</th>
<th>% of NRC Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomized</td>
<td>1</td>
<td>410.8</td>
<td>7.27</td>
<td>1.36</td>
<td>0.45</td>
<td>4.21</td>
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<td>377.9</td>
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<td>55.45%</td>
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<td>372.1</td>
<td>2.27</td>
<td>0.00</td>
<td>0.45</td>
<td>1.16</td>
<td>26.36%</td>
</tr>
<tr>
<td>Lactating</td>
<td>1</td>
<td>407.1</td>
<td>7.27</td>
<td>1.36</td>
<td>0.45</td>
<td>4.21</td>
<td>89.57%</td>
</tr>
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<td>392.3</td>
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<td>0.45</td>
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<tr>
<td></td>
<td>3</td>
<td>371.9</td>
<td>2.27</td>
<td>0.00</td>
<td>0.45</td>
<td>1.16</td>
<td>26.36%</td>
</tr>
<tr>
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<td>0.45</td>
<td>0.88</td>
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<td>0.00</td>
<td>0.45</td>
<td>3.16</td>
<td>87.78%</td>
</tr>
<tr>
<td></td>
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<td>409.5</td>
<td>3.64</td>
<td>0.00</td>
<td>0.45</td>
<td>1.72</td>
<td>52.12%</td>
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<tr>
<td></td>
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<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
<td>26.67%</td>
</tr>
</tbody>
</table>

a Supplement composition: 48% grain, 25% cottonseed meal, 5% molasses, 12% Dical, 10% urea and 0.88 g Vitamin A-10-P per kg of feed.
Samples were then collected for 3 hours at 10-minute intervals. Thirty days after the beginning of this period, cows in block 2 of the study were treated similarly to the cows in block 1. The challenge with GnRH injections and the blood collections were repeated at approximately 30-day intervals.

Period 3 (Period of Second Energy Reduction)

The cows were fed a ration that supplied 26% or 27% of the NRC requirements for TDN, for the lactating and non-lactating cows, respectively. This change in ration was 100 days from the first reduction for cows in block 1. Seven days later the cows were cannulated and bled at 10-minute intervals for 1 hour, injected with GnRH and then samples were collected for 3 hours at 10-minute intervals. Cows in block 2 had this ration change 63 days after the first reduction in energy, and were challenged with GnRH 25 days later. See Figure 1 for the study scheme.

Estimates of Milk Production

Milk production in the lactating cows was assessed by the weigh-suckle-weigh method. The 8-hour interval of calf separation was used in the study as this was found to provide less measurable error, had higher correlation with calf average daily gain and produced less observable irritation and discomfort in cows (Williams et al., 1979).
Figure 1. Scheme for events during the study, starting with estrus synchronization.

**BLOCK 1**

<table>
<thead>
<tr>
<th>Number of Cows Treated</th>
<th>Number of Cows Ovariectomized</th>
<th>Number of Cows Injected with GNRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (7)</td>
<td>L (4)</td>
<td>LI LO (3)(4)</td>
</tr>
<tr>
<td>NL (7)</td>
<td>NL (4)</td>
<td>NLI NLO (2)(4)</td>
</tr>
</tbody>
</table>

1st PGF$_{2a}$ Injection | 2nd PGF$_{2a}$ Injection | Estrus | 1st Reduction in TDN | 2nd Reduction in TDN |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Days</td>
<td>3 Days</td>
<td>10-12 Days</td>
<td>7 Days</td>
<td>43-44 Days</td>
</tr>
</tbody>
</table>

**BLOCK 2**

<table>
<thead>
<tr>
<th>Number of Cows Treated</th>
<th>Number of Cows Ovariectomized</th>
<th>Number of Cows Injected with GNRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (7)</td>
<td>L (3)</td>
<td>LI LO (3)(3)</td>
</tr>
<tr>
<td>NL (7)</td>
<td>NL (3)</td>
<td>NLI NLO (3)(3)</td>
</tr>
</tbody>
</table>

L = lactating  
NL = nonlactating  
LI = lactating intact  
LO = lactating ovariectomized  
NLI = nonlactating intact  
NLO = nonlactating ovariectomized
Milk production estimates were obtained in eight cows, four of which were to be ovariectomized 20 days later. Thirty-three and fifty-four days after the first reduction in TDN, levels of milk production in the same cows were again evaluated.

Paired "t" tests were performed to determine if any significant differences existed in milk production between the initial and later measurements.

At the end of the study three cows from each group were selected randomly for slaughter. Before slaughtering the animals were fasted for 24 hours, weighed and their hip height measured. At the time of slaughter the weights of the following endocrine organs were obtained: pituitaries, thyroids, adrenals and ovaries. The other organs weighed were kidney, heart, udder and the reproductive tract. From the ovaries the weights and sizes of the corpus lutea were measured where applicable, the size and number of follicles were recorded and the volume of follicular fluid measured. Cold carcass weight, fat thickness and ribeye area at the 12th rib were obtained. Laboratory analyses were performed to determine percent lipid, moisture and protein in the samples taken between the 12th and 13th rib of each animal. The fat and lean of this cut were ground through a 1.3 cm chopper, mixed thoroughly and a random 0.5 kg samples was
placed in a Hobart Model 10814 Food Cutter until a homogenized mixture was obtained. Two samples approximately 200 g in weight were taken from this, placed in separate plastic containers and stored at -25°C until analyzed. The analytical procedures have been previously described by Guimaraes (1981). Percent rib lipid and protein, with ribeye area were used to estimate percent carcass lipid (Marchello et al., 1979).

Radioimmunoassay for LH

Iodination Procedure

The procedure described by Greenwood et al. (1963) for labelling the LH molecule (LER-1716-2) with chloramine T was used. The I^{125} for iodination was purchased from Amersham Searle (preparation IMS 30) and allowed to react with the LH molecule for 90 seconds. Gel chromatography was used for separation of the labelled hormone from free iodine. Before using the labelled hormone it was tested for immunoreactivity as described by Williams (1979).

Antibodies and LH Standards

Bovine LH (NIAMD bLH-4) utilized for the preparation of standards had a biological potency of one unit being equivalent to one mg of NIH-LH-S1 determined by the Ovarian Ascorbic Depletion Assay of Parlow. The LH 1st antibody
used in this double antibody radioimmunoassay was rabbit antiovine LH (RABLH) (B 225) prepared as described by Niswander et al. (1969). The RABLH was used at a dilution of 1:60,000 in 1:400 normal rabbit serum in EDTA-phosphate buffered saline (EDTA-PBS) and bound 50% of labelled hormone in the absence of unlabelled hormone.

To prepare the Sheep Anti-Rabbit gamma globulin used as the second antibody, 200 mg of rabbit gamma globulin was dissolved in 2 ml saline and 2 ml of complete Freund's Adjuvant, and then injected subcutaneously, intradermally and intramuscularly into a mature wether. This injection was repeated after 4 weeks. The first blood collection was obtained 20 days after the second injection and subsequent collections were at weekly intervals. The sheep serum was used in the assay at a dilution of 1:5 in phosphate-buffered saline pH 7.0.

