EFFECTS OF CALCIUM SALTS OF LONG-CHAIN FATTY ACIDS, GROWTH FACTORS, AND ENERGY BALANCE ON OVARIAN FOLLICULAR DYNAMICS IN POSTPARTUM DAIRY COWS

By

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Dedicated to
Jacqueline S. Jamieson
and
Allison Helen Jamieson-Lucy
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EFFECTS OF CALCIUM SALTS OF LONG-CHAIN FATTY ACIDS, GROWTH FACTORS, AND ENERGY BALANCE ON OVARIAN FOLLICULAR DYNAMICS IN POSTPARTUM DAIRY COWS

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Copious milk production in dairy cattle during the early postpartum period requires mobilization of energy and nutrients from body tissue. Therefore, dairy cows are in negative energy balance (EB) in early lactation. Reproductive events, including estrus, ovulation, corpus luteum formation, and pregnancy, may be dependent on depth of negative EB. Physiological interactions of EB with reproductive organs may involve endocrine and metabolic events elicited by changes in EB. Feeding calcium salts of long-chain fatty acids (CaLCFA) to increase EB may improve reproductive function. The objective of experiments presented in this dissertation was to clarify relationships between CaLCFA, EB, growth factors, and ovarian function in postpartum dairy cows.

Initially, production characteristics were studied with respect to reproductive function. Cows having first ovulation vi
before 21 days postpartum consumed the greatest amount of dry matter and produced the greatest amount of milk compared with later ovulating cows. Primiparous cows were in greater negative EB and had delayed interval to first ovulation when compared to multiparous cows.

Subsequently, effects of EB and CaLCFA on ovarian follicular dynamics were investigated. Postpartum cows in negative EB had more small ovarian follicles and fewer large ovarian follicles compared to cows in positive EB. This suggested delayed interval to first ovulation in cows in negative EB. In addition, growth rate of the preovulatory follicles and the concentration of insulin-like growth factor-I in plasma were reduced by feeding cows to a negative EB. Feeding CaLCFA to postpartum cows did not increase the concentration of 15-keto-13,14-dihydro-prostaglandin F₂a in the plasma (demonstrated for lipid-infused heifers) but increased the incidence of large ovarian follicles in some but not all diets which included CaLCFA. Finally, treating cows with recombinant bovine somatotropin (bST; growth hormone) increased the number of 6 to 9 mm ovarian follicles. Other effects of bST on the ovary depended on diet, EB, and lactational status (lactating or nonlactating).

These data indicate that EB, dietary composition (CaLCFA), and growth factors influence ovarian follicular dynamics. These effects interact to determine patterns of ovarian follicular growth and development in postpartum cows.
CHAPTER 1
LITERATURE REVIEW

Introduction

In order to understand the interaction between nutrition and reproduction in the postpartum cow, it is necessary to explore numerous aspects of reproductive physiology, intermediary metabolism (nutrient mobilization) and animal nutrition. Morphological and endocrine events which occur prior to parturition in the cow dictate the fate of reproductive organ performance during the early postpartum period. The subsequent demands of lactation place further limitations on the function and development of ovary. The best method to manage this interaction between reproduction and lactation may be through unique dietary formulations targeted for the benefit of reproductive performance. This represents a challenging new area of postpartum research.

The focus of this dissertation is the postpartum cow. Therefore, this review will start with endocrine events during late pregnancy and parturition. The author will then review nutrient mobilization during early lactation and the recovery of reproductive organs from the previous gravid state. These events lead to the initiation of estrous cycles and the successful establishment of another pregnancy. Finally, the author will review the various known factors which affect this
return to normal fertility and those methods which are currently available to improve postpartum reproduction.

Endocrinology of Late Pregnancy

Progesterone and Estrogen

The endocrinology of late pregnancy of the cow centers, in part, around two steroid hormones--progesterone and estrogen. Concentrations of progesterone in the blood increase in pregnant cattle (compared with nonpregnant cattle) as early as day 12 of the estrous cycle and increase up to 40 days after insemination (Randel and Erb, 1971; Henricks et al., 1972). After this time, progesterone remains constant until the parturition-associated decline in progesterone in the blood (Randel and Erb, 1971; Henricks et al., 1972; Hoffman et al., 1973; Smith et al., 1973). The primary source of progesterone throughout pregnancy in the cow is the corpus luteum (CL; McDonald et al., 1954; Thornburn et al., 1977) and removal of the CL before 200 days of pregnancy will cause abortion in cattle (Estergreen et al., 1967). However, pregnancy is maintained in cattle if the CL is removed after 200 days of pregnancy (although gestation is slightly shortened; Estergreen et al., 1967). This finding suggests that a source of progesterone exists outside of the ovary in the late pregnant cow. The extraovarian source of progesterone appears to be the adrenal gland. This belief is based on evidence from Balfour et al. (1957) who found high concentrations of progesterone in the adrenal vein compared
with the systemic blood (suggesting a net production of progesterone in the adrenal). Furthermore, ovariectomy decreased circulating progesterone in pregnant cows, but ovariectomy and adrenalectomy completely eliminated detectable levels of progesterone in the blood of cows (Wendorf, 1983). Unlike other species (i.e., sheep), the placenta does not produce appreciable quantities of progesterone in the cow (Comline et al., 1974; Wagner et al., 1974; Ferrell et al., 1983). In the sheep, the CL of pregnancy yields to the placenta as the primary source of progesterone after about 50 days of gestation and maintains the pregnancy until parturition (Denamar and Martinet, 1955). In contrast, the cow placenta metabolizes progesterone during late pregnancy (Comline et al., 1974, Wagner et al., 1974; Ferrill et al., 1983). Progesterone appears to be the only hormonal secretion from the CL which is required for pregnancy since removal of the CL and replacement with exogenous progesterone will maintain pregnancy (Tanabe et al., 1968; Chew et al., 1979).

Estrone sulfate is the primary estrogen of pregnancy in the cow with lesser quantities of estrone and estradiol being present in the circulation (Hoffman et al., 1976). Estrone sulfate progressively increases during the first two-thirds of pregnancy (Eley et al., 1979; Robertson and King, 1979) and concentrations of estrone sulfate, estrone, and estradiol reach extremely high levels near term in cattle (Robertson and King, 1979) before declining at parturition (Robinson et al.,
1971). An unlikely source of these estrogens is the ovary since the activity of follicles on the ovary during late pregnancy is drastically reduced (Casida et al., 1943). The placenta of the cow has an active aromatase system suggesting that the placenta may be the primary source of these circulating estrogens in the cow (Ainsworth and Ryan, 1966). Indeed, studies in the cow show a net production of estrogens by the gravid uterus (Comline et al., 1974; Peterson et al., 1975; Hoffman et al., 1976) and in vitro perfusions suggests that the placental cotyledon is the primary source of these estrogens (Hoffman et al., 1979b).

Progesterone and estrogen secretion during late pregnancy is determined not only by the mother but also by the sire of the fetus. Guilbault et al. (1985b) demonstrated that the sire of the fetus could directly affect the concentration of progesterone and estrogens in plasma. Pregnancies sired by Angus males in Holstein heifers result in lower progesterone and lower estrogens compared with those sired by either Holstein or Brahman males. In addition, both estrogens and progesterone in plasma were positively correlated with calf birth weight. Therefore, sire of the fetus can influence not only the placental endocrinology (estrogen secretion; as might be expected given the genetic contribution of the male to the placenta), but also maternal endocrinology (progesterone secretion) suggesting a possible role for the fetus or placenta in controlling the maternal CL or adrenal.
The hypothalamus and the pituitary of the cow are controlled tightly by the concentration of estrogen and progesterone in the blood. Therefore, it is not surprising that the elevated concentration of progesterone and estrogens during late pregnancy in ruminants can affect dramatically how the pituitary functions. Luteinizing hormone (LH) from the pituitary is responsible for the maintenance of the CL of pregnancy (Heap et al., 1973), and the average concentration of LH remains low during late gestation in the cow (between 1.0 and 1.5 ng/ml; Hoffman et al., 1973; Eley et al., 1981). High concentrations of progesterone and estrogen result in decreased synthesis and secretion of LH in both ewes and cows during late pregnancy (Chamley et al., 1974; Schallenberger et al., 1978; Azzazi et al., 1983). The decrease in synthesis of LH leads to reduced quantities of LH in the pituitary in late pregnant cows (Nalbandov and Casida, 1940). The content of LH in the pituitary must increase in postpartum cows prior to the initiation of regular estrous cycles. This requirement represents a short-lived block to ovarian function since normal pituitary concentration of LH is restored within about 10 days (Fernandes et al., 1978). Apparently, high concentrations of progesterone cause a decrease in LH pulse frequency (Goodman and Karsch, 1980), probably due to a decrease in the amount of gonadotropin-releasing hormone (GnRH) released from the hypothalamus (Clarke and Cummins, 1982). Less LH synthesis may be a function of reduced GnRH
secretion since GnRH is required for the synthesis of LH in the pituitary (Fraser et al., 1975). Moss et al. (1981) found that the concentration of LH in the pituitary was decreased in ovariectomized ewes treated with estrogen alone or estrogen and progesterone (but not progesterone). This occurred in these animals despite the fact that the concentration of GnRH in the hypothalamus was unchanged and the number of GnRH receptors in the pituitary remained constant. Therefore, estrogen appears to either directly inhibit LH synthesis in the pituitary or reduce the amount of GnRH secretion (Nett, 1987). There may exist a molecular interaction between the estrogen receptor and the LH gene which shuts off LH gene expression in the late pregnant ewe or cow. The molecular biology of genes controlling the secretion of gonadotropins is now being clarified (Nett, 1990). Nett (1990) has found that while estradiol increases the number of receptors for GnRH in the pituitary, it also decreases the amount of mRNA for the subunits of follicle stimulating hormone (FSH) and LH. In addition, there appears to exist a mechanism by which estradiol prevents the GnRH induced increase in mRNA for gonadotropins. This type of negative regulation of transcription by a steroid molecule is unique and represents a novel method of steroid action.

Prostaglandins

The concentration of prostaglandin $F_{2\alpha}$ (PGF) in the blood of pregnant cattle remains low for most of pregnancy (Gimenez
et al., 1983) before increasing within 5 to 7 days prior to parturition (Fairclough et al., 1975). The primary source of preparturient PGF is the uterus (Guilbault et al., 1984a) and the concentration in the blood can reach 4 to 9 ng/ml. Prostaglandins are synthesized from fatty acids which must be consumed in the diet. The immediate precursor of the prostaglandins is arachidonic acid, which is a 20 carbon fatty acid with four double bonds (20:4). Linoleic acid (18:2) is desaturated to linolenic acid (18:3) which is further desaturated and undergoes chain elongation to arachidonic acid (Ramwell et al., 1977). Animals do not have the enzymes to introduce a double bond into the number 9 position in long chain fatty acids. Therefore, linoleic acid cannot be generated from oleic (18:1) or stearic acid (18:0) and is an essential fatty acid. The conversion of linoleic acid to arachidonic acid requires approximately 3 days. Arachidonic acid is incorporated primarily into the #2 position of membrane phospholipids (Moncader and Vane, 1979) with approximately 73% of arachidonic acid esterified to phospholipid in the cow (Lukaszewka and Hansel, 1980). Arachidonic acid is cleaved from membrane phospholipids by the enzyme phospholipase A₂ (Hall and Behrman, 1982) and the free arachidonic acid is acted upon by two enzymes (prostaglandin synthase and cyclooxygenase) to produce endoperoxides, PGG₂ and PGH₂ (Hemler and Lands 1976; White et al., 1978). Prostaglandin H₂ is then reduced to PGF or isomerized to PGE₂.
and PGD₂ and subsequently reduced to PGF by 9-keto reductase (Samuelson, 1978). The latter pathway has not yet been found to be active in cattle (Wlodauer et al., 1976). Most investigators studying prostaglandin secretion in late pregnant or postpartum cows measure 13-14-dihydro-15-keto-prostaglandin F₂α (PGFM) in the blood. This is the primary metabolite of PGF (Kindahl et al., 1976ab). Prostaglandin F₂α is metabolized to PGFM in the lung (Davis et al., 1980) with nearly 90% of PGF converted to PGFM in the cow. This high conversion rate in the lung is in contrast to the situation in the pig where a smaller percentage of blood PGF is converted to PGFM during each passage through the lung. Subsequent metabolism of PGFM results in the production of urinary metabolites (Kindahl, 1980). Although generally considered to be an inactive form of PGF, Milvae and Hansel (1983) demonstrated that 10 to 15 mg of PGFM injected into cattle could cause premature luteolysis of the CL.

The prepartum rise in PGF concentration in the blood is critical to the physiological events associated with parturition (Nathanielsz, 1978) and occurs in most species (reviewed by Thornburn et al., 1977). In the cow, PGFM concentration obtains a maximum within 2 to 3 days after parturition and then declines reaching a minimum by approximately two weeks after calving (Edquist et al., 1978; Eley et al., 1981; Guilbault et al., 1987ab). The elevated postpartum concentration of PGFM is a characteristic of the
cow and sheep (Lewis and Bolt, 1983) and is not generally observed in other species.

Additional Hormones

Besides progesterone, estrogens, and prostaglandins, several other hormones increase at the end of pregnancy. These include hormones which are involved in parturition (glucocorticoids) as well as hormones involved in the initiation of lactation (prolactin, growth hormone, and glucocorticoids; reviewed by Tucker, 1981). Prolactin increases dramatically 2 to 3 days prior to parturition and is believed to play some role in lactogenesis in the cow. Administration of ergot alkaloids to block this parturient rise in prolactin suppressed milk production in the cow, but lactation continued despite continued treatment to maintain low prolactin concentration (Akers et al., 1981). Administration of prolactin to ergot-treated cows resulted in normal milk secretion. These results provide further evidence for the role of prolactin in lactogenesis. There is a suspected role of increased growth hormone secretion at the time of parturition in the initiation of lactation. This is related to the ability of GH to stimulate lactation in later postpartum cows (Peel and Bauman, 1989). At this time, however, there is no defined role for GH during parturition in cattle. Glucocorticoids have a defined role in parturition (see below) and also are believed to be involved in the process of lactogenesis. Glucocorticoids remain low during
pregnancy until they increase approximately 1 to 2 days prior to parturition (Smith et al., 1973). There is a likely synergism between glucocorticoids and prolactin for the synthesis of \( \alpha \)-lactalbumin and casein in milk.

**Hormonal Control of Parturition in the Cow**

Normal gestation length in the cow ranges from 270 to 290 days and primarily depends on the breed, sex, and number of fetuses (Jainudeen and Hafez, 1980). In general, male calves and twin pregnancies are born earlier than female calves and single pregnancies, respectively. The breed of the calf and not the breed of the mother determines gestation length (Lampeter et al., 1980; King et al., 1982). This was demonstrated by reciprocal embryo transfer experiments that matched short gestation calves with long gestation dams, as well as, long gestation calves with short gestation dams (Lampeter et al., 1980; King et al., 1982). The cascade of events leading to expulsion of the fetus has been described by numerous reviews (First, 1979; Hoffman et al., 1979a; Johnson, 1981; Bazer and First, 1983). There are seven defined steps to parturition in the cow (Bazer and First, 1983). These are maturation of the fetus, termination of pregnancy, expansion of the birth canal, uterine contractions, maternal behavior, the synthesis of milk, and milk ejection. These processes occur in an ordered series which allows for efficient expulsion of the fetus. The fetus initiates parturition by hormonal secretions of the hypothalamus and pituitary (First,
1979). This was theorized because of the observation that calves with deformed or abnormal heads were not expelled at the end of the normal period of gestation but remained established in the uterus. Designed experiments demonstrated later that pituitary ablation prevented parturition (First, 1979) while replacement with adrenocorticotropic hormone (ACTH) would successfully initiate the parturient events. Growth of the fetal adrenals is stimulated by ACTH during the prepartum period, and the final surge of ACTH at parturition acts to switch adrenal steroid production to cortisol. Fetal cortisol causes the release of PGF from the uterus (First et al., 1982) and further stimulation of estrogen production from the placenta by cortisol also acts to enhance PGF secretion. The actions of PGF are threefold in the cow (Bazer and First, 1983). First, PGF causes the regression of the CL and a precipitous decline in progesterone concentration. Second, PGF causes the release of relaxin from the CL which is responsible for the softening of the cervix and pubic symphysis and allows for the expansion of the birth canal. Furthermore, PGF induces a release of oxytocin from the posterior pituitary which stimulates myometrial contractions necessary for labor and delivery of the fetus (First and Bosc, 1979). These are the hormonal events associated with the physical event of parturition. Other hormones (described in the previous section) increase at the time of parturition and
are responsible for the initiation of the subsequent lactation and the continued synthesis and secretion of milk.

**Early Lactation in the Cow**

The early postpartum cow represents a classic scientific example of goal oriented nutrient partitioning. The processes of nutrient partitioning in the cow have been reviewed by Bauman and Currie (1980). Although not coined by Bauman and Currie (they give credit to Kennedy, 1967), the term "homeorhesis" was reintroduced in this article to describe the physiological series of events which cows undergo in an effort to support the demands of early lactation. The first noted examples of this type of goal oriented metabolism were recorded by Mieshcer in 1880 who observed the migration of salmon up the Rhine river. He found that while the reproductive organs and gametes of these fish grew and proliferated during migration, they simultaneously lost 55% of their muscle weight due to energy mobilization caused by insufficient food consumption. Hammond (1947) later described a similar process in cattle in his discussion of the priority of body parts for nutrients in growing and gestating animals. These examples demonstrate the trafficking of nutrients in the body under certain physiological conditions.

Milk secretion in dairy cattle increases to a maximal amount within 4 to 8 weeks after calving and then subsequently undergoes a steady decline. During this period of maximum milk secretion, the function of the rumen and intestines of
the cow is undergoing adaptation from the relatively inactive period prior to calving to the most active period of nutrient digestion which occurs during early lactation. Since the production potential of the mammary gland increases at a faster rate than does the potential for the cow to consume feed, there exists an imbalance between nutrients consumed by the cow and nutrients exiting the cow in the form of secreted milk. Calculations of the energy required for maintenance and milk production compared with that consumed in feed show that early postpartum cows are in a nutrient deficit state (negative energy balance). The coordinated series of physiological processes which are elicited in response to early lactation are described by Bauman and Currie (1980). These include five different physiological functions which are supported by five different metabolic changes (involving different body tissues). First, the increase in milk production results in increased use of nutrients by the mammary gland. In support of this nutrient requirement, there is increased lipid metabolism through the coordinated decrease in lipogenesis and increase in lipolysis. This altered lipid metabolism is responsible for fulfilling the energy needs of lactation (to the mammary gland as well as the rest of the body) and for milk fat synthesis. The biochemistry of lipid mobilization from adipose tissue has been reviewed (Bauman and Davis, 1975; Bauman, 1976). The adipose tissue cell cycles lipid reserves toward either mobilization or storage. When
energy is being stored, glucose, acetate, and fatty acids (derived from the action of lipoproteins lipase on triglycerides within lipoproteins) are taken into the adipose tissue cell. Fatty acids are activated or formed de novo from acetyl CoA (enzymatic reaction of acetyl CoA carboxylase) and triglycerides are produced from fatty acyl CoA derivatives esterified to glycerol (generated by glucose metabolism). The accumulation of triglycerides in the adipose tissue cell occurs when the rate of production of triglyceride is greater than the rate of degradation. In contrast to this accumulation of the energy stores, the early postpartum cow mobilizes fat reserves. This occurs through the decreased synthesis of triglycerides and increased triglyceride breakdown. Chillard et al. (1977), working with goats, demonstrated that the late pregnant and lactating goat had reduced activity of lipoprotein lipase and acetyl CoA carboxylase (both responsible for triglyceride accumulation). Similar findings in lactating cows support these enzymatic shifts associated with decreased lipid synthesis and increased lipid mobilization (Shidhu et al., 1972; Shirley et al., 1973; Swann, 1976). Glucose metabolism is also altered relating to the requirement of glucose molecules for milk lactose (B 1-4 dimmer of galactose and glucose). During early lactation nearly 80% of the glucose turnover in the body is associated with the mammary gland and nearly 2 kg of glucose may be required daily by the mammary gland of high producing cows.
Glucose requirements are partially met by increased consumption of nutrients by postpartum cows (Lindsay, 1971). However, there is also an increase in gluconeogenesis and an increase in glycogenolysis in the liver. Further conservation of glucose occurs through reduced oxidation of glucose. Bennink et al. (1972) found that glucose oxidation decreased from 34% to 8% in lactating cows. The sudden imbalance of oxidizable substrate during this period is apparently corrected through increased use of fatty acids for energy. Final processes involved in the homeorhetic process include increased mobilization of protein reserves (from muscle and other tissues) and increased mineral absorption (from gut), mobilization (from bone), and retention (kidney). The specific involvement of vitamin D in the availability of calcium reserves has been reviewed (DeLuca and Schnoes, 1976).

Recovery of Reproductive Organs from Pregnancy

After calving and during the period of maximum milk production and energy and nutrient deficiency, the uterus, ovary, and hypothalamus/pituitary of the cow undergo a process of recovery and rebuilding for the establishment of subsequent pregnancy. This period is characterized by the hormonal maturation of the pituitary and hypothalamus, morphological and histological changes in the uterus, and the establishment of new follicular populations on the ovary leading to first ovulation.
Uterus

The process of uterine involution in the cow has been reviewed by several authors (Morrow et al., 1969; Moller, 1970; Kiracofe, 1980). Uterine involution is assessed grossly by rectal examination and estimation of the size of uterine horns in the cow on specific days postpartum. The time required for uterine involution (i.e., decrease in size of the uterus to a postpartum minimum) was estimated to be 28 to 56 days by Kiracofe (1980) who reviewed several papers on the subject. Similarly, Marion et al. (1968) found that an average of 39 days was required for dairy cattle to complete uterine involution.

The weight of the uterus decreases rapidly during the postpartum period, partially due to the accelerated loss of lochia from the uterine lumen during this time (Gier and Marion, 1968). Involution of the uterus is initially very rapid and then the rate decreases with days postpartum (Lauderdale et al., 1968). As might be expected, the rate of involution is faster in the larger gravid horn compared to the less extended nongravid uterine horn (Lauderdale et al., 1968). Uterine horn diameter and length decrease by 50% by 5 and 15 days postpartum, respectively.

There are several environmental, genetic, and clinical factors that can potentially influence the rate and extent of uterine involution in the cow. Most of these factors have a marginal effect on uterine involution with authors reporting
different results depending on the study. For example, the number of previous pregnancies may (Marion et al., 1968) or may not (Tennant et al., 1967) have an effect on the rate of uterine involution. Similarly, suckling may have no effect or delay uterine involution (Wilbank and Cook, 1958; Wagner and Hansel, 1969) or may speed up the process of uterine involution (Lauderdale et al., 1968). There is, however, a common belief in women that breast feeding will increase the rate of uterine involution. Finally, nutrition of the animal appears to have a minimal effect on the process of uterine involution (Dunn et al., 1969).

Several factors do affect the rate at which the uterus returns to the prepregnant state and some of these have important implications to the postpartum dairy cow. First, there is evidence that breeds of cattle differ in terms of the time required for complete involution (Casida, 1968). In addition, in nonheat-stressed cattle, the season at which the animal calves seems to affect involution with cows calving in the warm season involuting sooner compared to similar animals calving in the cold weather months (Marion et al., 1968). More dramatic, however, is the effect of parturient clinical problems on the rate and extent of uterine involution. Uterine involution is delayed in cows with dystocia, retained placenta, uterine infection, milk fever, and ketosis (Gier and Marion, 1968; Morrow et al, 1969; Fonseca et al., 1983). This reduced fertility in postpartum cows suffering from these
clinical problems could partially be related to a delayed or abnormal process of uterine involution.

Guilbault et al. (1985a) showed that the daily rate of uterine involution depended on the sire of the fetus. The rate of reduction in size of the uterine horn and cervix diameter is high in Holstein pregnancies sired by Holsteins or Brahmans compared to Angus bulls. This finding relates to previous work by Casida (1968) on uterine involution in different breeds of cattle. In addition, Guilbault et al. (1985a) found a positive correlation between the concentration of plasma PGFM and the rate of involution of the reproductive tract. This finding agrees with others who have studied the relationship between PGF and uterine involution (Eley et al., 1981; Lindell et al., 1982).

The process of uterine involution is more complex than a simple reduction in size of the reproductive tract of the female. Extensive degeneration and absorption of maternal tissues, as well as, regeneration of maternal epithelium must occur for subsequent pregnancy. Marion et al. (1968) described three stages of uterine involution. These were the reduction in size of the uterus, the loss of tissue from the previous pregnancy, and the repair and regeneration of tissue for subsequent pregnancy. The histology of involution is illustrated in several articles (Gier and Marion, 1968; Wagner and Hansel, 1969). In order for the uterus to return to the prepregnant state, the extensive musculature required for the
expulsion of the calf must be lost. Archibald et al. (1972) have described the degeneration of the myometrium as a three step process including degeneration of the sarcoplasm, vacuolation of the muscle cells, and nuclear atrophy. These processes appear to be completed by about 30 days postpartum. Coincident with this process is the loss of the maternal portion of the placentome (caruncles) which have sloughed off into the uterine lumen by the second week after calving. The remaining uterine structures are completely regressed by 40 to 60 days after calving.

The repair of the endometrium is a critical process for the restoration of normal estrous cycles and fertility in cattle. During the period immediately after calving the endometrium both degenerates and regenerates at the same time (Archibald et al., 1972). This process is accomplished through the highly proliferative nature of the endometrial epithelium. Eventually as the degeneration of the endometrium is completed, the regenerative process predominates (Archibald et al., 1972). It is clear that the regenerative process occurs first in the intercaruncular areas of the uterus and then the endometrium is regenerated over the caruncles (Gier and Marion, 1968; Wagner and Hansel, 1969). This process by which endometrial cells grow and cover the caruncles is completed by 19 to 25 days after calving (Gier and Marion, 1968; Archibald et al., 1972).
Several weeks are required after calving for postpartum cows to regain maximum fertility. Of interest is the fact that cows regain most of the potential fertility prior to completed uterine involution (Kiracofe, 1980). Clearly, however, cows inseminated before 30 days postpartum are less fertile compared with cows inseminated after 40 days postpartum (Perkins and Kidder, 1963). Two potential blocks to early postpartum fertility exist. First, there may be a lack of embryonic development due to fertilization failure or abnormal oocyte maturation within the early postpartum follicle, or second, the uterus may simply be unable to maintain an embryo during this rebuilding period. Casida (1968) examined embryos from cows inseminated between 4 to 6 weeks after calving and embryos from cows inseminated later postpartum. He found no difference in the rate of preimplantation embryo mortality between early and later postpartum cows. This suggested that lower fertility in cows bred early in the postpartum period may be related to the repair process occurring in the uterus.

The Ovary

The ovary of the cow during pregnancy becomes inactive. This process is initiated very early during the pregnancy and can be observed by ultrasonography within 35 days of conception (Pierson and Ginther, 1987). At the time of parturition, the ovaries of the cow are devoid of large follicles. Casida (1968) found that the largest follicle on
the ovary of a cow decreased from 12 mm on day 60, to 9 mm at 5 months of pregnancy and 4 mm at 9 months of pregnancy. This process of follicular decline is completely reversed at parturition when follicular growth is initiated in most cattle (Marion and Gier, 1968). Development of large (>10 mm) follicles usually occurs within 14 days (Wagner and Hansel, 1969; Stevenson and Britt, 1979). The growth of follicles on the ovary during this period is a prerequisite for first ovulation which can occur within 21 days in nonsuckled dairy cows (Marion et al., 1968; Stevenson and Britt, 1980; Staples et al., 1990). Large nonovulatory follicles normally grow and regress on the ovary prior to first ovulation and this may represent the inability of all postpartum follicles to secrete estrogen required for an LH surge. Spicer et al. (1986b) characterized the follicular development of anestrous postpartum beef cattle. They found that the number of 4 to 7.9 mm follicles increased fourfold between the first and sixth week postpartum while the number of small (1 to 3.9 mm) and large (>8 mm) follicles did not change during this time period. It was suggested that the large increase in the number of medium follicles represented a pool of follicles from which the first ovulatory follicle would be selected. When postpartum follicles were analyzed biochemically, Braden et al. (1986) found that numbers of LH and FSH receptors in granulosa cells from large follicles collected at different times during the postpartum period were similar. Therefore,
no specific deficiencies in the ability of the follicle to respond to gonadotropins was suggested. Braden et al. (1986) did find, however, that lower follicular fluid estradiol concentrations existed in follicles during the postpartum period compared to later during the estrous cycle. It does appear that the position of the CL or gravid uterine horn of the previous pregnancy influences the location of the largest follicle during the postpartum period. Spicer et al. (1986b) found that most large follicles were located on the ovary opposite to the previous CL-bearing ovary. This work is supported by Dufour and Roy (1985) who also found that the location of the CL and associated gravid uterine horn of the previous pregnancy had an effect on populations of follicles measured by histology.

The Pituitary and Hypothalamus

The maturation of the reproductive system occurs during distinct stages, the first of which is the return of the ability of the hypothalamus and pituitary to support and maintain normal estrous cycles. The content of LH and FSH in the pituitary is inversely related during the postpartum period (i.e., LH is low, FSH is high; Saiduddin et al., 1968). The amount of FSH that is secreted into the blood is high in early postpartum cows (Dobson, 1978; Schams et al., 1978). For this reason, the amount of FSH reaching the ovary is not considered to be a limiting factor in terms of follicle growth in postpartum cows (Schams et al., 1978; Lamming et al.,
In contrast to FSH, LH appears to be the primary hormone required for the growth and development of ovarian follicles after calving (Lamming et al., 1981). It is clear that prior to day 10 postpartum the pituitary of the cow does not contain significant amounts of releasable LH (Kesler et al., 1977; Fernandes et al., 1978; Schallengerger et al., 1978). This situation is reversed with progressive days after calving and the frequency of LH pulses increases with time (Schallengerger and Peterson, 1982). Restoration of pulsatile secretion is slow, however, and 10 to 30 days may be required to achieve consistent patterns of LH release which are characteristic of the estrous cycle (Lamming et al., 1981). Postpartum suckled beef cows have low concentrations of GnRH and LH in the blood and do not have pulsatile secretion of LH early during the postpartum period (Nett, 1987). This phenomenon, however, does not seem to be related to deficiencies of the hypothalamus or pituitary in terms of LH or FSH content, or number of GnRH receptors (measured on day 30 postpartum; Parfet et al., 1986).

There exists a circular dependence of the follicle and the hypothalamus/pituitary which dictates the timing of first ovulation (initially described by Stevenson and Britt, 1980). Secretion of LH stimulates the development and growth of follicles and in turn stimulates the development of LH and FSH receptors in follicles and the secretion of follicular estrogen. The secretion of estrogen further acts to increase
the frequency of LH pulses. Estrogen alone can also induce an LH surge in ovariectomized cows (Kesner et al., 1982) and is primarily responsible for steroidal regulation of ovulatory gonadotropin release. Eventually, when a critical level of estradiol secretion and pituitary priming is reached, the preovulatory LH surge occurs and, in the ideal case, this LH release is followed by first postpartum ovulation (Stevenson and Britt, 1980).

First ovulation in dairy and beef cattle is often not accompanied by behavioral signs of estrus (Morrow et al., 1966; Saiduddin et al., 1967; Kiracofe, 1980). These "silent heats" are true physiological events and are not the same as "missed heats" resulting from poor heat detection. Casida (1968) indicated that the behavioral signs of estrus increased with the postpartum interval, suggesting a possible need for prior progesterone exposure for normal estrus. In addition, fertility of cattle improves with the number of postpartum estrous cycles prior to breeding (Thatcher and Wilcox, 1973). First ovulations usually occur on the ovary opposite to the side of the previously pregnant uterine horn in cows ovulating early postpartum (<15 days; Marion and Gier, 1968). This trend is reversed after 20 days postpartum when a more random pattern of ovulation between the ovaries is found (Marion and Geir, 1968). Corpora lutea formed from the first postpartum ovulation in dairy and beef cattle are small, short-lived, and secrete less than normal amounts of progesterone (Morrow et
Short cycles occur even though follicles leading to these CL ovulate within the expected diameter range and have a normal level of gonadotropin support (Garverick et al., 1988). White et al. (1987) studied individual ovine follicles which were destined to form subfunctional CL. Their data suggested that these follicles were subnormal in terms of steroidogenic capacity. The abnormal function of the CL in postpartum cattle is probably related to prior exposure to progesterone since pretreatment of postpartum cows with progesterone results in CL with normal life span (Smith et al., 1987). Furthermore, Savio et al. (1990a) found that follicles that ovulated after day 20 postpartum were likely to result in short-lived CL. However, follicles ovulating before day 20 postpartum produced CL with normal life spans. This might suggest that follicles developing late in gestation (and high progesterone) can form normal CL. A less likely, but often suggested, cause of short-lived CL is the postpartum release of PGF which could regress developing CL. This is an unlikely physiological mechanism because the majority of PGF has been released from the uterus prior to the period during which cattle experience short cycles. It is clear, however, that the uterus is responsible for the destruction of the CL during the short estrous cycle. Cows expected to have short estrous cycles do not have premature luteolysis if the uterus is removed (Copelin et al., 1987; Wright et al., 1988). In addition,
luteolytic patterns of PGF secretion occur around the time of luteolysis in cows experiencing short estrous cycles (Garrett et al., 1988).