Statistical Analysis

When a cow in a particular treatment group at a specific date was challenged with GnRH its response was characterized by the following equation:

\[ Y = \beta_0 + \sum_{k=1}^{5} \beta_k X^k + \epsilon \]

where
\[ \beta_0 = \text{intercept on } Y \]
\[ \beta_k = \text{regression coefficient for the } k\text{-th-degree polynomial} \]

Graphs were plotted using the response obtained from the appropriate degree polynomial for each of the four treatment groups on a specific date. Similarly, the responses obtained for cows for each treatment group for the three dates were plotted on a single figure. The highest-degree polynomial with a significant increase in the \( R^2 \) value was utilized in the graphs.

From the model for the response curve, an orthogonal polynomial regression was derived such that,

\[ Y_{ijk} = A_0 + A_1 P_1(x) + A_2 P_2(x) + \ldots + A_5 P_5(x) \]

where

\[ A_0 = \text{constant} \]
\[ A_1 = \text{regression coefficient} \]
\[ P_1(x) = \text{first-degree polynomial, etc.} \]

At each of the times the cows were challenged with GnRH the model used was:

\[ Y_{ijk} = \mu + O_i + L_j + O_{ij} + \epsilon \]

This two-way analysis of variance was performed on \( A_0 \) to \( A_5 \), \( H \) (peak LH release), \( T \) (time to peak LH) and \( A \) (total LH.
release measured by area under the curve, using a compensating polar planimeter measured in square inches) for the four treatment groups. One-way analyses of variance were also performed and the means of these parameters for each treatment group were compared using Duncan’s Multiple Range Test.

An analysis of variance was performed to evaluate the overall effect of lactation, ovariectomy, date and their interactions on pituitary response to GnRH stimulation, using \( A_0, A_1 \) to \( A_5, H, T \) and \( A \). The model used was,

\[
Y_{ijkl} = \mu + O_i + L_j + LH_{ij} + A_k(ij) + D_l + \]

\[
OD_{iil} + LD_{jl} + OLD_{ijl} +
\]

where

\( \mu = \) overall mean

\( O_i = \) ovariectomy treatment \( i = 1, 2 \)

\( L_j = \) lactating treatment \( j = 1, 2 \)

\( A_k(ij) = \) animal within subgroup

\[
k = 1, 2, 3 \ldots 6 \text{ for } i = 1, j = 1 \\
= 1, 2, 3 \ldots 7 \text{ for } i = 1, j = 2 \\
= 1, 2, 3 \ldots 5 \text{ for } i = 2, j = 1 \\
= 1, 2, 3 \ldots 7 \text{ for } i = 2, j = 2 \\
\]

\( D_l = \) date \( l = 1, 2, 3 \)

Data obtained at ovariectomy were analyzed using the Least Significant Difference test and the data obtained at slaughter were analyzed using Duncan's Multiple Range Test.
CHAPTER 4

RESULTS AND DISCUSSION

Ovarian data and cow weights at ovariectomy are presented in Table 3. The cow weights were not different. The number of cows that had ovulated in each group was not significantly different also, although ovulations occurred predominantly on the right ovary for the nonlactating cows, while half of the ovulations occurred on the right and the other half on the left for the lactating cows. The weight of the right ovary was significantly heavier (.033 versus .022 g/kg, P < .05) in the nonlactating cows than in the lactating cows. This might have been because there were twice as many ovulations on this ovary in nonlactating cows than there were in lactating cows.

Estimates of Milk Production

Milk production was assessed 20 days before ovariectomy and 33 and 54 days after the first reduction in TDN. An equal number of intact lactating cows had their milk production evaluated. The results are shown in Tables 4 and 5. The initial milk production in the intact cows was 4.0 kg, which is in agreement with earlier findings (Corah et al., 1975;
Table 3. Ovarian data and cow weights at ovariectomy.

<table>
<thead>
<tr>
<th></th>
<th>Lactating (range)</th>
<th>Nonlactating (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cow weight (kg)</td>
<td>425.3&lt;sup&gt;a&lt;/sup&gt; (386-379)</td>
<td>438.7&lt;sup&gt;a&lt;/sup&gt; (371-488)</td>
</tr>
<tr>
<td>Weight of right ovary (g)</td>
<td>9.4&lt;sup&gt;a&lt;/sup&gt; (2.7-12.6)</td>
<td>13.9&lt;sup&gt;b&lt;/sup&gt; (6.4-26.4)</td>
</tr>
<tr>
<td>% cows with CL on right ovary</td>
<td>42.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight of right ovary/wt of cow (g/kg)</td>
<td>0.022&lt;sup&gt;a&lt;/sup&gt; (0.006-.030)</td>
<td>0.033&lt;sup&gt;b&lt;/sup&gt; (.014-.071)</td>
</tr>
<tr>
<td>Size of CL (mm)</td>
<td>26.3&lt;sup&gt;a&lt;/sup&gt; (17.0-36.0)</td>
<td>17.8&lt;sup&gt;b&lt;/sup&gt; (16.0-21.0)</td>
</tr>
<tr>
<td>Weight of CL (g)</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt; (0.7-3.6)</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt; (1.5-3.0)</td>
</tr>
<tr>
<td>Weight of left ovary (g)</td>
<td>9.3 (3.2-16.0)</td>
<td>10.5 (6.2-14.1)</td>
</tr>
<tr>
<td>% cows with CL on left ovary</td>
<td>42.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Size of CL (mm)</td>
<td>20.0 (17.0-22.0)</td>
<td>-</td>
</tr>
<tr>
<td>Weight of CL (g)</td>
<td>2.7 (1.8-3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Weight of left ovary/wt of cow (g/kg)</td>
<td>0.022 (.008-.041)</td>
<td>0.024 (0.3-.035)</td>
</tr>
</tbody>
</table>

Cows ovariectomized 8 to 10 days after estrus.

<sup>a, b</sup> Values within each row not sharing a common superscript are significantly different, P < .05
Table 4. Milk production in intact Brangus cows.

<table>
<thead>
<tr>
<th>Initial Milk Production (kg) (A)</th>
<th>Milk Production 33 Days after 1st Reduction in TDN (B) (B-A)</th>
<th>Milk Production 54 Days Later (kg) (C)</th>
<th>d_2 (C-A)</th>
<th>Calf's ADG A&gt;B</th>
<th>Calf's ADG A&gt;C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3</td>
<td>3.6</td>
<td>3.2</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>4.1</td>
<td>1.4</td>
<td>5.9</td>
<td>1.8</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>3.6</td>
<td>2.3</td>
<td>2.3</td>
<td>-1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>5.9</td>
<td>3.2</td>
<td>5.0</td>
<td>-0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>4.0</strong></td>
<td><strong>4.1</strong></td>
<td><strong>+0.1</strong></td>
<td><strong>1.1</strong></td>
<td><strong>1.1</strong></td>
</tr>
</tbody>
</table>

\[ S^2_d = 0.9 \]

\[ S^2_d = 0.9 \]

\[ t = \frac{\bar{d}}{S_d} = 1.4 \]
Table 5. Milk production in ovariectomized Brangus cows.