The Estrous Cycle of the Cow

The physiology of the estrous cycle of the cow represents the coordination of the hypothalamus/pituitary, ovary, and uterus. In farm animals, no structure dominates the other and the cyclical nature of the estrous cycles requires the interaction of these three organs. This can be contrasted with the human female, where the ovary and hypothalamus/pituitary act independently of the uterus during the menstrual cycle. Therefore, the estrous cycle of the cow is unique and represents a highly developed physiological system.

Follicular Growth and Development

The micromorphology of the ovary has been classically presented by Rajakoski (1960). Primordial follicles containing the oocyte surrounded by a single layer of flattened granulosa cells represent the predominant follicle type on the ovary at any time. The growth and development of primordial follicles follows a classical pathway which is initiated by the division of the granulosa cell layer and the differentiation of the surrounding ovarian connective tissue into the theca layer of the follicle. As the follicle grows, the zona pellucida is deposited around the oocyte and fluid accumulates between patches of granulosa cells. These processes of early follicular growth are believed to be
The process by which the female germ cell becomes established on the ovary and encased in the fluid filled follicle is complex and is initiated early during embryonic life. The essential steps involved have been summarized by Wassarman (1988) and include migration of extragonadal germ cells to the presumptive gonads, differentiation of the primordial germ cells into oogonia, cessation of mitosis of oogonia, initiation of meiosis of these same cells, prolonged arrest of meiosis, oocyte growth, and finally reinitiation of meiosis at puberty. The movement of the primordial germ cells has been described by several authors (Mauleon, 1978; Richards, 1980; Baker, 1982; Byskov, 1982; Spicer and Echternkamp, 1986). Primordial germ cells are initially located in the epithelium of the embryonic yolk sac. This is supported by histological data showing intense alkaline phosphatase activity (characteristic of both primordial germ cells as well as oogonia) in the cytoplasm of cells in this area. Their presence in the yolk sac precedes the development

stimulated by FSH. The accumulation of fluid eventually sets up a microenvironment for follicular function which includes granulosa and theca cell layers encasing a fluid filled chamber. The oocyte, sitting within granulosa cells forming the corona radiata and on top of the cumulus oophorus, remains attached to the follicle wall. When this morphology is obtained the follicle is called a mature Graafian follicle in honor of the first scientist to describe these structures.
of the genital ridge of the embryo. The genital ridge is a protrusion of mesenchymatous tissue encased in coelomic epithelium. In cattle, the genital ridge develops from day 28 (Gier and Marion, 1970) to day 32 (Erickson, 1966) of embryonic life and represents the embryonic origin of the ovary. Apparently, a diffusible substance directs the ameboid movement of germ cells from the yolk sac to the genital ridge (Eddy et al., 1981). Of interest is the fact that this substance is not species specific since germs cells of the mouse will migrate toward the developing chick gonad following cross-species transplantation of the yolk sac (Byskov, 1982).

Mitotic division of the germ cells proceeds subsequent to the movement of the cells to the genital ridge. In cattle, the number of germ cells occupying the embryonic ovary reaches a maximum at 110 to 130 days of fetal life (Erickson, 1966). After this time the population of germ cells within the ovary decreases. The existence of a large population of germ cells on the ovary during fetal life is consistent with other species (including the sheep [Mauleon, 1969] and the pig [Black and Erickson, 1968], as well as rat, human, monkey, and guinea pig [Byskov, 1982]). The term oogonia is used to refer to mitotically dividing germ cells before the initiation of meiosis. In cattle, meiosis in the female ovary begins at approximately 60 to 80 days of embryonic life (Ohno and Smith, 1964; Erickson, 1966; Marion and Gier, 1971; Vigier et al., 1976). However, meiosis is not completed in the embryonic
gonad and germ cells arrest in the diffuse diplotene stage of prophase I. They remain at this meiotic stage until puberty and are referred to as primary oocytes until meiosis continues. Most oogonia have completed meiotic arrest by day 170 to 175 of embryonic life (Erickson, 1966).

The establishment of the intimate relationship between the granulosa cell and the oocyte begins at about 90 to 100 days of fetal life. It is at this time that primordial follicles are first observed on the ovary in the cow (Erickson, 1966; Marion and Gier, 1971; Vigier et al., 1976). The primordial follicle is defined as an oocyte surrounded by a single layer of flattened granulosa cells. Eventually the granulosa cells of the follicle become more cuboidal giving rise to the primary follicle. The follicle is termed a secondary follicle once the cells surrounding the oocyte divide and form several layers. Finally, subsequent cell division and the initiation of fluid secretion into the extracellular space results in the formation of a fluid filled antrum. These follicles are considered morphologically distinct and now termed tertiary or Graafian follicles (Rajakowski, 1960). Apparently, the initiation of further development of individual primordial follicles does not occur until all germ cells on the ovary have meiotically matured and become encased in a single layer of granulosa cells (Mauleon, 1978). Once these processes are complete, antral follicles (those containing an organized cavity of follicular fluid)
appear on the ovary (approximately 210 days of fetal life [Mauleon, 1961]). The secretion of follicular fluid into the extracellular space is critical because it sets up the micromorphology of the follicle which is important to its known physiology. In addition, the developing antral follicle directs the differentiation of the surrounding cells into the theca cell layer, which eventually provides aromatizable substrate during the process of steroidogenesis.

The development of gonadotropin receptors on the theca and granulosa cell layers of the follicle is critical to its eventual growth, development, and function. It is clear that gonadotropin support is not required for follicles to reach the primordial stage of development. However, antrum formation seems to be dependent on the presence of gonadotropins (Richards, 1980). Experiments in rats show that, following hypophysectomy, large follicles on the ovary become atretic. In addition, after several days, only primordial follicles occupy the ovary (Richards et al., 1978). These follicles can however, be stimulated to grow if provided with estradiol or gonadotropins (FSH). Follicle stimulating hormone appears to be necessary for folliculogenesis at all times (Richards, 1980). Granulosa cells of small follicles contain appreciable quantities of FSH receptors but no LH receptors (Richards, 1980). This is supported by several lines of evidence including the inability of small follicles on the ovary to ovulate in response to the LH surge (Richards, 1980),
differences in the binding of radiolabelled LH or FSH to granulosa cell preparations (Richards et al., 1978), and the fact that granulosa cells of these small follicles do not luteinize in response to LH. In contrast, granulosa cells from larger follicles contain receptors for both LH and FSH. This has been shown in the rat (Richards, 1980) and cow (Ireland and Roche, 1982). Ireland and Roche (1982) showed appreciable binding of $^{125}$I-hCG to theca cell preparations from follicles of all sizes. However, similar binding to granulosa cells was not evident for follicles under 10 mm. Therefore, it appears that there are significant functional differences between follicles above and below 10 mm in diameter in the cow. Furthermore, binding of $^{125}$I-FSH to granulosa cells diminished appreciably with increasing follicle size suggesting a general shift of receptors away from FSH and toward LH.

The steroidogenic capacity of the follicle is critical to its function as an endocrine gland. The classical description of steroid production by the follicle is the two cell theory (summarized by Hansel and Convey, 1983). Granulosa cells of cattle produce estradiol after stimulation with substrate or being placed in coculture with thecal cells (Moor, 1977; Hansel and Fortune, 1978). Therefore, there exists a two cell interaction for the production of estradiol by the follicle. Luteinizing hormone (acting via the adenylate cyclase second messenger system [Ireland, 1987]) is believed to stimulate the
conversion of cholesterol to pregnenolone as a first step in the steroidogenic process (Hansel and Fortune, 1978). The subsequent metabolism of pregnenolone to androstenedione (and not progesterone) leads to the available substrate for aromatization. In the granulosa cell, estradiol is produced through FSH stimulation (adenylate cyclase second messenger system [Ireland, 1987]). The cellular mechanisms for the two cell hypothesis have recently become clear (see review by Richards and Hedin, 1988). In the thecal cell the action of LH is to increase the activity of gene expression for P450 side chain cleavage enzyme and P450 17α lyase and promote the production of androstenedione. The conversion of androstenedione to estradiol occurs in the granulosa cell (P450-aromatase). Estradiol subsequently regulates gene expression within the granulosa cell (increasing transcription of the LH receptor, second messenger components, P450 enzymes) but this regulation is dependent on the actions of FSH on the granulosa cell. Therefore there seems to be a synergism between the actions of FSH and estradiol which leads to the proper function of the ovarian follicle.

The dynamics of the growth of small follicles on the ovary of cattle have been clarified by one study. Lussier et al. (1987) studied the growth rate of follicles across several small follicular size classes. They found that antrum formation in the cow begins with follicles ranging in size from .12 to .16 mm in diameter with a distinct antrum being
present by the time most follicles reach .28 mm in diameter. Increases in size were mainly attributed to the division of granulosa cells for follicles below 2.5 mm in diameter. Above this diameter range, however, increased follicle size was mainly a result of accumulation of fluid within the antrum. They also found that mitotic index was greatest in follicles in the .68 to 1.52 mm size range which supported the experimental observations mentioned above (i.e., growth of larger follicles is not due to granulosa cell division). Based on their calculations (derived from number of granulosa cells within different size classes and time required for cell division) they determined that it takes 27 days for a follicle to grow from .13 to .67 mm, 6.8 days to grow from .68 to 3.67 mm and 7.8 days to grow from 3.68 to 8.56 mm. Therefore, over 40 days are required for a follicle to grow from the early antral phase to the preovulatory size.

The growth of large follicles on the ovary of cattle remains a curiosity for researchers. For unknown reasons, large follicles grow and regress on the ovary at all times during the estrous cycle. Cyclical changes in bovine follicles on the ovary were first described by Choudary et al. (1968). Initial investigations into the growth patterns of follicles were conducted using India ink marking and sequential laparotomy (Matton et al., 1981). Matton et al. (1981) found that large follicles on the ovary at different times of the estrous cycle were constantly being replaced.
Because of the development of ultrasound technology (Pierson and Ginther, 1984), large follicles on the ovary can be observed and followed easily. It is now clear that follicles grow in a wave-like pattern on the ovary with small follicles increasing in size to a maximum diameter and then slowly declining (Sirois and Fortune, 1988; Savio et al., 1988). Cows will have anywhere from one to four follicular waves during an estrous cycle (Sirois and Fortune 1988; Savio et al., 1988). When studying heifers, Sirois and Fortune (1988) found that heifers had two to four waves with the three wave cycle being most predominant. The initiation of each wave started on day 1.9, 9.4, and 16.1, respectively, with the final wave leading to the ovulatory follicle. Savio et al. (1988), also working with heifers, reported that the first, second, and third waves were detected on days 4, 12, and 16 of the estrous cycle. Differences in the day of detection of the specific wave reported in these papers probably relates to individual definitions of initiation versus detection of a follicular wave. Postpartum dairy cattle also display follicular development in wave-like patterns. Savio et al. (1990a) reported that cows having short estrous cycles had a single follicular wave, while cows with normal length and long estrous cycles had 2 and 3 follicular waves respectively.

The biochemical changes in follicles which result in these patterns of wave-like growth are complex and at this time unclear. Some of the biochemical aspects relating to
ovarian follicular turnover and growth have been reviewed (Spicer and Echternkamp, 1986). There are no obvious changes in gonadotropin secretion to describe the growth and regression of the first dominant follicle though the normal switch in pulsatility of LH found late in the estrous cycle can explain partially the growth of the preovulatory follicle. Skyler et al. (1987) sampled follicles from days 4, 12, and 19 of the estrous cycle and biochemically classified follicles in relation to the stage of the estrous cycle. As might be expected based on the theory of follicle waves, estrogen secretion of large follicles in vitro was greatest on day 19 of the estrous cycle and least on day 12 (when large atretic follicles are normally found on the ovary). Ireland and Roche (1983) examined the specific biochemical changes in the follicle which occur late during the estrous cycle. They found that ovulatory follicles increased in diameter, follicular fluid volume, and number of granulosa cells after day 17 of the estrous cycle. In addition, these potentially ovulatory follicles had greater estrogen, progesterone, and androstenedione in the follicle fluid. This contrasted with atretic follicles which had little estrogen and more progesterone and androstenedione. Another biochemical marker within bovine follicles is acid and alkaline phosphatase. Henderson and Cupps (1990) found that while acid phosphatase activity varies inversely with the size of the follicle, there was no relationship of acid phosphatase with stage of the
estrous cycle or gestation. In addition, neither acid phosphatase or alkaline phosphatase were found to be accurate markers for atresia. High levels of phosphatases were, however, characteristic of small healthy antral follicles. An exhaustive study of the biochemistry of follicular fluid, including albumin, total protein, lysosomal enzyme content, steroids, ion concentrations, and ascorbic acid content for follicles on each day of the estrous cycle in beef cattle has been reported (Wise, 1987). Interestingly, acid and alkaline phosphatase activity, lactate dehydrogenase, and ascorbic acid concentrations in follicular fluid decreased as follicles increased in size. Also, albumin and total protein decreased as follicles got larger. Although numerous biochemical components of follicular fluid were measured in this study, no clear marker for atresia existed.

Polypeptides secreted by Graafian follicle in sheep were studied by Moor and Crosby (1987). There exists a class of polypeptides that are secreted by granulosa cells prior to the LH surge (46 to 60 kDa). The secretion of these polypeptides did not change immediately after the LH surge in vitro, but there was a switch to lower molecular weight species after 15 hours (30 kDa). Culture of intact follicles in vitro resulted in similar patterns of protein production. However, granulosa cells maintained in culture immediately switch to the secretion of the 30 kDa species unless stimulated by LH. Therefore the control of these proteinaceous products may be
a function of the ability of the granulosa cell to remain responsive to LH.

Single follicles grow and decline during each follicular wave. Since there are several potential follicles which could grow and become dominant at any one time, some mechanism must exist to control the number of follicles growing on the ovary of cows. Ireland and Roche (1987) have described three processes related to wave-like growth in cattle. First there is recruitment of follicles on the ovary to form a growing pool of follicles. Next, a single follicle is selected to grow beyond all others during the selection phase. Finally, the selected follicle gains physiological maturity and obtains the ability to prevent the continued growth of other follicles (a process known as follicular dominance). One of the earliest potential biochemical regulators of follicular growth in cattle is inhibin. Inhibin is found in follicular fluid of cattle and other farm animals and controls the secretion of follicle stimulating hormone from the pituitary (see reviews by Steinberger and Ward, 1988; and Ying, 1988). Ireland et al. (1983) found that when follicle fluid was injected into ovariectomized heifers the concentration of FSH in the blood decreased. Furthermore, when cattle were injected with follicular fluid, follicle maturation and estrus was delayed (Johnson and Smith, 1985; Quirk and Fortune, 1986). Finally, ovulation rate in sheep was increased by immunization with follicular fluid (McNeilly, 1985). These data have led to the
hypothesis that inhibin, secreted from the dominant follicle may actually suppress FSH and therefore control follicular growth on the ovary. The chemical structure and amino acid sequence of inhibin is nearly equivalent to transforming growth factor-β (TGF-β; Mason et al., 1986). The inhibin molecule is a protein dimer consisting of an α subunit and β subunit. It has been purified from bovine follicular fluid (Robertson et al., 1986; Fukuda et al., 1986) and is found in two forms (31,000 and 58,000 dalton, Robertson et al., 1985; Robertson et al., 1986). A related peptide—Activin has been isolated from porcine follicular fluid and consists of a dimer of β subunits from inhibins (Mason et al., 1985). Activin stimulates the release of FSH from cultured pituitary cells (Ling et al., 1986; Vale et al., 1986) and may potentially represent the peptide responsible for counteracting the actions of inhibin on FSH secretion (Ying, 1988).