<table>
<thead>
<tr>
<th>Initial Milk Production (kg) (A)</th>
<th>Milk Production 33 Days After 1st TDN Reduction (B)</th>
<th>Milk Production 54 Days After 1st TDN Reduction (C)</th>
<th>Calf's ADG A+B</th>
<th>Calf's ADG A+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>0.9</td>
<td>-2.3</td>
<td>1.8</td>
<td>-1.4</td>
</tr>
<tr>
<td>7.3</td>
<td>3.2</td>
<td>-4.1</td>
<td>3.2</td>
<td>-4.1</td>
</tr>
<tr>
<td>6.4</td>
<td>2.3</td>
<td>-4.1</td>
<td>4.8</td>
<td>-1.6</td>
</tr>
<tr>
<td>8.2</td>
<td>3.6</td>
<td>-4.6</td>
<td>5.5</td>
<td>-2.7</td>
</tr>
<tr>
<td>Mean 6.3</td>
<td>2.5</td>
<td>-3.8</td>
<td>3.8</td>
<td>-2.5</td>
</tr>
</tbody>
</table>

\[ S_d^2 = 0.2 \quad \text{and} \quad S_d = 0.4 \]
\[ t = \frac{-\bar{d}}{S_d} = -8.6^* \quad \text{and} \quad -4.0^* \]

* Significantly different from initial production (P < .025).
Guimaraes, 1981). Although production fell 53 days later, the difference was not significant. The mean production 54 days later was 4.1 kg. The lack of detectable differences in milk production was also reflected in the calves' average daily gains calculated for the same time periods.

The values for milk production in the cows that were ovariectomized are presented in Table 5. The average initial milk production was 6.3 kg, much higher than the values reported by Corah et al. (1975), Robison et al. (1978) and Guimaraes (1981). In a study using Brahman cows in a tropical beef production system in Venezuela, Neidhardt et al. (1979) estimated the average 24-hour milk yield to be 6.2 kg during 216 days. Thirty-three, as well as 54 days after 1st energy reduction, milk production was significantly lower (P < .05) than initial production. Ovarian steroids play a significant role in the development and growth of the mammary gland. However, once lactation has been initiated ovarian steroids have not been shown to affect milk production (Cowie and Butler, 1974). Concomitant with the depression in milk production, a decrease in average daily gain of the calves was observed for the period A to C, shown in Table 5.

### Condition Scores and Cow Weights

The weights and condition scores obtained during the various stages in the study are presented in Table 6. All
Table 6. Body weights and condition scores.

<table>
<thead>
<tr>
<th></th>
<th>Reduced TDN Intake</th>
<th>Normal TDN Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactating Cows</td>
<td>Non-Lactating</td>
</tr>
<tr>
<td></td>
<td>Intact (range)</td>
<td>Ovariectomized (range)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Condition Score (0 to 30 days after 1st TDN Reduc.)</td>
<td>9.5&lt;sup&gt;a&lt;/sup&gt; (9-11)</td>
<td>9.3&lt;sup&gt;a&lt;/sup&gt; (9-10)</td>
</tr>
<tr>
<td></td>
<td>11.0&lt;sup&gt;abc&lt;/sup&gt; (10-12)</td>
<td>10.3&lt;sup&gt;b&lt;/sup&gt; (9-12)</td>
</tr>
<tr>
<td>Condition Score (31 to 60 days after 1st TDN Reduc.)</td>
<td>8.8&lt;sup&gt;a&lt;/sup&gt; (8-10)</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt; (7-9)</td>
</tr>
<tr>
<td></td>
<td>10.8&lt;sup&gt;b&lt;/sup&gt; (10-12)</td>
<td>10.6&lt;sup&gt;b&lt;/sup&gt; (10-12)</td>
</tr>
<tr>
<td>Condition Score (61 to 90 days after 1st TDN Reduc.)</td>
<td>7.8&lt;sup&gt;a&lt;/sup&gt; (7-9)</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt; (6-9)</td>
</tr>
<tr>
<td></td>
<td>10.0&lt;sup&gt;b&lt;/sup&gt; (9-11)</td>
<td>10.0&lt;sup&gt;b&lt;/sup&gt; (9-12)</td>
</tr>
<tr>
<td>Condition Score (7 to 25 days after 2nd TDN Reduc.)</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt; (6-8)</td>
<td>6.7&lt;sup&gt;a&lt;/sup&gt; (6-8)</td>
</tr>
<tr>
<td></td>
<td>9.6&lt;sup&gt;b&lt;/sup&gt; (7-11)</td>
<td>9.6&lt;sup&gt;b&lt;/sup&gt; (7-12)</td>
</tr>
<tr>
<td>Cow Weight (0 to 30 days after 1st TDN Reduction)</td>
<td>407.1&lt;sup&gt;a&lt;/sup&gt; (350-445)</td>
<td>411.4&lt;sup&gt;a&lt;/sup&gt; (372-473)</td>
</tr>
<tr>
<td></td>
<td>446.1&lt;sup&gt;a&lt;/sup&gt; (399-477)</td>
<td>443.9&lt;sup&gt;a&lt;/sup&gt; (374-507)</td>
</tr>
<tr>
<td>Cow Weight (31 to 60 days after 1st TDN Reduction)</td>
<td>410.8&lt;sup&gt;a&lt;/sup&gt; (374-450)</td>
<td>419.9&lt;sup&gt;a&lt;/sup&gt; (389-481)</td>
</tr>
<tr>
<td></td>
<td>433.8&lt;sup&gt;a&lt;/sup&gt; (369.666)</td>
<td>436.6&lt;sup&gt;a&lt;/sup&gt; (374-503)</td>
</tr>
<tr>
<td>Cow Weight (61 to 90 days after 1st TDN Reduction)</td>
<td>392.3&lt;sup&gt;a&lt;/sup&gt; (334-442)</td>
<td>377.9&lt;sup&gt;a&lt;/sup&gt; (368-392)</td>
</tr>
<tr>
<td></td>
<td>409.5&lt;sup&gt;a&lt;/sup&gt; (375-436)</td>
<td>408.5&lt;sup&gt;a&lt;/sup&gt; (349-460)</td>
</tr>
<tr>
<td>Cow Weight (7 to 25 days after 2nd TDN Reduction)</td>
<td>371.9&lt;sup&gt;a&lt;/sup&gt; (321-392)</td>
<td>372.1&lt;sup&gt;a&lt;/sup&gt; (350-432)</td>
</tr>
<tr>
<td></td>
<td>402.2&lt;sup&gt;a&lt;/sup&gt; (361-433)</td>
<td>407.8&lt;sup&gt;a&lt;/sup&gt; (348-462)</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Values within each role not sharing a common superscript are significantly different, P < .05.
the cows on reduced energy intake were losing condition during the course of experimentation. The lactating cows, both ovariectomized and intact, lost more condition than their nonlactating counterparts. These differences were significant (P < .05) from 30 days after the first reduction in TDN until the end of the study. These results are in agreement with an earlier study by Guimaraes (1981) who also observed more severe decreases in condition in lactating cows than in nonlactating cows.

The cows that were on normal TDN intake maintained their condition during the study and scored higher (P < .05) than the cows that were on reduced TDN intake. All the cows on reduced TDN intake were losing weight during the course of the study. The total losses in weight were 35.2, 39.3, 44.0 and 36.1 kg for treatments 1, 2, 3 and 4, respectively. These losses amounted to 9%, 10%, 11% and 10% of the average initial body weight of the cows in treatments 1, 2, 3 and 4, respectively. There were no significant differences in the means of the weights at each of the designated periods in Table 6 among the 4 treatment groups on reduced energy intake. At all the periods the weights of treatments 5 and 6 (cows on normal TDN) were significantly higher (P < .05) than cows on reduced TDN (treatments 1, 2, 3 and 4).
Organ and gland weights are presented in Table 7. No differences were obtained in the weight of the organs among the cows on reduced energy intake. Although no statistical differences were detected in the weights of the reproductive tract, the intact cows (both lactating and nonlactating) tended to have higher weights than their ovariectomized counterparts. Ovarian hormone play a substantial role in regulating uterine metabolism. Growth of the uterus (both protein synthesis and cell division) is induced by estrogen. Estrogens also cause hyperemia. One of the functions of progesterone is to stimulate growth and development of the uterine endometrium (Ashdown and Hancock, 1974). The absence of uterine stimulation would account for the low values of reproductive tract weight observed in the current study. The udder weight was not different between the lactating and nonlactating cows. This is in agreement with Guimaraes (1981) and contradicts the findings of Butler (1980). The weight of the thyroid in intact nonlactating cows was significantly higher (P < .05) than the nonlactating ovariectomized cows. The significance of this particular finding is not apparent. Pituitary weights of lactating ovariectomized cows was higher than that of treatment 4. This difference was significant (2.3 versus 1.4, P < 0.5) between the lactating ovariectomized and nonlactating intact
Table 7. Cow weights, condition scores, organ, and gland weights at slaughter.

<table>
<thead>
<tr>
<th></th>
<th>Reduced TDN Intake</th>
<th>Normal TDN Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactating Cows</td>
<td>Non-Lactating</td>
</tr>
<tr>
<td></td>
<td>Intact (range)</td>
<td>Ovariectomized</td>
</tr>
<tr>
<td>Number of cows</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Condition Score</td>
<td>7.0 (6-8)</td>
<td>6.3 (6-7)</td>
</tr>
<tr>
<td>Cow Weight (kg)</td>
<td>355.1 (334-368)</td>
<td>364.3 (347-396)</td>
</tr>
<tr>
<td>Udder Weight (kg)</td>
<td>4.7 (3.1-6.1)</td>
<td>3.6 (2.9-4.9)</td>
</tr>
<tr>
<td>Heart Weight (kg)</td>
<td>2.1 (1.7-2.4)</td>
<td>1.9 (1.8-2.1)</td>
</tr>
<tr>
<td>Adrenal Weight (g)</td>
<td>15.3 (13.4-17.4)</td>
<td>20.6 (18-24)</td>
</tr>
<tr>
<td>Thyroid Weight (g)</td>
<td>166.3^a (135-183)</td>
<td>204.9^ab (175-223)</td>
</tr>
<tr>
<td>Reprod. Tract Weight</td>
<td>0.70 (.450-1.070)</td>
<td>0.26 (.21-.29)</td>
</tr>
<tr>
<td>Pituitary Weight (g)</td>
<td>1.7^ab (1.4-2.1)</td>
<td>2.3^b (1.7-2.9)</td>
</tr>
<tr>
<td></td>
<td>1.03 (0.08-1.20)</td>
<td>0.93 (0.60-1.30)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Kidney Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ovary Weight (g)</td>
<td>11.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Right Ovary Weight (g)</td>
<td>8.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Follicular Fluid Volume (ml)</td>
<td>4.07</td>
<td>6.77</td>
</tr>
</tbody>
</table>

Values within each row not sharing a common superscript are significantly different, $P < .05$.
cows. Removal of ovarian steroids which have an effect on pituitary function may explain the increase in weight obtained in the lactating ovariectomized cows. There were no statistical differences obtained in the weights of the ovaries and follicular fluid volume. This is consistent with earlier findings (Spitzer et al., 1978; Guimaraes, 1981).

Table 8 presents the organ and gland weights obtained at slaughter per unit of body weight. No significant differences were found in any of the parameters except for the adrenal glands. The ovariectomized lactating cows had significantly higher value (.057 g/kg versus .04 g/kg, P > .05) than the nonlactating ovariectomized cows. Though this value was not significantly higher than that from intact lactating cows and nonlactating intact cows (treatment 3), it still tended to be higher. This increase in the size of the adrenal could have been compensatory in nature, increasing steroid secretion to partially replace the ovarian steroids or in response to experimental stress. Adrenal steroids have been shown to depress milk production (Cowie and Butler, 1974). Although the levels of circulating adrenal steroids were not measured in this study, the larger adrenal in the lactating ovariectomized cows might have been a reflection of hyperplasia or hypertrophy and a
Table 8. Organ and gland weights per unit of body weight.

<table>
<thead>
<tr>
<th></th>
<th>Reduced TDN Intake</th>
<th>Normal TDN Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact Ovaryctomized</td>
<td>Lactating Non-Lactating</td>
</tr>
<tr>
<td>Number of Cows</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Udder Weight (kg/kg)</td>
<td>0.013 (0.009-.017)</td>
<td>0.013 (0.011-.017)</td>
</tr>
<tr>
<td>Heart Weight (kg/kg)</td>
<td>0.006 (.005-.007)</td>
<td>0.005 (.004-.005)</td>
</tr>
<tr>
<td>Adrenals Weight (g/kg)</td>
<td>0.043&lt;sup&gt;a&lt;/sup&gt;b (0.040-.047)</td>
<td>0.040&lt;sup&gt;a&lt;/sup&gt; (0.035-.045)</td>
</tr>
<tr>
<td>Thyroids Weight (g/kg)</td>
<td>0.470 (.371-.549)</td>
<td>0.447 (.265-.645)</td>
</tr>
<tr>
<td>Reprod. Tract Weight (kg/kg)</td>
<td>0.002 (.001-.003)</td>
<td>0.001 (.001-.001)</td>
</tr>
<tr>
<td>Pituitary Weight (g/kg)</td>
<td>0.005 (.004-.006)</td>
<td>0.004 (.004-.006)</td>
</tr>
<tr>
<td>Kidney Weight (kg/kg)</td>
<td>0.003 (.002-.003)</td>
<td>0.002 (.002-.003)</td>
</tr>
<tr>
<td>Right Ovary Weight (g/kg)</td>
<td>0.032 (.019-.048)</td>
<td>0.044 (.011-.025)</td>
</tr>
<tr>
<td>Left Ovary Weight (g/kg)</td>
<td>0.024 (.015-.035)</td>
<td>0.038 (.009-.035)</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Values within each row not sharing a common superscript are significantly different (P < .05).
resultant increase in hormone secretion. The results for milk production of ovariectomized cows substantiates this observation as the levels of milk production were significantly (P < .05) lower after ovariectomy.