Another peptide which may directly control follicular dominance is follicle regulatory protein (FRP). FRP is found in follicle fluid (diZerga et al., 1982) and is secreted by granulosa cells grown in culture (diZerga et al., 1983; Tonetta et al., 1988). The ability of FRP to inhibit aromatase activity of granulosa cells grown in vitro suggests it may be a local or systemic regulator of the growth of follicles (diZerga and Wilks, 1984). Indeed, when FRP was infused into guinea pigs, follicular growth was prevented (Katsuhiko, 1987). One model, outlined by Ireland and Roche, 1987,
suggests follicular dominance is established when a single follicle grows and secretes both FRP and inhibin. These two compounds acting together potentially block FSH secretion and inhibit aromatase activity of neighboring follicles. The loss of aromatase activity and associated estrogen in the follicular fluid will likely lead to atresia of subordinant follicles due to a decline in estrogen induced gonadotropin receptors on granulosa cells (reviewed by Richards and Hedin, 1988). Ireland and Roche (1987) also suggest that atresia of the dominant follicle may be a result of a build up of FRP in follicles which are not destined to ovulate. The death of the granulosa cell layer (indicated by development of pycnotic bodies and low estrogen to progesterone ratio, Moor et al., 1978; Carson et al., 1979) results in declining inhibin secretion and the initiation of a new follicular wave.

**Growth Factors and Follicular Function**

As might be expected for a structure undergoing rapid growth and differentiation, the follicle and follicular fluid contain numerous local acting growth factors. The role of these growth factor in the physiology of the follicle has been recently reviewed (Carson et al., 1989; Schams, 1989). The growth factors in the follicle do not necessarily promote cell growth and division but may have more important roles in cell differentiation. The follicle contains receptors for IGF-I, IGF-II, EGF and TGF-β (Schams, 1989) and local acting growth factors are often produced by the cells of the follicle and
act in a paracrine manner (Davoren and Hsueh, 1986; Hammond et al., 1985; Hsu and Hammond, 1987). The role of IGF-I is apparently regulatory and IGF-I has been shown to increase the activity of side-chain cleavage enzyme (Velhuis et al., 1986), potentiate the action of FSH through enhancing FSH induced cAMP levels in the cell (Adashi et al., 1986), and increase LH receptors on the cell surface (Adashi et al., 1985ab). In addition, IGF-I can stimulate the production of androgens (estrogen precursors) from the thecal layer of the follicle (Hernandez et al., 1988). Therefore IGF-I seems to function as a growth factor which potentiates and complements some of the actions of FSH (Carson et al., 1989). The mitogenic activity of IGF-I on granulosa cells has been established in farm animals (Savion et al., 1981; Baranao and Hammond, 1984) but is not consistent across species (Adashi et al., 1984). Another peptide growth factor—TGF-β—also has a positive effect on the differentiation of granulosa cells (Carson et al., 1989). These effects include enhancement of the actions of FSH on LH receptors (Knecht et al., 1986; Dodson and Schomberg, 1987) and enhancement of FSH associated aromatase activity (Hutchinson et al., 1987). TGF-β seems to have the opposite effect on granulosa cell division and blocks EGF induced mitoses (Moses et al., 1985).

Epidermal growth factor has the opposite effect to that of IGF-I and seems to be inhibitory to the differentiation of the granulosa cell. Most notably is the ability of EGF to
inhibit the basal secretion of inhibin by granulosa cells (Zhang et al., 1987). In addition, EGF inhibits FSH-induced adenylate cyclase activity (FSH second messenger; Dodson and Schomberg, 1987), the development of LH receptors within the follicle (Mondschein and Schomberg, 1981; Knecht and Catt, 1983), and FSH dependent aromatase activity (Hsueh et al., 1981; May et al., 1982). These represent major blocks to normal follicular growth and development and may suggest that EGF is acting to control the growth and development of the dominant follicle. Intravenous infusion of EGF into ewes has a dramatic effect on the health of follicles. Radford et al. (1987) infused Merino ewes with either 60 or 100 ug EGF/kg of body weight on days 4, 9, or 14 of the estrous cycle and found that all follicles on the ovaries of these ewes were atretic after 2 days. Subsequent to EGF treatment, 12 days were required for the EGF-treated ewes to obtain patterns of follicular health and atresia which were similar to control animals. In contrast to the negative regulatory effects of EGF on follicle function, EGF has been found to stimulate the division of granulosa cells in vitro (Gospodarowicz et al., 1977; Gospodarwicz and Bialecki, 1979; Skinner et al., 1987b). This indicates that there exists an inverse relationship between the ability of growth factors to induce mitoses of granulosa cells and the ability to simulate differentiation (Carson et al., 1989). This is suggested by the fact that IGF-I and TGF-β stimulate the differentiation of the granulosa
cell function in vitro but do not stimulate mitotic division. Opposite to these growth factors is EGF which inhibits differentiation, but is stimulatory to granulosa cell division (Carson et al., 1989). Finally, the ability of the theca cell layer to secrete growth factors which affect the granulosa cell layer needs to be recognized. This was initially discovered by McNatty et al. (1980) who demonstrated enhanced growth of granulosa cell cultures treated with theca cell preparations. It is now believed that the EGF and TGF-B are produced locally by the thecal cell and act in a similar manner to what has been described above (Skinner et al., 1987ab).

Although there is an apparent need for cell division within the granulosa cell, follicle fluid contains growth factor inhibitors which prevent the proliferation of mouse fibroblasts in vitro (Carson et al., 1988). Carson et al., showed that there apparently exists a follicle fluid growth factor inhibitor in the 10,000 and 180,000 MW range. These inhibitors may act to block the effects of mitogens (i.e., EGF) in follicular fluid. A more specific role for mitogenic inhibitors in follicle fluid is unclear at this time (see Chapter 6).

Growth of the Preovulatory Follicle

As discussed above, follicles grow and regress on the ovary at several times during the estrous cycle. The pattern of development is the similar at all times, however, late in
the estrous cycle large follicles do not regress but continue to grow and ovulate. Ovulation is the result of an interaction of the growing follicle with the hypothalamus and pituitary in the absence of the CL (and associated progesterone). As the preovulatory follicle grows on the ovary it secretes greater quantities of estrogen (McCraken et al., 1971; Hansel et al., 1973) with a maximum amount of estradiol being secreted around the time of estrus. The growth of the preovulatory follicle is largely dependent on the changes which occur in pituitary gonadotropin secretion which switches to a high frequency, low amplitude pattern of pulses after progesterone decline (Rahe et al., 1980; Walters and Schallenberger, 1984). Increased LH secretion has a dramatic effect on the follicle and enhances estradiol secretion. Indeed, Baird et al. (1976a) reported that each LH pulse was associated with a pulse in estradiol (perhaps related to surges in substrate availability in the follicle). The mechanism by which steroids control the nature of LH pulses has been elucidated in heifers (Imakawa et al., 1986c). Under progesterone influence, the pituitary releases LH pulses at a low frequency and when luteolysis occurs (and progesterone concentration declines) the frequency of LH pulsing increases. Estrogen maintains the output of LH as the frequency increases during the preovulatory period. The control of LH secretion by steroids has also been demonstrated using immunological approaches. Chang et al. (1987) showed
increased LH pulse amplitude and increased basal LH secretion in heifers actively immunized against progesterone and estrogen. This treatment had no effect on serum FSH concentrations, but increased the size of ovaries and number of follicles greater than 15 mm. Other studies employing immunization against steroids show similar effects (Thomas et al., 1987). An exhaustive review of the actions of ovarian steroids on LH and the LH pulse generator has been written (Karsch, 1987). The fact that estradiol is responsible for the LH surge in cattle has been unequivocally established. Martin et al. (1978) blocked estrogen secretion in cattle and prevented the LH surge. Furthermore, exogenous estradiol can elicit an preovulatory-like LH surge in cows (Beck and Convey, 1977). In order for estradiol to be effective in eliciting the LH surge, progesterone must be low. Both Bolt et al. (1971) and Short et al. (1973) found that changes in gonadotropin secretions characteristic of the preovulatory period could not be initiated in animals that maintained a CL during estrogen treatment. In addition, cows treated with low levels of progesterone have large estrogen active follicles, with highly pulsatile LH secretion, but do not initiate an LH surge (Roberson et al., 1989). There are three mechanisms by which estrogen elicits an LH surge in sheep and cattle (reviewed by Hansel and Convey, 1983). Estradiol increases the ability of the pituitary to release LH and FSH in response to GnRH, up regulates GnRH receptors on the pituitary, and
sets up the timing of the release of GnRH associated with the LH surge. The large release of LH at estrus (responsible for ovulation) occurs because of these mechanisms which increase the responsiveness of the ovary to GnRH and increase the release of GnRH from the hypothalamus (Kesner and Convey, 1982).

The Corpus Luteum

There are two main functions of the LH surge in cattle. First the LH surge is responsible for initiating the final maturation of the oocyte (Moor and Seamark, 1986) as well as initiating the process of ovulation and, second, the LH surge causes the differentiation and luteinization of granulosa and theca cells which will form the subsequent CL of the estrous cycle. Corpus luteum formation proceeds rapidly after ovulation and the CL reaches maximal size by about 7 days after estrus (Duncan et al., 1960; Donaldson and Hansel, 1965; Deane et al., 1966). Two cell types occupy the CL and have unique morphological characteristics (Koos and Hansel, 1981) including large and small sizes (Alila and Hansel., 1984; Fields et al., 1985; Chegeni et al., 1984). The size of the cell has been the primary distinction between the two cell types with small cells ranging from 15 to 18 microns and large cells being larger and ranging up to 45 microns (Chegeni et al., 1984). In order to identify the source of large and small cells, Alila and Hansel (1984) produced monoclonal antibodies to granulosa and thecal cells of the follicle.
They found that their monoclonal antibodies for granulosa cells recognized most often the large cells and that their monoclonal antibodies for thecal cells recognized small cells. These results, although seemingly definitive, have been surrounded by some controversy because of lack of scientific proof that surface epitopes on follicular cell types are necessarily similarly expressed in the CL.

The large cells of the CL are the cell type that primarily produces progesterone (Harrison et al., 1987; Alila et al., 1988). In addition, LH stimulated the secretion of progesterone from small and not large cells (Fritz et al., 1982; Alila et al., 1988). Other differences between large and small cells of the CL include differences in PGF receptor number and affinity (higher in large cells; Fitz et al., 1982). This suggests that the cells of the CL are responding differently to PGF induced luteolysis. In support of this concept, Rao et al. (1979) noted more PGF binding sites with higher affinity later in the estrous cycle when luteolysis normally occurs.

The CL is maintained by the basal secretion of LH from the pituitary and this endocrine regulation of the CL has been reviewed (Keyes and Wiltbank, 1988). The secretion of LH is characterized by low frequency and high amplitude pulses of LH (Rahe et al., 1980). This type of secretion maintains the production of progesterone in a truncated steroidogenic pathway. The hormonal stimulation of the luteal cell has been
summarized by Niswender et al. (1981) and is similar to other cAMP second messenger systems (i.e., binding of LH to the receptor, stimulation of adenylate cyclase, production of cAMP, activation of protein kinases, phosphorylation of substrates, protein synthesis, and finally receptor internalization and recycling). Ovariectomy results in an increase in the concentration of LH secreted by the pituitary and provides a clear example of the steroidal control of GnRH and LH secretion (Hobson and Hansel, 1972). Replacement therapy with both estradiol and progesterone results in a return of the precastration concentrations of LH in the blood (Beck et al., 1976; Karsch et al., 1980).

Other protein molecules critical to reproductive function are now known to be present in the CL. These are most notably oxytocin and relaxin. Oxytocin in found in the CL (Fields et al., 1983; Wathes et al., 1983) and has been specifically localized to the large luteal cells (Harrison et al., 1987). The release of oxytocin parallels the secretion of both progesterone (Walters and Schallenger, 1984; Walters et al., 1984) and PGF. Therefore, there seems to be some involvement of oxytocin in the PGF induced luteolysis in the cow (see below).

Luteolysis

Luteolysis in the cow, sheep, and pig is dependent on the presence of the uterus. Loeb (1923) was the first to demonstrate that removal of the uterus in the guinea pig
caused an extended lifespan of the CL. This finding was substantiated 30 years later in cattle when Wiltbank and Casida (1956) showed that hysterectomy extended CL lifespan.

Of interest in cattle is the local mechanism by which PGF is transferred from its uterine origin to the ovary. This transfer is necessitated because of the high rate of PGF metabolism which occurs in the lung resulting in the inactive metabolite PGFM (Davis et al., 1980). Ginther et al. (1967) were the first to establish the need for local transfer of PGF to the ovary. They demonstrated this by removing the horn of the uterus ipsilateral to the CL and attempting to induce luteolysis with oxytocin (uterine PGF releaser). Their finding, that this preparation prevented luteolysis, demonstrated an apparent need for local PGF transfer. The specific anatomy allowing for the transfer of PGF from the uterine vein to the ovarian artery was first established by Ginther et al. (1973). The two blood vessels are in close apposition in the ewe and cow and it is believed that anastomoses exist or direct transfer of PGF occurs across the vessels. Direct physiological evidence for this transfer is provided by Wolfenson et al. (1985) who measured the amount of PGF in the uterine vein, ovarian artery, and a peripheral artery around the time of luteolysis. They found concentration of PGF equal to 565, 228, and 106 pg/ml in each of these vessels, respectively. The fact that the concentration of PGF in the ovarian artery was 122 pg/ml
higher than the peripheral artery suggests a local transfer of PGF. Earlier evidence that PGF from the uterus was the luteolysin included the finding that exogenous PGF is luteolytic in cattle (Hansel et al., 1973; Lauderdale, 1974), and that high concentration of PGF existed in the uterine and surrounding venous vasculature at the time of luteolysis (Nancarrow et al., 1973; Shemesh and Hansel, 1975b; Lamothe et al., 1977; Bartol et al., 1981a).

The mechanism of luteolysis in cattle has been elucidated recently. It is believed that luteolysis is initiated partially by the ovarian follicle. This concept is supported by the fact that destruction of ovarian follicles late in the estrous cycle results in delayed luteal regression (Milvae et al., 1984). Previous to this finding it was known that estradiol, administered late in the estrous cycle would cause the release of PGF (Barcikowski et al., 1974; Bartol et al., 1981b) and could cause luteolysis (Thatcher et al., 1986). In contrast, estradiol administered early in the estrous cycle does not cause luteolysis and does not result in the release of PGF (Loy et al., 1960). Therefore, there is an apparent need for the uterus to be primed (i.e., stage of the estrous cycle) for the release of estradiol to terminate the estrous cycle. In addition, Silvia and Taylor (1989) have shown that the actual ratio of estradiol to progesterone at the time of an oxytocin challenge is also critical to the uterine release of PGF. Progesterone priming probably is responsible for the
maturation of this system (see Baird et al., 1976b; Ottobre et al., 1980) since administration of progesterone early during the estrous cycle will cause short estrous cycles (Ginther, 1968; Lawson and Cahill, 1983). Garrett et al. (1988) clearly showed that the short estrous cycles (16.7 days vs 21.6 days, in this study) after administration of progesterone during the early part of the cycle was due to an early release of PGF from the uterus. Oxytocin is the other hormone involved in luteolysis and is luteolytic when administered to cattle (Armstrong and Hansel, 1959; Auletta et al., 1972). This is probably a result of the ability of oxytocin to release PGF from the uterus (Milvae and Hansel, 1980). Estradiol may be primarily responsible for the induction of oxytocin receptors in the uterus (Roberts et al., 1976; McCraken et al., 1984), and the content of oxytocin in the CL increases to its greatest amount on days 11 to 18 of the estrous cycle. These findings, taken together, have led to a model for PGF induced luteolysis which was first proposed for the sheep and may also apply to the cow (McCraken et al., 1981, 1984; Thatcher et al., 1989a; Helmer et al., 1989). Luteolysis is initiated by estradiol from the follicle. The action of estradiol on the uterus is twofold. First, estradiol causes a delayed release of PGF (Thatcher et al., 1986) and second, estradiol enhances synthesis of oxytocin receptors (Roberts et al., 1976; McCraken et al., 1984). Secretion of estradiol results in initial release of PGF from the uterus which is transferred to
the ovarian artery by local transfer and causes the release of oxytocin from the CL. This oxytocin, in turn, releases more PGF from the uterus, which releases more oxytocin from the CL. This cycle continues until the oxytocin in the CL is depleted. Additional secretion of estrogen from the follicle could also release more PGF. This cascade of events which occurs between day 17 and 19 in the cow results in functional and structural destruction of the CL. Three to five pulses of PGF (measured by PGFM) are associated with luteolysis in cattle (Kindahl et al., 1976ab). Potential direct methods for the action of PGF on the CL include vasoconstriction of the blood supply to and from the CL (Pharris et al., 1970), immunological rejection of the CL by eosinophils (Murdock, 1987) and macrophages (Poavola, 1979), and possible disruption of the second messenger system (cAMP) for LH (Lahav et al., 1976; Thomas et al., 1978).