**Body Composition Parameters at Slaughter**

Table 9 presents information obtained at slaughter. Carcass lipid percent was calculated using cold carcass weight, ribeye area, 12th rib fat thickness and plate lipid as described in a prediction equation by Marchello et al. (1979). There were no significant differences detected among the lactating and nonlactating cows on reduced energy for condition score, cow's height, hot carcass weight, cold carcass weight, ribeye area, fat thickness or percent kidney fat. For all these parameters the corresponding means from cows on normal TDN intake were much higher. Percent protein and percent moisture were significantly higher (P < .05) in nonlactating ovariectomized cows than in lactating intact and ovariectomized cows. Though treatments 1 and 2 were not significantly different from the nonlactating intact cows, their values for these parameters tended to be higher. The percent carcass lipid was significantly lower (P < .05) in lactating cows than in nonlactating cows on reduced TDN intake. Carcass lipid percent averaging 11.1, 12.0, 19.7 and 22.7 in lactating intact and ovariectomized and
Table 9. Body composition parameters at slaughter.a

<table>
<thead>
<tr>
<th></th>
<th>Reduced TDN Intake</th>
<th>Normal TDN Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactating Cows</td>
<td>Non-Lactating</td>
</tr>
<tr>
<td></td>
<td>Intact (range)</td>
<td>Ovariectomized (range)</td>
</tr>
<tr>
<td>Number of Cows</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cow's Height at Slaughter</td>
<td>51.0a (50-52)</td>
<td>55.6a (41-62)</td>
</tr>
<tr>
<td>Hot Carcass Weight (kg)</td>
<td>217.3a (206-225)</td>
<td>207.7a (188-234)</td>
</tr>
<tr>
<td>Cold Carcass Weight (kg)</td>
<td>212.4a (198-225)</td>
<td>201.0a (179-228)</td>
</tr>
<tr>
<td>Rib Eye Area (inches^2)</td>
<td>9.7a (7-11.7)</td>
<td>7.9a (6.8-9.0)</td>
</tr>
<tr>
<td>Fat Thickness (inches)</td>
<td>0.083a (0.50-10)</td>
<td>0.070a (.01-.1)</td>
</tr>
<tr>
<td>% Kidney Fat</td>
<td>1.8a (1.2-2.8)</td>
<td>2.6a (1.4-5.0)</td>
</tr>
<tr>
<td>% Protein</td>
<td>18.6a (17-20)</td>
<td>18.4a (17.1-20.6)</td>
</tr>
<tr>
<td>% Moisture</td>
<td>66.4a (63.3-70.9)</td>
<td>66.3a (64.1-70.3)</td>
</tr>
<tr>
<td>% Carcass Lipid</td>
<td>11.1a (6.6-14.1)</td>
<td>12.0a (7.2-14.6)</td>
</tr>
</tbody>
</table>

a Values within each row not sharing a common superscript are significantly different, P < .05.
nonlactating intact and ovariectomized cows, respectively. Amount of fat cover over the 12th rib was not significantly different among the cows on reduced TDN, though the lactating cows tended to have much lower values (.08 inches and .07 inches versus .18 inches and .14 inches). These results are in agreement with Butler (1980), Pendlum et al. (1977), and Guimaraes (1981), who obtained significantly higher percent carcass lipid and higher fat cover values in nonlactating versus lactating cows. The lactating cows evidently mobilized their fat reserves to supply the required nutrients for synthesis of milk.

The condition of the nonlactating cows on reduced energy was higher than that of their lactating counterparts. This was true as well for the percent carcass lipid. Lactating cows continued to cycle even though they had half the body fat observed in nonlactating cows at the end of the study. Butler (1980) showed that in Brangus cows at 60 days postpartum the minimum body lipid percent necessary for pregnancy to occur was approximately 13 percent. In the current study cows continued to exhibit normal estrous activity even though the percent carcass lipid was down to 11 percent.

It was concluded from the present study that the concept of a 13% minimum percent lipid requirement for
normal reproductive activity in early stages of lactation did not apply to cows during late lactation.

Serum Luteinizing Hormone Profiles

Table 10 presents the polynomial equations and $R^2$ values representing the "best fit" of the GnRH response curves for each treatment group and each period. The intact cows had a single peak in their response curves after GnRH injections and were fitting the quadratic and cubic equations best. The ovariectomized cows had a more complex pattern, having more than one peak in the response curve and fitted the quadratic and quintic equations.

Pituitary response to GnRH 30 to 40 days after the cows had been subjected to a reduction in TDN is plotted in Figure 2, and the statistical evaluation is presented in Table 11.

Prior to the GnRH injection the basal LH levels were not statistically different, although the ovariectomized cows tended to have higher values. After the GnRH injection the ovariectomized cows appeared to reach peak LH values at about the same time, whereas peak values were observed in the intact cows approximately 30 minutes later. Maximum LH values at this period tended to be higher in the ovariectomized than in the intact cows. Lactating cows tended to release more total LH than their nonlactating
Table 10. Best fitting LH response curves following GnRH stimulation.

<table>
<thead>
<tr>
<th></th>
<th>- 30-40 Days -</th>
<th>- 60-70 Days -</th>
<th>- 7-25 Days -</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 1st</td>
<td>After 1st</td>
<td>After 2nd</td>
<td>Reduction in TDN</td>
<td>Reduction in TDN</td>
<td>Reduction in TDN</td>
</tr>
<tr>
<td></td>
<td>Degree</td>
<td>Degree</td>
<td>Degree</td>
<td>Polynomial</td>
<td>Polynomial</td>
<td>Polynomial</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>R²</td>
<td>R²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact Cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Lactating</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0.47</td>
<td>0.51</td>
<td>0.57</td>
</tr>
<tr>
<td>b. Nonlactating</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0.79</td>
<td>0.72</td>
<td>0.57</td>
</tr>
<tr>
<td>Ovariectomized Cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Lactating</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0.70</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>b. Nonlactating</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>0.49</td>
<td>0.53</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Figure 2. Pituitary response in Brangus cows to GnRH stimulation 30-40 days after first reduction in TDN intake.
Table 11. Orthogonal polynomial regression coefficients, magnitude of LH peak, time to peak and area under LH release curve after GnRH injection 30-40 days after 1st reduction in TDN intake.\(^a\)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Orthogonal Polynomial Regression Coefficients</th>
<th>Basal LH (ng/ml)</th>
<th>Magnitude of LH Peak (ng/ml)</th>
<th>Time to LH Peak</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating Intact (6)</td>
<td>[A_0, 5.86; 1, 4.42; 2, -1.79; 3, -0.85; 4, 0.28; 5, -0.31]</td>
<td>1.2</td>
<td>10.80</td>
<td>142.0</td>
<td>4.13</td>
</tr>
<tr>
<td>Nonlactating Intact (5)</td>
<td>[A_0, 4.81; 1, 4.32; 2, -2.14; 3, -0.77; 4, 0.83; 5, -0.75]</td>
<td>0.7</td>
<td>7.03</td>
<td>115.0</td>
<td>1.75</td>
</tr>
<tr>
<td>Lactating Ovariec- tomized (7)</td>
<td>[A_0, 8.74; 1, 3.41; 2, -2.10; 3, -0.38; 4, 0.93; 5, -0.88]</td>
<td>1.4</td>
<td>17.11</td>
<td>92.86</td>
<td>3.83</td>
</tr>
<tr>
<td>Nonlactating Ovariec- tomized (7)</td>
<td>[A_0, 6.66; 1, 2.92; 2, -4.96; 3, -0.40; 4, 2.72; 5, -1.06]</td>
<td>1.3</td>
<td>9.11</td>
<td>120.0</td>
<td>2.68</td>
</tr>
</tbody>
</table>

\(^a\) No significant differences detected in any of the parameters, \(P > .05\).
counterparts. No differences of statistical significance were detected in any of the parameters measured.