Maternal Recognition of Pregnancy

Estrus occurs within 2 to 3 days after the loss of the CL. This system, devised to destroy the CL, does not operate if the animal is pregnant. Early conceptuses secrete proteinaceous products into the uterine lumen. These have been characterized in several species including sheep (Godkin et al., 1982), pigs (Godkin et al., 1982b), and cattle (Bartol et al., 1985). While the products of the sheep (secreted on days 12 to 21) and the cow (secreted on days 16 to 24) are involved in the prevention of luteolysis (Valet et al., 1988;
Helmer et al. (1989), the porcine conceptus proteins (secreted on days 10.5 to 16) are not required to prevent luteolysis (Harney et al., 1989). Knickerbocker et al. (1986a) infused secretory products of the conceptus into the uterine lumen of nonpregnant cow and found that the lifespan of the CL was extended. Helmer et al. (1989) furthered the work of Knickerbocker et al. (1986ab) by purifying and identifying a product of the conceptus which prevented luteolysis when administered into the uterus of cows. This low molecular weight, acidic protein (called bovine trophoblastic protein-1 [bTP-1]) is immunologically related (Helmer et al., 1987) to the sheep protein (ovine trophoblastic protein-1 [oTP-1]) which has been shown to prevent luteolysis in the ovine species (Valet et al., 1988). The action of bTP-1 on the uterine endometrium is being elucidated at this time. Apparently, bTP-1 prevents the synthesis of PGF by the uterine endometrium through a prostaglandin synthetase inhibitor which has been identified (Gross et al., 1987). Recent studies of the molecular biology of the bTP-1 complex (reviewed by Roberts et al., 1990) have demonstrated that the message for bTP-1 is synthesized as early as day 12 of pregnancy and continues through day 25 of pregnancy. However maximal secretion of bTP-1 occurs around the time of luteolysis in the cow (day 15 to 19). The recent finding that bTP-1 is a member of the alpha interferon family (Imakawa et al., 1987a; 1989) has initiated a series of experiments designed to test the
biological activity of associated recombinant bovine interferon α1 in sheep and cattle. Plante et al. (1989) showed that bovine interferon alpha-I-1 could extend interestrus intervals in cattle. At this time, the specific physiological mechanism by which bovine recombinant interferon alpha-I-1 prevents luteolysis is unclear, but bovine recombinant interferon alpha-I-1 injections may have practical application in the improvement of fertility for cattle carrying embryos not secreting enough bTP-1 to prevent normal luteolysis.

**Exogenous Growth Factors and Follicular Function**

The presence of numerous growth factors in the follicular fluid of laboratory and farm animals suggests that there may be a role for exogenous growth factors in regulating follicular growth. Evidence for this in laboratory animals comes from the fact that mice selected for high growth rate and body weight have greater GH and IGF-I concentrations in the serum (Blair et al., 1988; O'Sullivan et al., 1986) and also have greater litter size (Eisen et al., 1973; Eisen and Leatherwood, 1978). The role of IGFs in regulating the number of ovulations in farm animals is inferred in that cows selected for high twinning rate having higher concentrations of IGF-I in the follicular fluid of ovulatory follicles compared to single ovulating cows (Echternkamp et al., 1990). In addition, crossbred sheep with higher IGF-I concentrations in the serum had higher ovulation rates (Zavy et al., 1989).
Finally, women treated with exogenous growth hormone during superovulation prior to in vitro fertilization procedures had shorter follicular phases (17.5 days vs 22 days) and required fewer doses of human menopausal gonadotropin to achieve superovulation (Matson et al., 1989). Therefore a role for exogenous GH (and associated release of IGF-I) in increasing rate of growth of follicles was suggested.

An increase in ovulation rate occurs when additional energy is included in the diet of estrous cycling gilts and sows (reviewed by Anderson and Melampy, 1972). This effect is believed to moderated by insulin which is an ovarian growth factor related to the IGFs. The gonadotropic effect of insulin has been recognized for many years (reviewed by Poretsky and Kalin, 1987). There are two lines of evidence for a link between insulin and the ovary. First, diabetic patients that are not supplemented with insulin have classic symptoms of ovarian hypofunction including amenorrhea, late onset of puberty, anovulation, and low pregnancy rates (Poretsky and Kalin, 1987). In contrast, hyperinsulinemic women, suffering from insulin resistance, characteristically have hyperstimulated ovaries. The action of insulin on the ovary is believed to act primarily through a cross reaction with ovarian IGF-I receptors (evidence reviewed by Poretsky and Kalin, 1987). Cox et al. (1987) tested the effect of dietary flushing and insulin on ovulation rate in pigs. They found that both increased energy (5.7 vs 9.9 Mcal ME/d) and
exogenous insulin (0 vs .1 IU/kg body weight every 6 h) increased ovulation rate (14.0 vs 17.6 and 14.6 vs 17.0, respectively) and the effects of insulin were greatest in gilts fed the high energy diet. However, they found that these effects were independent of gonadotropin secretion or estradiol. This work is contrasted partially by Flowers et al. (1989), who found that higher insulin concentration in nutritionally flushed gilts was associated with greater ovulation rate. However, Flowers et al. (1989) found higher mean FSH concentration and greater number of LH pulses near estrus in flushed gilts.

A biochemical and ultrastructural basis for the effects of insulin on the ovary in swine has recently been reported. Matamoros et al. (1990) found that the amount of follicular atresia during the preovulatory period decreased in gilts treated with insulin when compared to saline treated control gilts. Furthermore, estrogen to progesterone ratio in medium-sized follicles was greater in gilts treated with insulin. Dietary energy appeared to complement the actions of insulin in this study by enhancing the estradiol production of larger follicles. Therefore, both dietary energy and the energy associated hormone--insulin--seem to have synergistic effects on the follicle in the gilt.

The number of ovulations per estrous cycle also is affected by nutritional treatments and/or insulin in cattle. Fasting cows treated with PMSG was effective in reducing
ovulation rate in one study (Lamond, 1970). Furthermore, Harrison and Randel (1986) showed that in energy restricted beef heifers ovulation rate was increased from 1.0 to 7.8 CL per heifer after insulin treatment. This effect was not detected in heifers fed a high plane of nutrition (ovulation rate = 1.7 vs 1.3, control vs insulin, respectively). Therefore the role for insulin is still speculative in adequately fed cattle. Other studies, not involving the use of exogenous hormones, also demonstrated that nutrition affected follicular development in cyclic cattle. Maursse et al. (1985) found that the size of the largest nonatretic follicle was greatest on day 12 for cyclic heifers on a high plane of nutrition compared with control-fed animals.

Growth factors in serum may be critical to our understanding of how nutrition affects the growth of follicles. It has been established clearly that the concentration of IGF-I in the serum of cows in poor nutritional status is low. The reason for low IGF-I in undernourished cattle is a lack of responsiveness of the liver to GH (Elsasser et al., 1989). Elsasser et al. (1989) found that the IGF-I release in response to GH challenge was very small in underfed steers. This underresponsiveness of the liver to GH occurs even though GH is elevated in underfed cattle (Carstairs et al., 1980; Houseknecht et al., 1988). The level of IGF-I seems to be somewhat responsive to the concentration of glucose in the blood (infused glucose
increased IGF-I by 30%) and is very responsive to acute changes in feed intake (Rutter et al., 1989). When cows were fed 50% of required dietary nutrients, serum IGF-I dropped by 50% in 4 days (Rutter et al., 1989). Feed restriction in heifers, as well as humans, has a similar effect on IGF-I levels (Houseknecht et al., 1988; Underwood et al., 1986).

Although growth factor administration has been generally associated with increased follicular development, some evidence in prepubertal gilts suggest a negative effect on the growth of the follicle. Bryan et al. (1989) found that a higher proportion of gilts treated with GH remained acyclic during the prepubertal period (noncyclic gilts = 9 out of 12 for GH, 4 out of 12 for control). This occurred even though the concentration of IGF-I in the follicular fluid and serum of GH treated gilts was higher than control gilts. In addition, proportion of gilts expressing estrus was lower in GH treated pigs (1 of 3 cyclic for GH-treated gilts vs 7 out of 8 for control). The effects of GH on the follicles of these gilts appeared to be related to functional differences in the granulosa cells. Therefore, the anabolic effects of exogenous GH (also demonstrated in this study) do not necessarily complement the ovarian follicular function. McShane et al. (1989) studied the effect of dietary energy and GH treatment on growth rates and puberty in heifers. Treating heifers with GH resulted in increased rate of growth (as expected). Although dietary energy increased the incidence of
heifers reaching puberty before 13 months of age (12 out of 20 for high energy vs. 4 out of 20 for low energy), there was no beneficial or detrimental effect of GH (10 out of 20 for GH-treated vs. 6 out of 20 for vehicle). In addition, although it appeared that a greater proportion of GH-high energy heifers reached puberty earlier than other groups, there were no diet-by-treatment interactions detected for the number of heifers reaching puberty by 13 months of age. Similar studies of GH-treated heifers around the time of first breeding have shown no detrimental effect of GH treatment on conception rate, pregnancy rate, or services per conception (Grings et al., 1990).

As in the gilts described above, growth hormone does not necessarily promote follicular function or fertility when administered to lactating dairy cows. Generally there is a slight reduction in overall fertility in groups of cattle treated with growth hormone (Aguilar et al., 1988; Chalupa et al., 1988; Hard et al., 1988; Huber et al., 1988; Lamb et al., 1988; Palmquist, 1988; Pell et al., 1988; Samuels et al., 1988). The decrease in fertility associated with GH treatment may be due to either a direct effect on the ovary or greater metabolic load on animals treated with growth hormone (Peel and Bauman, 1987). Evidence for an ovarian effect is suggested by Schemm et al. (1990) who found that GH-treated cows had higher plasma progesterone concentration than cows treated with placebo. Furthermore, Schemm et al. (1990)
reported that GH did not alter estradiol in cyclic dairy cattle. However, the number of LH pulses near estrus was increased by GH. Therefore, GH, and/or the altered metabolism induced by GH, acts at the level of the brain or ovary to alter some hormone known to be critical to reproductive function (i.e., progesterone and LH). Given these changes in hormonal function, the authors did not note any difference in the diameter of the largest follicle prior to ovulation for first or second estrous cycles. However, there did appear to be a trend for GH-treated cows to have smaller preovulatory follicles.

**IGF Binding Proteins**

The IGFs are known to be bound in blood and follicular fluid by binding proteins (termed IGF binding proteins [IGF-BP]). The first evidence for low molecular weight binding proteins was found in early reports which suggested that the serum of starved or diabetic rats was inhibitory to the incorporation of sulfate in cartilage (i.e., had a negative effect on sulfation assays; Salmon, 1975; Avasthy et al., 1986). This effect was believed to be a result of a specifically induced IGF-I inhibitor. Early attempts (Kuffer and Herington, 1984) at purification of IGF-I inhibitors suggested that the inhibitor existed in the 16 to 18 kDa range. Later, Ooi and Herington (1986) purified and cross-linked two molecular weight species of IGF-I inhibitors from human serum. They found the major bands binding IGF-I were a
21.5 and 25.5 kDa molecular weight species of binding proteins. Minor binding species were found at 37, 34, and 18 K. Other laboratories (Phillips et al., 1983) working with streptozotocin-induced diabetic rats, reported inhibitor activity in the 24 kDa range which likely corresponds to the inhibitors isolated by Kuffer and Herington (1986). Interest in the action of these inhibitors in diabetic patients relates to the reduced rate of anabolism in diabetics. Therefore, the potential exists for these inhibitors (commonly found in serum of diabetic patients) to mediate this facet of the disease (Phillips et al., 1983). In addition, altered growth of fetuses of diabetic patients may also be a result of these inhibitors acting on growth factors (Sadler et al., 1986).

Besides the presence of the IGF-I binding protein inhibitors in diabetic patients, these proteins can be induced by acute starvation. Acute starvation will not only decrease serum concentrations of IGF-I (Isley et al., 1983; Soliman et al., 1986; Underwood et al., 1986), but will also cause a shift in the type and pattern of IGF-I binding proteins in the serum. McCusker et al. (1989) reported that fasting of newborn pigs resulted in a shift of the intensity of IGF-I binding protein bands. In fasted piglets the amount of binding protein at 43, 39, 34, and 24 kDa was reduced by 11.5, 7.2, 69.8, and 5.2% of control levels while the 29 kDa binding protein increased by 180%. The 29 kDa binding protein which increased in starved individuals probably corresponds to the
inhibitor which was reported in diabetic and starved rats. In actuality, the hormonal control of metabolism is nearly equivalent in starvation and diabetes. This is because both conditions involve low insulin secretion and the mobilization of body nutrients. Therefore, it is not surprising that these binding proteins are induced in both situations. Indeed, insulin does regulate the level of one binding protein (Suikkari et al., 1988). Suikkari et al. (1988) reported that patients with type 1 and 2 diabetes had elevated amounts of the 34 kDa binding protein while patients with insulinoma (pancreatic insulin-producing tumor) had a reduced amount of this species. A characterization of the amount of other IGF-BP was not reported in this study. Taylor et al. (1987) did report that insulin treatment of diabetic rats eliminated the presence of a low molecular weight IGF inhibitor (estimated in the 30 to 50 kDa range) which probably corresponds to the lower molecular weight species identified by Phillips et al (1983) and Ooi and Herrington (1986). The discrepancy in molecular weight is probably related to the use of ultrafiltration membranes for molecular weight estimation in the Taylor et al. (1987) study.

The relevance of IGF-BP to the postpartum cow relates to the fact that postpartum cows are mobilizing nutrients and have very low insulin concentrations in the blood. Therefore, it would not be surprising if postpartum cattle express IGF-I inhibitors as part of their full complement of IGF-BP (Hossner
et al., 1988). In addition, these binding proteins are expressed in the ovary. Mondschein et al. (1989) reported, that although the dominant binding protein in follicular fluid of pigs was the 45 kDa molecular weight species, cultured granulosa cells secreted primarily the 28 and 34 kDa species. Therefore, the origin of the 45 kDa species was left unclear. Human ovary granulosa cells also secrete IGF-BP which further supports the cross-species nature of these proteins in the follicle (Suikkari et al., 1989). The exact role of the IGF-BP in the ovary is unclear. However, they may have an inhibitory role in the ovary as well. Ui et al. (1989) showed that a protein with high amino acid sequence homology with the human 53 kDa IGF-BP was inhibitory to the FSH-stimulated estrogen production in granulosa cells. This may suggest a role for IGF-BP in regulating follicle growth. Furthermore, Adashi et al. (1990) showed that the array of IGF-BP secreted by granulosa cells in vitro was altered by FSH treatment. In this study, there was a substantial reduction in the 23 kDa and the 28 kDa binding proteins with FSH treatment. They suggest that this represents a mechanism by which FSH (which is considered stimulatory to the granulosa cell) acts to reduce the amount of a substance which is inhibitory to growth factor function in the cell. At this time, however, further evidence for this hypothesis needs to be presented.
Factors Affecting Fertility and Return to Estrus in Cattle

The previous sections of this review have dealt primarily with the classical physiological events which occur in cattle during late pregnancy and during the early postpartum period. The author has tried to review a normal series of events which eventually leads to pregnancy in cattle. The following sections of this review will address more specifically the physiological mechanisms which exist in postpartum cattle and may be involved in the extent with which cows return to estrus and become pregnant. Obviously, the nutrition of the postpartum cow is of primary importance to the successful return to estrus. This subject has been reviewed extensively (see reviews by Butler and Smith, 1989 and Short, 1990). The remainder of the literature review will examine several factors including nutrition which may be critical to solving problems associated with reproductive abnormalities in the postpartum cow.