The response to GnRH stimulation 60 to 70 days after a reduction in TDN, and the statistical evaluation of this response in each treatment group are presented in Figure 3 and Table 12, respectively.

No differences were detected in basal LH levels among the treatment groups before the GnRH injection. Ovariectomized cows, however, tended to have higher levels than the intact cows. After the GnRH injection the ovariectomized cows attained peak LH values earlier than the intact cows (130 minutes versus 67 minutes, P < .05) in lactating intact and ovariectomized (136 minutes versus 87 minutes, P < .05) in nonlactating intact and ovariectomized cows showed a different response pattern from intact cows as seen in the coefficients $A_1$, $A_4$ and $A_5$ (P < .05). The lactating cows at this period were still tending to release more total LH than their nonlactating counterparts.

The results from the GnRH challenge 7 to 25 days after the second reduction in TDN intake are presented in Figure 4 and Table 13. Prior to the GnRH injection no differences were detected in the basal LH levels among the four treatment groups, although the levels were higher in ovariectomized than in intact cows. After the GnRH injection the ovariectomized cows were responding in a similar
Figure 3. Pituitary response in Brangus cows to GnRH stimulation 60-70 days after first reduction in TDN intake.
Table 12. Orthogonal polynomial regression coefficients, LH peak, time to peak and area under LH release curve after GnRH injection 60-70 days after first reduction in TDN.

<table>
<thead>
<tr>
<th>Treatment Group (n)</th>
<th>Orthogonal Polynomial Regression Coefficients</th>
<th>Basal LH (ng/ml)</th>
<th>Magnitude of LH Peak</th>
<th>Time to LH Peak</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant $A_0$</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Lactating Intact (6)</td>
<td>5.77</td>
<td>4.55$^{ab}$</td>
<td>-2.21</td>
<td>-0.68</td>
<td>-.76$^a$</td>
</tr>
<tr>
<td>Nonlactating Intact (5)</td>
<td>6.33</td>
<td>5.61$^b$</td>
<td>-1.76</td>
<td>-0.63</td>
<td>-.03$^c$</td>
</tr>
<tr>
<td>Lactating Ovariectomized (6)</td>
<td>8.92</td>
<td>2.51$^a$</td>
<td>-2.73</td>
<td>-0.05</td>
<td>1.29$^b$</td>
</tr>
<tr>
<td>Nonlactating Ovariectomized (7)</td>
<td>8.62</td>
<td>2.34$^a$</td>
<td>-2.03</td>
<td>-0.03</td>
<td>1.02$^{ab}$</td>
</tr>
</tbody>
</table>

**Significant Main Effects**

| Lactating (L) | * |
| Ovariectomized (O) | * * | * * | * |

$^{ab}$ Values under each column not sharing a common superscript are significantly different (P < .05).
Figure 4. Pituitary response in Brangus cows to GnRH stimulation 7-25 days after second reduction in TDN intake.
Table 13. Orthogonal polynomial regression coefficients, LH peak, time to peak and area under LH release curve after GnRH injection 7-25 days after second reduction in TDN.

<table>
<thead>
<tr>
<th>Treatment Group (n)</th>
<th>Orthogonal Polynomial Regression Coefficients</th>
<th>Basal LH (ng/ml)</th>
<th>Magnitude of LH Peak (ng/ml)</th>
<th>Time to LH Peak</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant A_0 1 2 3 4 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating Intact (6) 7.47_{ab}</td>
<td>4.86 -2.17_{a} -0.83_{ab} 0.68_{ab} -0.31 1.2</td>
<td>14.30</td>
<td>118.33</td>
<td>3.98</td>
<td></td>
</tr>
<tr>
<td>Nonlactating Intact (5) 5.42_{a}</td>
<td>2.64 -1.30_{a} -1.10_{a} 0.13_{a} -0.26 0.8</td>
<td>11.13</td>
<td>126.00</td>
<td>3.24</td>
<td></td>
</tr>
<tr>
<td>Lactating Ovariectomized (7) 7.65_{ab}</td>
<td>4.53 -2.66_{ab} -0.10_{ab} 1.39_{bc} -0.94 1.5</td>
<td>13.20</td>
<td>78.57</td>
<td>2.92</td>
<td></td>
</tr>
<tr>
<td>Nonlactating Ovariectomized (7) 10.29_{b}</td>
<td>2.59 -3.69_{b} 0.13_{b} 1.97_{c} -0.94 1.8</td>
<td>40.44</td>
<td>74.29</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>Significant Main Effect</td>
<td>* * *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovariectomized (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

_{abc} Values under each column not sharing a common superscript are significantly different, P < .05.
manner, as shown by the shapes of their curves. The lactating and nonlactating intact cows also had similarly shaped response curves. The ovariectomized cows continued to peak earlier than the intact cows. These differences were shown to be significant. The results of the statistical analysis are presented in Table 13. Differences were noted in $A_0$, $A_2$, $A_3$ and $A_4$ in ovariectomized versus intact cows ($P < .05$). Total LH release in lactating cows continued to be higher than in their nonlactating counterparts.

Ovarian steroids have been shown to play an important role in the regulation of LH secretion, estrogens being stimulatory (Reeves et al., 1971; Beck and Convey, 1977) and progesterone inhibitory in nature (Tillson et al., 1970; Christensen et al., 1974). During this study the intact cows, both lactating and nonlactating, were in various stages of the estrous cycle when they were challenged with GnRH (Table 14). Progesterone levels are generally high between days 6 and 15 of the estrous cycle, resulting in a maximum inhibitory influence on LH release.

In the current study, stage of the cycle did not appear to markedly influence the time required to attain peak LH values in both lactating and nonlactating intact cows. Total serum LH released did not appear to be influenced by the stage of the estrous cycle as well, although
Table 14. Effect of stage of estrous cycle on pituitary response to GnRH stimulation.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Stage of Cycle</th>
<th>Lactating Intact Cows</th>
<th>Nonlactating Intact Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time to Peak After GnRH (minutes)</td>
<td>Total LH Released Measured by Area Under Curve (square inches)</td>
</tr>
<tr>
<td>a. 1-5 days (n = 3)</td>
<td>133</td>
<td>3.93</td>
</tr>
<tr>
<td>b. 6-15 days (n = 12)</td>
<td>121</td>
<td>3.93</td>
</tr>
<tr>
<td>c. 16-21 days (n = 2)</td>
<td>135</td>
<td>4.12</td>
</tr>
<tr>
<td>a. 1-5 days (n = 3)</td>
<td>137</td>
<td>2.14</td>
</tr>
<tr>
<td>b. 6-15 days (n = 6)</td>
<td>145</td>
<td>4.30</td>
</tr>
<tr>
<td>c. 16-21 days (n = 3)</td>
<td>130</td>
<td>0.96</td>
</tr>
</tbody>
</table>

\textsuperscript{a} No differences of statistical significant were detected in time to peak and total LH.
nonlactating cows that were in the 6-15 day stage of the 
estrous cycle when challenged with GnRH released the least 
amount of LH. This difference was not of statistical 
significance. These findings are in agreement with those 
of Webb et al. (1977) and Schams et al. (1974). From our 
results, stage of the cycle did not alter release of LH in 
the presence of adequate exogeneous GnRH. The presence or 
absence of ovarian steroids did influence the rapidity with 
which GnRH could induce release of LH.