Suckling

Beef cows that are nursing young have a delayed first ovulation compared with dairy cows that are mechanically milked. This phenomenon, known as suckling induced anestrus, has been intensely studied in beef cows. Anestrus occurs in suckled cows even though the pituitary gland remains responsive to GnRH throughout the postpartum period (Paret et al., 1986; Jaeger et al., 1987). Opiod peptides (associated with maternal behavior) apparently modulate suckling-induced
anestrus and various forms are found in high concentrations in the suckled-cow hypothalamus (Malven et al., 1986). Infusion of naloxone (an opioid antagonist) will increase serum LH in suckled cows (Gregg et al., 1986; Whisnant et al., 1986). Furthermore, binding sites for naloxone in the brain were higher in anestrous suckled cows compared with cyclic suckled cows (Trout and Malven, 1988). A similar mechanism of opioid induced anestrus appears to exist in lactating sows. Infusion of morphine into sows around the time of weaning will delay postweaning estrus and decrease LH secretion (Armstrong et al. (1988). As with the sow, infusion of morphine in cattle decreased LH secretion, but did not affect FSH (Peck et al., 1988). Therefore, opioid modulation of reproductive activity in lactating farm animals seems to occur across several species. The additional possibility that oxytocin is a mediator in postpartum anestrus has been tested (Stewart and Stevenson, 1987). Suckled beef cattle are exposed potentially to more surges of oxytocin compared with dairy cows milked twice daily. However, six daily injections of oxytocin into dairy cattle did not delay interval to postpartum ovulation (Stewart and Stevenson, 1987).

In addition to opioid modulation of GnRH secretion in postpartum suckled cows, increased sensitivity of the pituitary and hypothalamus to the negative feedback effects of estradiol apparently plays some role in this phenomenon. This is supported by work of Acosta et al. (1983) who found that
suckling increased the sensitivity of LH release to estradiol. In addition, Chang and Reeves (1987) decreased postpartum anestrus from 96 days (control) to 45 days (treated) in postpartum suckled cows given an antiestrogen (enclomiphene).

**Plasma Metabolites and Reproductive Function**

The mobilization of body nutrients which occurs in postpartum cattle results in an array of circulating metabolites which are characteristic of an animal that is undergoing body nutrient depletion. The possibility exists that the concentration of various metabolites in the blood may affected directly the function of the ovary. Huszenicza (1988) studied the metabolic characteristics of cows with different classifications of postpartum reproductive function (i.e., early ovulation, late ovulation, cystic, anestrus, or irregular ovulation). Cows with late ovulation, anestrus, or irregular ovulation had a higher concentration of nonesterified fatty acids, and ketone bodies in the blood, whereas these same cows had lower total cholesterol and carotene. In contrast, plasma metabolites were similar in normal and cystic cows. Therefore, certain metabolites are associated with some cases of abnormal ovarian function. It is not clear, however, whether they directly influence ovarian processes.

Glucose availability seems to be an important factor in determining the level of reproductive function in mammals. It is believed that glucose and the associated hormone--insulin--
are critical determinants of an animal's overall energy status. Recently, insulin was found in the cerebral spinal fluid and insulin receptors are known to exist in the brain of laboratory animals (reviewed by Baskin et al., 1987). Therefore, insulin is a likely hormonal link between the metabolic status of the animal and pituitary hormones (i.e., gonadotropins). Direct evidence of the importance of glucose and insulin on reproductive function exists in laboratory and farm animals. For example, Schneider and Wade (1989) found that when they blocked glycolysis and fatty acid oxidation in food-restricted Syrian Hamsters they induced anestrus. Hamsters remained cyclic as long as one of these pathways remained functional (even though they were food deprived). Therefore, functional anestrus which occurs normally in food deprived hamsters is not a result of the loss of body tissue, but to the eventual loss of metabolizable substrates.

Evidence exists in farm animals for a link between glucose and postpartum reproductive function. Patil and Deshpande (1979) reported that anestrous Gir cows had lower blood glucose and serum protein concentration than cows exhibiting estrus during the postpartum period. In addition, when Carstairs et al. (1980) fed postpartum dairy cows at different levels of nutrition, they found that serum glucose was correlated negatively with interval to first ovulation. This is not always reported consistently because Kappel et al. (1984) found no relationship between blood glucose and
interval from calving to conception. However, serum cholesterol was related inversely to interval from calving to conception in this study. Low glucose and insulin concentrations also are characteristic of beef cows induced into anestrus by long term nutrient restriction (Richards et al., 1989b). Maintaining low concentrations of glucose in the blood of beef cows during the postpartum period by infusion of phloridzin (a chemical which increases glucose clearance from the blood) decreased the secretion of LH (Rutter and Manns, 1987). Rutter and Manns (1987) demonstrated that postpartum suckled beef cows and phloridzin-treated beef cows had similar LH profiles (i.e., small amplitude LH pulses) which were different from nonsuckled, saline-infused postpartum cattle examined in this study (large amplitude LH pulses). Later studies (Rutter and Manns, 1988) showed that phlorizin treatment during the preovulatory period resulted in lower mean concentration of LH (1.17 vs 1.53 ng/ml) and lower LH pulse amplitude (1.69 vs 2.47 ng/ml) in cycling beef cows. However, most phlorizin-treated cows eventually ovulated. Finally, McCann and Hansel (1986) found that heifers fasted during the preovulatory period had lower plasma LH concentrations which were associated with reduced plasma insulin and glucose concentrations. Two of the four fasted heifers subsequently had normal ovulations and CL development, while the remaining two heifers experienced either delayed ovulations or abnormal CL development (based on progesterone
concentrations). Therefore, based on this study and the phlorizin work of Rutter and Manns (1987; 1988), a plausible link between glucose and gonadotropin secretion was established. However, it is not clear if these studies, using beef cattle and heifers, apply to early lactation dairy cattle at a time when large quantities of glucose are required for milk production. Certainly, a tremendous amount of glucose (approximately 2 kg daily into milk) is utilized in the postpartum cow, whereas concentrations of glucose in the blood remain low and fairly stable (see Chapter 4). Therefore, similar studies need to be performed in dairy cows in early lactation.

Although this work has concentrated on direct effects of glucose on gonadotropin secretion and CL formation, it should be recognized that glucose is critical to normal embryonic development. Clinical evidence for this is found in the reduced fertility of diabetic women (Poretsky and Kalin, 1987). Diamond et al. (1989) tested the effects of streptozotocin and alloxan-induced diabetes on oocyte maturation and embryo development in the mouse. Oocytes from diabetic mice had a lower percentage of germinal vesicle breakdown and slower embryonic development. Both of these deficiencies were corrected by insulin treatment. Therefore, embryo survival in cycling cows also may be dependent on adequate glucose.
Diet-Derived Modulators of Ovarian Function

Cattle consume dry matter in excess of 2 to 4% of their body weight on a daily basis. Therefore, feeds and feed ingredients should be examined as one possible modulator of ovarian function. The use of ionophores in ruminant rations may affect the ovary. Ionophores act within the rumen to shift volatile fatty acid production towards greater propionate and less acetate. These chemicals are used commonly in growing beef cattle rations to increase rate of gain and in finishing rations to improve conversion of feed intake to body weight gain, but are rarely used in dairy rations because of reduced milk fat percentage associated with reduced acetate production. Interestingly, the use of ionophores can alter ovarian and hypothalamic responses (recently reviewed by Sprott et al., 1988). Increased sensitivity of the pituitary to GnRH challenge (Rutter et al., 1983) and greater follicular growth in superovulated cattle were reported in heifers and cows fed ionophores. The possibility that ratios of volatile fatty acids directly affect ovarian function may be one area of nutrition/reproduction research which will receive increased attention in the future. In addition, potential direct effects of ionophores on the ovary, including tissue permeability and fluidity, need to be examined.

More direct environmental modulators of ovarian function exist in nature. The most notable of these is the plant
compound 6-methoxybenzoazolinone (6-MBOA) which is found in young growing seedlings of rye, corn, and wheat. This compound, when consumed by rats or pine voles, will stimulate follicular development on the ovary (Butterstein and Schadler, 1988; Schadler et al., 1988). The mechanism by which 6-MBOA stimulates the ovary is unclear. However, evidence exists that this compound either stimulates FSH secretion (Schadler et al., 1988) or interacts with FSH to enhance its action (Butterstein and Schadler, 1988). These naturally occurring cyclic carbamates are believed to be environmental cues which synchronize the initiation of reproductive activity with the springtime growth of new grasses (Sanders et al., 1981). There are no reports of the activity of these compounds in farm animals at this time. However, the cross-species evidence presented here suggests their potential for action in nonrodents.

Attempts to control the reproductive performance of lactating cows using various dietary ingredients may yield important economic benefits to producers. It is obvious that the first step in adequate reproductive performance in lactating cows is correct ration formulation (see Swanson, 1989). Certainly, gross deficiencies in dietary minerals (reviewed by Hurley and Doane, 1989) or improperly balanced protein in dairy rations (reviewed by Ferguson and Chalupa, 1989) or inadequate energy (reviewed by Butler and Smith, 1989), will affect reproductive function adversely. The
future goal of nutrition/reproduction research should be to evaluate how different formulations and ration ingredients affect ovarian function so that dietary ingredients beneficial to reproductive performance can be identified and credited for their specific roles in reproduction.

Currently, one of the most often mentioned nutritional factors thought to be beneficial to reproductive performance is dietary fat supplementation. Dietary fats are believed to enhance reproductive performance of postpartum dairy cattle through the alleviation of energy deficiencies commonly experienced during the postpartum period (reviewed by Palmquist and Jenkins, 1980). Dietary fats can be fed either prilled (i.e., granulated) or as a calcium salt of long-chain fatty acids (CaLCFA). Either form was shown to have little effect on the function of the rumen. This was tested by in vitro (Jenkins and Palmquist, 1982; Chalupa et al., 1984) and in vivo methods (Chalupa et al., 1984; Grummer, 1988; Jenkins and Palmquist, 1984). Calcium salts of long-chain fatty acids are insoluble in the rumen because the calcium remains bound to the fatty acid as an insoluble soap at neutral or slightly acidic pH. Upon passage to the abomasum (and exposure to lower pH) the calcium and the fat are dissociated and unesterified fatty acids move into the small intestine. Intestinal digestion and absorption of fatty acids have been reviewed (Palmquist and Jenkins, 1980).
Different compositions of dietary fats can be prepared for feeding depending on the method of formulation. Most prilled fats and CaLCFA are primarily saturated because the physical nature of the fats are better suited for the mechanical processes involved with their production (i.e., polyunsaturated fats are liquid at room temperature, while most monounsaturated or saturated fats are solids). Methods do exist to incorporate polyunsaturated fats into dairy rations. Scott et al. (1971) outlined a procedure for the preparation of polyunsaturated fats which are resistant to rumen degradation, but degraded in the acidic environment of the abomasum. He used a casein/formaldehyde matrix to encase safflower oil droplets. Supplementing the ration of sheep with these particles resulted in a shift in the amount of linoleic acid in the depot fat (from 2.8% to 28.9% linoleic acid in the depot fat for basal and supplemented diets, respectively). In addition, the amounts of oleic and stearic acid in the body were reduced. A similar shift in the composition of fats can be achieved by feeding dairy or beef animals protected polyunsaturated fats. For example, Garret et al. (1976) increased the amount of linoleic acid in the tailhead fat of steers from 2.0 to 12.1% by feeding protected lipids.

The composition of milk fat in dairy cows generally reflects the composition of fats which exit the rumen. Therefore, feeding protected fats will increase the amount of
polyunsaturated fats in milk. Tove and Mochrie (1963) acutely altered the composition of milk fat by infusing an intravenous emulsion of soybean oil. In cows infused with 900 g of soybean oil, the linoleic acid composition of milk fat increased to over 20% for 2 days. Longer periods of supplementation of cattle with polyunsaturated fats also will stably elevate polyunsaturated fats in milk (Mattos and Palmquist, 1975). These data concerning the manipulation of linoleic acid content of the diet may be pertinent to future attempts to manipulate prostaglandin dynamics in postpartum or cyclic cattle (see Chapter 2 and below).

The specific effect of fat feeding on reproductive performance is not clear. Rhodes et al. (1978) fed prepubertal heifers protected tallow and delayed puberty. They reported that only 34% (10/29) heifers reached puberty while being fed the fat product while 80% (24/30) of control heifers reached puberty during the same time period. Therefore, altering the lipid intake of the prepubertal animal may have detrimental effects on the growth and development of the ovary by an undetermined mechanism. In cycling animals, Talavera et al. (1985) found that feeding hypercholesterolemic diets either reduced (early estrous cycle) or increased (late estrous cycle) the concentrations of progesterone in the blood. Similarly, Carroll et al. (in press) reported greater progesterone concentrations during the luteal phase in cows fed 5% prilled long chain fatty acids. This suggests a stage
specific relationship between fat feeding and ovarian function. There does seem to be a positive effect of high fat feeding on the postpartum performance of beef cattle. Williams (1989) reported that feeding a high lipid diet (containing 30% whole cottonseed) caused a higher incidence of low-level progesterone release prior to first ovulation compared to nonlipid fed animals (81.2% vs 37.5%, lipid vs control, respectively). Subsequent calf removal and GnRH treatment resulted in longer luteal phase of induced corpora lutea in lipid-fed cows (15.3 days vs 7.2 days). Therefore, feeding a high lipid diet to beef cattle during the postpartum period can, under these conditions, reduce the incidence of short cycles after first ovulation. The mechanism for this probably is related to short-term elevations in progesterone experienced by these lipid-fed cattle. This could result in progesterone priming of the uterus or follicle before first ovulation and lead to estrous cycles of normal length. The role of cholesterol and lipoprotein metabolism in ovarian function has been reviewed (Grummer and Carroll, 1988).

The evidence for an effect of lipid supplementation on dairy cattle performance is less clear. Extra dietary lipid may or may not improve reproductive performance and this may depend upon whether or not the lactating animal responds to fat feeding with greater milk production. Ferguson (1988) found that feeding prilled fat (.45 kg/d) resulted in a 20% increase in first service conception rate and a slight
reduction in days open. Alternatively, when CaLCFA were fed to a different group of cattle reproductive performance was unchanged (although nonsignificant declines in first service conception rate and overall conception rate were associated with fat feeding). The lack of an effect of fat on reproductive performance in this study may have been related to the greater milk production in these cows. Additional evidence for a positive effect of hard fats (prilled fats) on reproductive performance and a negative effect of CaLCFA on reproductive performance is provided by Chalupa and Ferguson (1988). Further evidence suggesting a negative effect of CaLCFA on reproductive performance are presented in Chapter 2. 

Uterine PGF Release and Ovarian Function

The postpartum release of PGF from the uterus has been studied as one modulator of reproductive efficiency during the postpartum period. As reviewed earlier, the uterus of the postpartum cow releases significant amounts of PGF and this release is correlated with the rate of uterine involution (Eley et al., 1981; Lindell et al., 1982; Guilbault et al., 1985a). There is also evidence that the release of PGF in postpartum cattle can influence how the ovary functions during this period. Direct evidence for this comes from several sources. First, injection of PGF into postpartum cattle improved first service conception rates in some studies. Young et al. (1984) found that when 25 mg PGF was injected into cows during the early postpartum period (14 to 28 days
postpartum), first service conception rate was increased from 43% to 64%. This increase occurred even in cows that did not have a functional CL at the time of injection (first service conception rate = 70% vs 44%, PGF treated vs control, respectively). Therefore, they concluded that the effect of PGF at this time was not related to the additional estrous cycle that a PGF-treated cow might experience after PGF. In contrast to these data, Benmrad and Stevenson (1985) did not show significantly higher fertility at first service in cows treated with PGF at a similar time postpartum (first service conception rate = 42 vs 29%, PGF-treated vs control, respectively). However, cows treated with PGF had a shorter interval from calving to conception (days open; 86 vs 115 days; treated vs control, respectively).