The pituitary responses for each treatment group 
during the three dates the cows were challenged with GnRH 
are presented in Figures 5, 6, 7 and 8. In the lactating 
intact cows the peak LH levels tended to increase over the 
period of experimentation (Figure 5). The peak LH values 
in lactating ovariectomized cows were similar at all periods 
of TDN reduction, and so were the times to peak LH (Figure 
6). No consistent trends were observed in the nonlactating 
cows during the course of the study (Figure 7). Time to LH 
peak and its magnitude stayed about the same in the nonlac­
tating ovariectomized cows (Figure 8).

Table 15 presents the mean squares of the various 
LH parameters measured during the study, and how they were 
affected by lactation, ovariectomy, date and their inter-
actions. Ovariectomized cows responded significantly earlier
Figure 5. Pituitary response in lactating intact Brangus cows after being on reduced TDN intake.
Figure 6. Pituitary response in lactating ovariectomized Brangus cows, to GnRH stimulation, after being on reduced TDN intake.
Figure 7. Pituitary response in nonlactating intact Brangus cows, to GnRH stimulation, after being on reduced TDN intake.
Figure 8. Pituitary response in nonlactating ovariectomized Brangus cows, to GnRH stimulation, after being on reduced TDN intake.
Table 15. Pituitary response to GnRH injection (stimulation) during reduced energy intake.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>A0</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>H</th>
<th>T</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactation (L)</td>
<td>1</td>
<td>0.99</td>
<td>0.58</td>
<td>3.3</td>
<td>0.19</td>
<td>0.73</td>
<td>0.03</td>
<td>0.03</td>
<td>928.5</td>
<td>22.2</td>
</tr>
<tr>
<td>Ovariectomy (O)</td>
<td>1</td>
<td>118.1*</td>
<td>66.4**</td>
<td>18.5</td>
<td>8.8*</td>
<td>18.7*</td>
<td>4.6*</td>
<td>2.7</td>
<td>38601.4**</td>
<td>3.5</td>
</tr>
<tr>
<td>L by O</td>
<td>1</td>
<td>12.3</td>
<td>1.0</td>
<td>4.915</td>
<td>0.24</td>
<td>3.4</td>
<td>0.06</td>
<td>0.23</td>
<td>360.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Error (1)</td>
<td>21</td>
<td>16.5</td>
<td>4.5</td>
<td>5.5</td>
<td>12.1</td>
<td>2.0</td>
<td>0.97</td>
<td>1.3</td>
<td>2258.3</td>
<td>12.4</td>
</tr>
<tr>
<td>Date (D)</td>
<td>2</td>
<td>5.3</td>
<td>0.34</td>
<td>1.0</td>
<td>0.80</td>
<td>0.63</td>
<td>0.04</td>
<td>0.89</td>
<td>2259.2</td>
<td>6.5</td>
</tr>
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<td>L by D</td>
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<td>3.7*</td>
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<td>1.5</td>
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<tr>
<td>D by O</td>
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<td>0.43</td>
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* Significance (P < .05)
**Significance (P < .01)

PO = Constant
P1 = 1st Degree Polynomial Regression Coefficient
P2 = 2nd Degree Polynomial Regression Coefficient
P3 = 3rd Degree Polynomial Regression Coefficient
P4 = 4th Degree Polynomial Regression Coefficient
P5 = 5th Degree Polynomial Regression Coefficient
H = Magnitude of LH peak
T = Time to LH peak
A = Area under LH release curve
(P < .05) than the intact cows. Time on reduced TDN intake had no effect on pituitary response to GnRH.

**General Discussion**

Cows were in late lactation during the current study. Other research dealing with effects of nutrition and LH secretion has been done almost entirely with cows during early lactation. Serum LH levels in suckled cows during early stages postpartum have been reported to be lower than in nonsuckled cows (Short et al., 1972; Carruthers et al., 1978). The lactating cows in the current study tended to release more total serum LH than the nonlactating; therefore, lactation did not suppress LH release but appeared to enhance it during late lactation. The weights of the pituitaries obtained at the time of slaughter were higher in the lactating than in the nonlactating cows as well.

Time on reduced energy intake did not seem to change the response pattern to GnRH challenge. In cows of a particular genetic background, there is a body weight below which one can expect lowered fertility (Lamond, 1970). Topps (1977) reported that cows may lose from 20 to 30% of their mature weight before cyclic ovarian activity ceases. In the present study the nonlactating cows had the highest percent loss in body weight, 11%.
The lactating cows' low body condition and their body composition as measured by percent carcass lipid was considerably lower (P < .05) than the nonlactating cows. Luteinizing hormone plays a significant role in reproduction, and it was assumed that adverse changes in weight and condition which affect reproduction might be reflected in changes in circulating serum levels. In the current study there were no significant effects of time on reduced energy on pituitary responsiveness to GnRH stimulation, as measured by serum LH. These results are in agreement with those of Spitzer et al. (1978), who found that level of nutrition had no significant effect on systemic blood levels of LH over two and one-half estrous cycles. Other workers have reported similar findings (Hill et al., 1970; Dunn et al., 1974; Beal et al., 1978).

In order to be able to induce enough loss in body weight, it might be necessary to use more severe dietary energy restrictions. The cows used in this study appeared to be highly tolerant to energy deprivation and did not show expected losses in body weight. To fully document the effects of energy deprivation on hormonal profiles it may be necessary to include various breeds of beef cattle in a single study.
CHAPTER 5
SUMMARY AND CONCLUSIONS

Four-year-old Brangus cows, lactating and nonlactating, were used to study the effect of reduced TDN intake on pituitary response to gonadotropin releasing hormone (GnRH) stimulation. Milk production was also evaluated before and after ovariectomy. Seven cows from each group were ovariectomized and the rest left intact. The study was carried out in two blocks: 4 lactating ovariectomized (LO), 3 lactating intact (LI), 4 nonlactating ovariectomized (NLO), and 3 nonlactating intact (NLI) cows were used in the first block, and the rest in the second block. The cows in the first block were put on a ration that supplied 55% of the NRC requirements for TDN for the lactating cows (LO and LI) and 52% of the NRC requirements for TDN for the nonlactating cows (NLI and NLO) 130 days after the study was initiated. The cows in the second block were put on this ration 40 days later. The second ration change was 100 days and 63 days for cows in blocks 1 and 2, respectively, after the first ration change, to supply 26% of the requirements for TDN in all the treatment groups. Forty days after the first ration change, the cows in block 1 were challenged
with GnRH, while this was done 30 days later for the cows in block 2. This was repeated every 30 days and up to the point where the study was terminated. At the end of the study, cows were slaughtered to determine body composition and comparison of the organs and glands among the four groups was conducted.