A second line of evidence which implicates postpartum PGF in the functioning of the ovary is derived from the relationship between postpartum PGFM concentrations and various measures of ovarian activity. For example, Madej et al. (1984) reported that there was a significant correlation between postpartum PGFM and interval to first ovulation followed by a normal luteal phase (r=-.37). A more definitive test of the relationship between PGF and uterine and ovarian responses was performed by Guilbault et al. (1987ab). These researchers blocked the postpartum release of PGF using flunixin meglumine (FM) and measured uterine and ovarian morphology with or without PGF replacement. An untreated
group was also included. These treatments resulted in a similar rate of uterine and cervical involution. In addition, percentage of active ovaries (defined as those having a CL and/or follicle by palpation) was lower in FM treated cows. However, those cows which received PGF in addition to FM had a greater percentage of active ovaries on the side ipsilateral to the previous pregnancy (44.8% vs 27.8%, treated vs saline, respectively). Further analysis of blood progesterone and estradiol supported the concept that the PGF replacement may have enhanced the activity of the ovaries in FM-treated cows (Guilbault et al., 1987ab).

Histological evidence also supports the concept of improved follicular growth in cows treated with PGF. Villeneuve et al. (1989) infused postpartum beef cows with PGF from days 2 to 13 after parturition and measured changes in follicle populations by histology. Prostaglandin infusion resulted in an increased diameter of the largest follicle on the ipsilateral (day 15 and 35 postpartum) and contralateral (day 15 only) ovaries. They also found that PGF infusion increased the size of the largest, second largest, and third largest nonatretic follicle during this period. Therefore, this direct histological evidence demonstrates the effect of PGF on ovarian follicular growth.

There may be alternative means of increasing ovarian follicular growth through enhanced prostaglandin synthesis. The previous studies relied on intravenous infusion or
intramuscular injection of PGF. Linoleic acid is the essential fatty acid precursor of arachidonic acid and the prostaglandins (reviewed by Oliw et al., 1983; Rivers and Frankel, 1981). It is possible to feed diets high in linoleic acid to increase the amount of arachidonic acid in membrane lipids and enhance prostaglandin in the circulation of humans and laboratory animals (Dupont et al., 1978; Mathias and Dupont, 1979; Adam et al., 1982). Furthermore, feeding specific long chain fatty acids can alter plasma cholesterol content (Garg et al., 1988) which has been related to ovarian function in some studies (Kappel et al., 1984). Therefore, ratios of specific fatty acids fed ruminants may have dramatic effects on arachidonic acid, prostaglandins, and cholesterol. The ability of fatty acids which were either infused into the circulation or fed as CaLCFA to alter prostaglandin dynamics and ovarian function has been tested in cattle (see Chapters 3 and 5). There were no detrimental effects of feeding different fatty acids on fetal development, pregnancy, or lactation in one study in pigs (Opstvedt et al., 1988).

An additional method to increase the release of prostaglandins in the postpartum cow is to manually stimulate the uterus. This was demonstrated to be an effective method to increase postpartum prostaglandin in one study (Wann and Randel, 1990). While this manipulation was found to increase PGFM on day 35 postpartum, multiparous and primiparous cows
that had their uterine horns manually stimulated on day 35 postpartum had similar intervals to first estrus.

Use of GnRH to Stimulate Postpartum Ovarian Function

Anestrous cows typically have an adequate amount of pituitary LH, but this LH is not released and is not available to stimulate ovarian function. Therefore, several studies have been implemented to attempt to simulate GnRH release patterns and increase the amount of LH released in postpartum cows. The first report related to this concept was in prepubertal heifers. Skaggs et al. (1986) treated prepubertal heifers with either continuous infusion of LHRH (via minipumps), pulsatile infusion of LHRH (manual injection), or control (saline infused). Both LHRH infusion and pulsatile injection resulted in estradiol increases, preovulatory LH surges, and subsequent development of a short-lived CL. However, these effects were not sustained and LHRH-treated heifers reached puberty at the same time or later than control heifers. Spicer et al. (1986a) were initially unsuccessful in inducing ovulations in anestrous postpartum suckled beef cows which were treated with LHRH every 2 hours for 48 or 96 hours. Their treatment did not alter populations of follicles on the ovary. This suggested that the LHRH treatment may have been administered for too short a time period. Longer term administration of LHRH (.4 to 25.6 ug of LHRH released per day) using microencapsulation was also unsuccessful in shortening postpartum anestrus in postpartum suckled beef cows.
(Roberts et al., 1989) although mean concentrations of LH were increased by the higher LHRH dosage. D'Occhio et al. (1989) tested the same concept using either GnRH or GnRH agonist (Buserelin). Postpartum suckled beef cows were treated with GnRH or agonist for 28 days using osmotic minipumps. This treatment induced short-lived CL in 8 out of 9 GnRH treated cows. However, there was no sustained effect and cows returned to anestrus after this initial short cycle. A similar inability to change postpartum interval using exogenous hormonal treatments was found in estradiol-treated suckled beef cattle (Garcia-Winder et al., 1986). Therefore, these studies suggest that although it is possible to simulate the hormonal events (i.e., GnRH and LH secretion) which are characteristic of puberty and the reinitiation of estrous cycles after calving, there seems to be other contributing factors (including suckling) which limit sustained follicular growth during this period. Therefore, the ability to control postpartum anestrus and puberty may lie within these undiscovered control mechanisms as well as a better understanding of how suckling affects GnRH secretion. In work related to GnRH-associated anestrus, Traywick and Esbenshade (1988) have actively immunized gilts against GnRH. This immunization results in anestrus because of loss of LH secretion from the pituitary. Interestingly, when immunized gilts were pulsed with GnRH, the induced anestrus could not be overridden and gilts remained anestrus (Traywick and
Therefore, immunologically-induced barriers to normal follicular growth and development can potentially be used to prevent unwanted pregnancy in farm animals.

Prepartum Body Condition and Fertility

There exist a potential relationship between the size, body composition, and weight of an animal during the prepartum period and her reproductive performance after she calves. This concept is based on the need for body nutrients to support lactation and the idea that there is a critical amount of body stores required to maintain reproduction. Richards et al. (1986) tested this concept in postpartum beef cows and found that body condition at calving was the most important factor determining postpartum reproductive performance. Calf weight, however, was more dependent on postpartum nutrition (Richards et al., 1986). In addition, Corah et al. (1975) found that calf mortality was least and percentage of cows showing estrus by 40 days postpartum greatest in animals fed 100% of NRC requirements compared with animals fed 65% of NRC requirements for the 100 days prior to calving. The prepartum body condition (specifically overconditioning) may not be as critical in dairy cattle. Butler and Smith (1989), working with dairy cattle, found that overconditioned, control, and overconditioned cows that were losing weight during the dry period, had similar postpartum reproductive performance and were not different in terms of their susceptibility to
postpartum metabolic disorders or disease. This contrasts to the findings of other researchers. Others have concentrated on the change in body condition during the postpartum period and fertility. Selk et al. (1988) studied range cattle for 5 years and concluded that the major factors which affect pregnancy rate in range beef cows were body condition score at calving and at the initiation of the breeding season. This work resulted in the development of a regression equation for the sigmoidal relationship between pregnancy rate and body condition score (BCS; \( \% \text{ preg} = 1.28 - 0.986 \times \text{BCS} + 0.248 \times \text{BCS}^2 - 0.016 \times \text{BCS}^3 \)).

Cows with severely reduced body condition during the postpartum period can recover from the effects of nutrient restriction (Richards et al., 1989a). Therefore, postpartum feeding regimens designed to increase nutrient intake in these beef animals should allow for full recovery. Houghton et al. (1990) found that whereas thin cows had a longer interval to first insemination, their first service conception rate was actually higher than for cows calving with average or above average body condition. Their study suggested that cows that are thin or moderately obese at parturition should be managed to reach an average body condition during the breeding season. This is because thin or moderately obese cows either losing or gaining weight (respectively) as they approached the breeding season had lowest overall fertility. It is clear at this time, however, while several studies demonstrate the
importance of prepartum body condition to postpartum reproductive performance, other researchers find no effect of prepartum feeding on reproductive efficiency (Boyd et al., 1987). This may relate to relative body condition (based on subjective body score measurements) of cows across different studies.

Other factors which affect reproduction in postpartum beef cows include frame size and management. As might be expected, Buttram and Willham (1989) found that cows from different herds (i.e., management options) differed in terms of reproductive performance (calving rates varied from 75.3 to 68.4%). In addition, across herds, small frame beef cattle were more efficient reproductively than larger cows (percent calving = 85.0, 78.1, 70.7 for second parity, and 80.9, 66.1, and 68.7 for third parity, small, medium, and large frame, respectively). Therefore, the scientific evidence showing that smaller frame dairy cattle (i.e., Jersey breed) are reproductively more efficient than larger Holstein cows (Badinga et al., 1985) are supported by these data.

**Energy Balance and Reproductive Function**

Few topics in the nutrition/reproduction literature have received as much attention as the relationship between energy balance and reproduction. In general, it is believed that cows in lower energy balance have poorer reproductive performance. Postpartum energy balance is an arithmetic calculation based on dietary energy intake, and the nutrient
requirements for maintenance and lactation of the animal. It is advantageous to use energy balance instead of milk production when studying reproductive indices because nutrient deficiencies occur when cattle do not consume appropriate amounts of feed, and not necessarily when cows produce large quantities of milk. Therefore, some lower producing cows consume small amounts of feed and have equal or lower energy balance compared with higher producing individuals that have greater capacity to consume feed.

The concepts surrounding energy balance and reproduction have been reviewed (Butler and Canfield, 1989; Butler and Smith, 1989). The mechanism by which energy balance exerts its effects is believed to be related to changes in the secretion of LH from the pituitary. There have been numerous reports detailing the effect of energy and energy restriction on the secretion of LH. Tatman et al. (1990) found that nutrient restriction would decrease the amount of LH secreted from the pituitary in ovariectomized ewes. However, a critical level of adipose tissue must be lost before LH secretion and pituitary content of LH are affected. Similar dependence on adipose tissue mass has been suggested by other researchers. Richards et al. (1989a) found that nutrient restricted beef cows had reduced LH and became anestrous after the body condition score went below 3.5 (1 to 9 scale).

Prepartum nutrition can influence the postpartum resumption of gonadotropin secretion. One study suggests that
the postpartum release of LH to a GnRH challenge is greatest in cows fed to gain weight during the prepartum period compared with maintenance or below maintenance-fed cows (Killen et al., 1989b). These results were opposite to those obtained for cows during the prepartum period where cows losing body weight released the most LH in response to GnRH (Killen et al., 1989ab). It is interesting to speculate that cows losing body weight may have had different steroid concentrations during late pregnancy and this may have led to differential GnRH release. Boyd et al. (1987) did find differences in prepartum estrone sulfate and progesterone concentration in cows fed high and medium planes of nutrition. However, their findings (greater progesterone and estrone sulfate concentration in medium-fed cows compared with high-fed cows) suggest that underfed cows have less, not more LH secretion. In addition, Killen et al. (1989a) found no difference in prepartum concentrations of progesterone or estrogen in blood of control-fed compared with nutritionally-restricted beef cows.

Undernutrition may affect the secretion of LH by increasing the sensitivity of the pituitary gland to estradiol. Indeed, Imakawa et al. (1987b) found that when nutrient-restricted ovariectomized heifers were treated with estradiol, LH secretion was suppressed. This did not occur in control heifers. This work agrees with work in underfed sheep where undernutrition also increased the inhibitory effect of
estradiol on LH secretion (Davis et al., 1987). Therefore, the general mechanism of increased sensitivity of the hypothalamus or pituitary to steroids seems to exist in the underfed condition and is similar to other physiological states of anestrus (i.e., puberty [Kinder et al., 1987] and seasonal anestrus [Karsch et al., 1984]). Interestingly, when similar nutrient-restricted heifers were ovariectomized and treated with estradiol, the sensitivity of LH secretion to estradiol remained during a refeeding period designed to reinitiate estrous cycles (as occurred in contemporary control cows; Imakawa et al., 1986a). The effects of energy restriction on LH secretion can be elicited in prepubertal as well as postpartum animals. Day et al. (1986) found that energy restricted prepubertal beef heifers had decreased concentrations of LH in serum, as well as a reduced responsiveness to a GnRH challenge. Finally, unlike other scenarios of postpartum anestrus (i.e., suckling), opioid peptides may not play a role in decreasing LH secretion in nutrient-restricted animals. Canfield and Butler (1989) did not show enhanced LH secretion when anestrous postpartum dairy cows were infused with naloxone. Therefore, other mechanisms (including enhanced sensitivity to estrogens) seem to be mediating postpartum anestrus associated with negative energy balance in farm animals.

One of the clearest suggestions that there is a negative association between energy balance and reproduction is the
steadily declining fertility of dairy cows because the development and implementation of artificial insemination (Butler and Smith, 1989). While the amount of milk produced per cow has increased since 1952, conception rates to artificial insemination have dropped from 66% to about 50% (current rate). This has occurred despite constant conception rates in virgin heifers during this period.

A putative relationship between energy balance and reproductive function was first tested by Butler et al. (1981). They reported that there was a negative correlation between postpartum energy balance and interval to first ovulation. In addition, they found that first postpartum ovulation occurred 10 days after minimum energy balance was achieved. Stevenson and Britt (1980) proposed a hypothesis which was based on their multiple regression analysis of factors which affect the interval from calving to first ovulation. The most important factors determining interval to first ovulation were serum estrogen and number of follicles greater than 10 mm during the first 2 weeks postpartum. They concluded that milk production and energy intake during the first 2 weeks were not critical to interval to first ovulation based on their multiple regression approach. This interpretation of the data may be somewhat flawed because their model included certain overriding factors (i.e., number of large follicles by day 10) which may have masked the importance of other effects. The more critical question is
what controls the concentration of estrogen in the blood and the number of large follicles on the ovary by day 10 (i.e., these should have been the dependent not independent variables in this study). They did report a negative relationship between body weight change and interval to first ovulation which supports the concept that changes in energy balance (manifested in loss or gain of body weight) will influence time of first ovulation. In related work, Staples et al. (1990) reported an antagonistic relationship between energy balance and postpartum reproductive function. They found that when they divided cows into three groups based on interval to first ovulation, anestrous cows (not ovulating for 60 days after calving) consumed less feed and produced less milk than cows ovulating earlier during the postpartum period. These cows also had lower energy balance during week 2 postpartum. Their conclusion was that these anestrous cows utilized a greater amount of body reserve during the first 2 weeks of lactation than other cows, possibly leading to a lack of ovarian function. This study is unique because it demonstrates that energy balance is not necessarily inversely related to milk production and that lower producing cows, consuming less feed, can be in greater negative energy balance compared with higher producing cows consuming more feed. Other classic papers have presented similar evidence that milk production is not necessarily negatively associated with interval to first ovulation (Marion and Gier, 1968).
The relationship between milk production and reproduction is often studied using large data sets available through the Dairy Herd Improvement Association. These records were analyzed by Laben et al. (1982). A small but antagonistic relationship between milk production and reproduction was detected when they examined 130,000 records. Days to first insemination, days open, and services per cow increased by .27, .61, and .014, respectively, for each 100 kg increase in milk production. However, highest producing herds did not always have the poorest reproductive statistics suggesting that better management can overcome the otherwise negative relationship between milk production and reproduction. Hillers et al. (1984) examined 2800 first inseminations and found that first service conception rate did not depend on milk production but interval to first service increased from 71 days to 81 days as milk production increased from 5444 to 9981 kg. These data agree with those of Laben et al. (1982) because there was an increase of about .2 days to first service for each 100 kg increase in milk production in these cows. Finally, Badinga et al. (1985) examined 2263 Holstein and Jersey records and showed that the genetic correlation between milk yield and services per conception was .38 and that the genetic correlation between services per conception and body weight was .37. Therefore, a large genetic antagonism appeared to exist among milk yield, body weight, and reproductive efficiency.
It is not clear how negative energy balance causes a decline in cattle fertility. Certainly a direct effect of negative energy balance on the embryo may exist. Krackow (1989) found that energy restriction during lactation in mice caused preimplantation death of embryos. In addition, diabetic mice were found to have embryos with a decreased rate of germinal vesicle break-down, as well as abnormal development (Diamond et al., 1989). These abnormalities were corrected with insulin replacement. Whether low insulin in postpartum dairy cows is having a similar effect is unclear. One hypothesis for farm animals (suggested by Butler and Smith, 1989) is that because cows in more negative energy balance ovulate later during the postpartum period they are disadvantaged at the time of first insemination. This is based on the work of Thatcher and Wilcox (1973) who found that cows that had more postpartum estruses prior to 60 days postpartum were more fertile (services per conception = 2.60, 2.58, 2.32, 2.21, and 1.75, for cows having 0, 1, 2, 3, and 4 heats respectively). The time of first ovulation may be best estimated from time of energy balance nadir during the postpartum period (Butler and Canfield, 1989). Energy balance nadir is defined as the time of lowest energy balance (Mcal/day) during the early postpartum period. Cows seem to ovulate about 10 days after they pass this point of lowest energy balance and this relationship has been described (days to first ovulation = 10.4 + 1.2 x [days to nadir]). Validity
of this relationship is questionable for the following reasons. First, there is very little variation in the time when cows reach EB nadir (usually within 5 to 15 days after calving, see Butler and Smith, 1989; Chapters 2 and 4) and second, most Holstein cows ovulate between 15 and 25 days postpartum. Therefore, the finding that cows ovulate 10 days after EB nadir may be related to timing of changes in energy balance and the timing of events of reproduction which may be independent of each other. Certainly, cows ovulating late during the postpartum period have an interval to EB nadir which is similar to earlier ovulating cows (see Chapter 2).