The mean milk production before ovariectomy was significantly higher (P < .025) than the production 33 and 54 days after the first energy reduction (6.3 versus 3.8 and 2.5). No significant differences were found in initial milk production after 53 and 74 days later for intact cows.

At ovariectomy the nonlactating cows had body condition significantly higher (10.3 versus 9.2, P < .05) than the lactating cows, but their weights did not differ.

The mean of the weights of the cows among the 4 treatment groups were not different at each of the times the cows were weighed. Cows in all the treatment groups were losing weight and by the end of the study, lactating intact, and ovariectomized, nonlactating intact and ovariectomized, had lost 9, 10, 11 and 10% of their initial body weight. The nonlactating cows' condition scored significantly higher (P < .05) 31 days after the ovariectomies were performed till the end of the study.

The weights of the heart, adrenal, kidney, udder, and reproductive tracts were not different amount the
treatment groups at slaughter. The weight of the pituitary in lactating ovariectomized cows was significantly greater (P < .05) than that of the nonlactating intact cows. There were no differences in the weight of the left and right ovary in lactating and nonlactating intact cows. Follicular fluid volume did not differ in the intact cows. When the organs were expressed as a function of cow's weight, adrenals were significantly different only between lactating ovariectomized and nonlactating ovariectomized cows (0.057 g/kg versus 0.040, P < .05).

Lactating cows had significantly lower (P < .05) carcass lipid than nonlactating cows. Although the differences in fat cover over the twelfth ribs were not significant, lactating cows both intact and ovariectomized had much lower values than their nonlactating counterparts. Percent protein and moisture were significantly higher (P < .05) in lactating than in nonlactating cows.

When the cows were first challenged with GnRH, no significant differences were noted in the values of the constant AO, the orthogonal polynomial regression coefficients 1 through 5, the magnitude of the LH peak, time to peak LH release after GnRH stimulation and total area under LH release curve. The nonlactating cows at the time they were challenged with GnRH tended to release less total LH than
their lactating counterparts. Thirty and sixty days later, when the cows were challenged with GnRH the time to attainment of LH peak was significantly shorter (P < .05) in ovariectomized cows, lactating and nonlactating than their intact counterparts. The lactating cows (LI and LO) released more LH at both times than the corresponding nonlactating treatment group (NLI and NLO). This is consistent with the sizes of the pituitaries obtained at the times of slaughter.

An analysis of variance was performed to examine the effect of time on reduced energy on the constant A0, the orthogonal polynomial regression coefficients 1 through 5 (A1, A2, A3, A4 and A5), magnitude of LH peak, time to LH peak and area under LH release curve. Length of time on reduced TDN did not appear to affect any of these parameters. Ovariectomy was the only factor that significantly influenced time to attainment of LH peak.

From these data, though the numbers were small, it showed ovariectomized cows reduced milk production as early as thirty days after ovariectomy.

The length of time the cows had been on reduced TDN intake in this study did not affect magnitude of LH release in either lactating or nonlactating cows. The loss in body weight during the duration of this study was not large enough
to induce differences in response to GnRH stimulation at the beginning of the study and at the end.

Cows in late lactation, either ovariectomized or intact, tended to release more LH in response to GnRH stimulation than the corresponding nonlactating cows, although their carcass lipid percent was significantly lower (11.4 and 12.0 versus 19.7 and 22.7) in LI, LO, NLI and NLO, respectively.
APPENDIX A
BLOOD COLLECTION SCHEDULE

Three syringes were used for blood collection. With the first syringe, 10 cc of blood was drawn and discarded. A clean and sterile 10 cc syringe was then used to draw out 10 cc of blood which was transferred to a 16 x 100 mm glass test tube containing 1 ml of heparin (14.4 mg/ml of 0.9% saline) which was then mixed immediately. Ten ml of heparinized saline was flushed back into the cannula. Precautions were observed at all times so as to maintain the column of fluid in the cannula.

At the beginning of the experiment, all the cows were cannulated and bled via the hugular cannulas every 10 minutes for 1 hour, then cows were injected with GnRH and bled at 10 minute intervals for 3 hours. Serum was separated from the samples and frozen for radioimmunoassay (RIA) later, for LH levels.
OVARIECTOMY PROCEDURE

The operation was done with the cow standing up in a chute. The area of the triangle made by the loin ribs and hind leg was shaved to the back near the hip joint. This area was scrubbed with betadine and rinsed with clean water. This cleaning procedure was repeated three times. Betadine solution in a wash bottle was applied to the area along which the incision is to be made. About 10 ml of lidocaine was injected subcutaneously and intradermally along the incision line (6 inches long). Sterility was maintained by scrubbing hands with betadine scrub and using sterile gloves and surgical equipment that was also aseptic. An incision wide enough for the entry of a clenched fist was made. The hide was incised and followed by a smaller incision through the muscular layers. The latter can be widened by blunt dissection to allow an arm to be introduced into the peritoneum. The hand was inserted through the incision, the ovaries were located and then, using an ecraseur (instrument for spaying), the ovary was cut free and removed by hand from the peritoneum. The point of ovarian detachment was clamped to prevent excessive hemorrhage. Each ovary was removed individually, one at a time. The abdominal wall was then sutured using an uninterrupted continuous suture. The
animals were post-surgically observed for any discharge from the operative site. Sutures were removed 10 days later.
CANNULATION AND BLOOD COLLECTION

One hour before ovariectomy, the cows were cannulated using the procedure described below.

Procedure for Jugular Cannulation

The animal was placed in a chute, haltered and the head pulled to one side and tied securely to expose the jugular area. The hair was then clipped around the proposed site of cannulation, thoroughly cleansed with betadine scrub and then rinsed with water. This was repeated three times. Betadine solution from a wash bottle was applied to the cannulation site. Using a 20 gauge needle, 5 ml of a local anesthetic lidocaine hydrochloride was injected intradermally and subcutaneously around the surgical site located over the jugular. A scalpel with a number 20 sized blade was used to make a 10 cc incision over and parallel to the vein, followed by insertion of a 10 gauge trocar through the incision and into the jugular. A 36 inch length of sterile, Silastic medical grade tubing, (.062 x .125 Dow Corning, Midland, Michigan) was then threaded through the needle and into the vein, taking care to direct it downward toward the vena cava and leaving 18 inches of tubing exposed. This tubing was filled with heparinized saline and before removing the trocar, an easy flow of blood during withdrawal
through the tubing was insured. To maintain the heparin-filled column, a sterile adapter and plug are placed onto the end of the cannula to seal the tubing. The incision through which the tubing was placed was closed with one suture (Vetafil, size 00) and the exposed portion of the cannula sutured at a $90^\circ$ angle to the jugular depression with the end placed on the back of the cow between the scapulae. Adhesive glue (kamar adhesive) on the site where the end of the cannula was to be placed was applied, and then a piece of medical adhesive was then placed over the first layer.
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