Changes in energy balance are often associated with decreased luteal function in cows and heifers (Beal et al., 1978). Villa-Godoy et al. (1988) found that postpartum cows in more negative energy balance had lower serum progesterone during the second and third postpartum estrous cycles. In addition, cows usually showed increasing concentrations of progesterone in plasma for each successive estrous cycle (associated with greater energy balance). In vitro studies using CL from nutrient-restricted animals support this work. Apgar et al. (1975) found that the CL from under-fed cows was less responsive to LH (as determined by progesterone production) compared with normal-fed cows. The authors suggest that this effect may be related to substrate availability in the underfed CL. Imakawa et al. (1986b) reported a positive correlation between serum progesterone and
body weight change in underfed sexually mature beef heifers. Finally, Knotson and Allrich (1988) found that heifers fed at 80% of NRC requirement had lower serum progesterone during the luteal phase compared with heifers fed either 100% or 120% of requirements. This decrease in progesterone occurred even though characteristics of LH secretion were similar in these cows. Of interest was the fact that there was no difference in the proportion of heifers exhibiting estrus, the interval to estrus, or the duration of estrus after PGF for heifers fed differently on this trial. There did appear, however, to be a delay in the onset of estrus after PGF in the 80% group (49 vs. 37 hours; 80% vs 120% of NRC respectively). Results of these studies agree with those of Spicer et al. (1990) who also found higher serum progesterone in cows in higher postpartum energy balance. Serum IGF-I concentrations also were measured in this trial and found to be higher in cows in more positive energy balance, as expected (Gluckmann et al., 1987). Therefore, the authors concluded that although they did not find any relationship between energy balance and interval to first ovulation, the higher concentrations of progesterone in cows in more positive energy balance might be related to higher circulating levels of IGFs. In agreement with this, Schemm et al. (1990) reported higher concentrations of progesterone during the luteal phase of GH-treated cattle. The work of Spicer et al. (1990) suggests that IGF-I can act on the follicle to enhance growth or differentiation (leading
to larger or more steroidogenic CL) or may act directly on the CL to increase progesterone synthesis. All data relating energy balance to steroid concentrations in the blood should be interpreted with the knowledge that metabolic clearance rates of steroids increase with increasing level of nutrition (Parr et al., 1987). Therefore, differences in the steroidogenic capacity of the CL may be estimated poorly by measurement of peripheral progesterone in cows consuming vastly different amounts of dry matter or being in different levels of negative energy balance.

Other Factors Affecting Postpartum Reproduction

Concentrations of progesterone in the blood may be related to the amount of B-carotene in the blood. Bindas et al. (1984) found subtle differences in progesterone response to hCG challenge in cows fed B-carotene. However, there was no dramatic effect of B-carotene on most gross measures of reproductive performance in these cattle. A similar relationship between B-carotene and progesterone has been reported by others (Graves-Hoagland et al., 1989). Effects of B-carotene on ovarian function may be related to level of vitamin A in the follicular fluid. Schweigert and Zucker (1988) found that although there was no statistical difference in the concentration of B-carotene in healthy and atretic follicles, the concentration of vitamin A (B-carotene metabolite) was twice as high in healthy compared with atretic follicles (.32 ug/ml vs .15 ug/ml).
Along with vitamin supplementation, minerals added to the diet may enhance luteal function. Lopez et al. (1989) found that mineral supplementation increased length of the luteal phase from 12.6 to 15.2 days in crossbred cows. In addition, subnormal fertility is sometimes corrected with mineral supplementation. Ingraham et al. (1987) increased first service conception rate from 33 to 57\% in cows given supplemental Mg and Cu. These types of data have supported the concept that the addition of chelated minerals (chemically protected from digestion in the rumen) to dairy cow diets will increase fertility. However, at this time, data on the relationship between chelated minerals and overall reproductive efficiency and fertility is limited to the popular press (Manspeaker et al., 1987).

Exposure of female animals to males of the same species has been shown to hasten the return to estrus or initiation of puberty in species like the pig and sheep ([termed "ram effect"], see Hudgens et al., 1987). This concept was tested in one study in suckled beef cows (Custer et al., 1990). In this study, exposure of first calf heifers to sterile bulls shortened the interval to first estrus in two trials and increased the number of cows having progesterone rise prior to first estrus from 31\% (control) to 51\% (bull exposed). Of interest was the fact that LH secretion was unaffected by bull exposure, suggesting that other mechanisms besides gonadotropin secretion may be mediating these effects.
It should be recognized that postpartum health has a major effect on reproductive performance. Hamudikuwanda et al. (1987) found that while milk production had a minor effect on services per conception and days open (.009 services and .6 days per 100 kg milk, respectively) there were more dramatic effects associated with reproductive disorders. This is supported by work of Fonseca et al. (1983) who found that Holstein cows with clinical problems averaged 8.8 more days to first ovulation, 17.0 more days to first detected estrus, 14.2 more days to first insemination, and 16.6 more days from calving to conception compared with clinically healthy cows. Research by Holt et al. (1989) agrees with these results. When cows were grouped according to postpartum uterine disorders, they found that cows with retained placenta had decreased plasma progesterone during the first luteal phase, greater days open, and greater services per conception compared with cows having a clinically normal postpartum period. It appears that the effect of uterine infections on cow reproduction may be related to reduced LH secretion associated with endotoxins, produced by the bacterial strains, acting on the pituitary and hypothalamus (Peter et al., 1989).

Methods to Increase Fertility in Dairy Cows

Genetic Selection

Although reproductive traits are traditionally not thought to be responsive to genetic selection, the growth and development of the follicle has been altered in some species
of sheep. Driancourt et al. (1990) studied the follicular dynamics associated with ovulation rate in sheep. Merino ewes selected for increased ovulation rate had fewer large atretic follicles during the preovulatory period compared to control Merino ewes. In contrast, Finn ewes selected for higher ovulation rate recruited a greater number of follicles during the preovulatory period than Finn control ewes. This study demonstrated that follicular dynamics can be altered by genetic selection. Furthermore, rapid genetic progress was made in the selection for multiple ovulation in cattle (Echternkamp et al., 1990). Therefore, follicular dynamics in cattle as well as sheep can be altered genetically. Given the need for more efficient reproduction in dairy cattle, genetic selection for optimal follicular dynamics may represent a means for improved efficiency of reproduction in the future.

Prebreeding Progestogens

Results of some studies have suggested that the concentration of progesterone in blood prior to insemination is critical to conception rate at the subsequent service (see Helmer et al., 1982). This concept was tested in a recent study where postpartum cows were pretreated with progesterone for 7 days prior to a PGF-induced estrus (Stevenson et al., 1989). While there was no effect of level of prebreeding progesterone on first service conception rates, the incidence of detected estrus was increased from 54% to 71% in progesterone-treated cows.
Pretreating beef cattle early during the postpartum period with norgestomet can increase the growth and development of follicles and decrease the incidence of short estrous cycles (Garcia-Winder et al., 1986). This may be related to greater estrogen content of preovulatory follicles in beef cattle pretreated with norgestomet during the postpartum period (Garcia-Winder, 1987). Britt et al. (1974) fed postpartum dairy cows melegesterol acetate (MGA, a synthetic progestogen) from 21 days to 35 days postpartum. He found that this regimen increased the number of large follicles and reproductive efficiency of treated cows was improved. The MGA-treated cows had a shorter intervals to first estrus, shorter intervals from calving to conception, and fewer services per conception.

GnRH Treatment

Several methods have been developed in an attempt to control or increase the level of fertility in dairy cattle. Gonadotropin-releasing hormone can be used at three stages during the postpartum period to influence the fertility in dairy cattle. First, GnRH can be used during the early postpartum period. Injection of GnRH after day 10 postpartum should be effective in releasing LH from the pituitary at a level equivalent to later postpartum periods (Fernandez et al., 1978). Therefore, GnRH can be injected between days 15 and 25 to induce an ovulation and initiate estrous cycles in cattle. This treatment, however, has inconsistently benefited
fertility. Etherington et al. (1984) found that GnRH (day 15 postpartum) increased intervals to first estrus and first service, while Stevenson et al. (1987) noted an increase in the number of days open associated with GnRH treatment. A beneficial effect of GnRH treatment on days open, first service conception rates, and/or services per conception has been reported in two studies (Nash et al., 1980; Benrad and Stevenson, 1986), and Britt et al. (1977) found that fewer GnRH-treated cows were culled for infertility (26% vs 57%, GnRH vs saline, respectively). Therefore, it is not specifically clear whether or not injection of GnRH will improve reproductive performance at this time. Some of the discrepancies reported in these studies may be related to overall herd health. Cows with uterine infections at the time of GnRH treatment may be disadvantaged because of the development of luteal tissue and progesterone secretion during the postpartum period. This can act to limit the movement of debris out of the uterus through the cervix (which is constricted under progesterone influence). This concept is not supported by Benmrad and Stevenson (1986) who found that cows with clinically abnormal postpartum periods benefited from GnRH treatment.

A second time when GnRH can be given to cattle is at the time of insemination. This type of treatment should be considered for either normal or "repeat-breeder" cows. It has been definitively demonstrated that injection of GnRH to cows
at the time of first insemination does not increase fertility (Chenault et al., 1990; Lewis et al., 1990). In a related study (Mee et al., 1990), in which the timing of GnRH and insemination relative to estrus were factorialized, no combination of GnRH treatment or time of insemination after estrus resulted in improved fertility. There may be benefits of GnRH injection in repeat-breeder cows. These are clinically normal cows that have been inseminated three or more times and have not conceived. Numerous investigations support the concept that GnRH improves fertility in repeat-breeder cows (Stevenson et al., 1981; Lee et al., 1983; Phatak et al., 1986; Stevenson et al., 1988; Pennington et al., 1985). Taken together these five studies represent over 2000 total inseminations and a 14.2% increase in fertility in repeat-breeders injected with GnRH (38.9% vs 53.1%, control vs GnRH, respectively). Therefore, there appears to be inherent differences in the physiology of repeat-breeder and normal cows which is specifically remedied by treatment with GnRH.

A third time when GnRH can be used to affect fertility is during the luteal phase. The use of GnRH at this time will extend the lifespan of the CL (Milvae et al., 1984). This is due to the luteinization of follicles on the ovary which can act as additional sources of progesterone (Milvae et al., 1984) or prevent the mechanism associated with normal termination of the luteal phase (Thatcher et al., 1989b). The latter effect is believed to be related to the loss of an
estrogen source (i.e., follicles) which trigger PGF secretion from the uterus. This is supported by the work of Fogwell et al. (1985) and Milvae et al. (1984) who destroyed follicles on the ovary and extended the lifespan of the CL. Extension of luteal lifespan during the estrous cycle may save some slower growing embryos which do not secrete enough bTP-1 to survive normal luteolysis (Helmer et al., 1989; Thatcher et al., 1989a). Use of GnRH during diestrus improved conception rate from 60.9 to 72.4% in one study in which injections were given to lactating dairy cows (Macmillan et al., 1986). However, those results have not been supported by subsequent work using GnRH during diestrus in either cows or heifers (Lewis et al., 1990).

Conclusions

The purpose of this review was to first examine the physiology of the late pregnant and early postpartum cow and then to relate this knowledge to methodologies available to increase reproductive efficiency. It is clear that while we have a reasonable understanding of those mechanisms that control the initiation of estrous cycles and ovarian activity, we do not have a clear understanding of what practical methods are available to improve postpartum fertility. Given the complexity of the postpartum period and the dynamic nature of the postpartum cow, these answers will have to be arrived at by scientific approaches which employ both in vivo and in vitro methodologies.
One of the most exciting areas of future research may be the integration of digestive function with ovarian function and the specific formulation of diets to enhance fertility. This may be accomplished through increasing energy balance or by altering the concentrations of certain metabolites. Once we have a clearer understanding of those metabolic signals that affect the ovary we can begin to integrate traditional knowledge of nutrition and reproduction. This dissertation addresses some aspects of this interaction. This work adds to existing knowledge of the nutrition and reproduction of the postpartum cow and also provides a basis for continued research.
CHAPTER 2
INFLUENCE OF DIET COMPOSITION, DRY MATTER INTAKE, MILK PRODUCTION, AND ENERGY BALANCE ON TIME OF POSTPARTUM OVULATION AND FERTILITY IN DAIRY COWS

Introduction

Advances in the feeding, management, and genetics of dairy cattle have led to increased milk production per cow (Butler and Smith, 1989). High producing cows cannot consume enough feed to support their potential for milk production. Therefore, energy expenditures for milk production are contributed by body fat reserves (Bauman and Currie, 1980; Coppock, 1985). High producing dairy cows can lose in excess of 50 kg of adipose tissue during early lactation in compensation for milk energy output (Berghorn et al., 1988; Villa-Goday et al., 1988). This mobilization of body fat reserves and the associated partitioning of nutrients toward milk production is one of the most striking examples of goal oriented shifts in metabolism available for study in any species (Bauman and Currie, 1980).

Active changes in the ovary, pituitary, hypothalamus, and uterus directed at reinitiation of estrous cycles coincide with these metabolic shifts associated with early milk production. Remarkably, in the face of tremendous energy expenditures, most dairy cows experience their first
postpartum ovulation about 15 to 21 days (d) after calving (Stevenson and Britt, 1979; Lamming et al., 1982). This ovulation sets up a series of ovarian cycles which potentially lead to the reestablishment of pregnancy. The importance of the timing of this first ovulation to subsequent fertility has been documented. Cows that ovulate early in the postpartum period are more fertile because they experience more estrous cycles prior to first insemination (Thatcher and Wilcox, 1973). This principle has made interval to first ovulation an important index of reproductive efficiency.

The metabolic factors which determine when a cow experiences her first postpartum ovulation and becomes pregnant are economically and scientifically important. Previous investigations have examined the effects of milk production and energy balance (EB) on interval to first ovulation (Butler et al., 1981). At this time, the effect of milk production has not been established clearly. Some investigators report finding a positive relationship between milk production and interval to first ovulation (Stevenson and Britt, 1979; Fonesca et al., 1983) whereas others have found the opposite relationship (reviewed by Butler and Smith, 1989). A more consistent effect is found when EB is examined. Cows in more negative EB ovulate later postpartum, possibly due to the presence of unidentified energetic signals associated with depletion of body reserves. Insulin is one potential metabolic signal which has been studied in nonfarm
species and may be mediating the effect of EB on the hypothalamus and ovary (Baskin et al., 1987; Poretsky and Kalin, 1987).

Lactational performance is extremely dependent on quality, quantity, and nutritional balance of feed consumed by postpartum dairy cows. Therefore, trends in the formulation of diets for postpartum cows are dynamic with the availability of new scientific data affecting industry feeding practices. Although not traditionally examined, it seems plausible that dietary ingredients which affect milk production and energy metabolism can influence the function of the hypothalamus, pituitary, and ovary. The interaction of diet with nutrient needs may be manifested as either enhanced or reduced fertility and likely will become an increasingly important factor in future feeding management decisions.

The objective of this study was to monitor the interval to first ovulation and fertility in a herd of high producing dairy cows and to clarify the importance of milk production, DM intake, and EB to interval to first ovulation and interval to pregnancy (days open). In addition, dietary treatments employed during the second year were evaluated for their effects on reproductive efficiency.

**Materials and Methods**

**Animals and Diets**

Holstein cows in the University of Illinois dairy herd were arranged into two groups according to calving date. Cows
Table 2-1. Diet composition (% of total dry matter) for two experimental calving groups of lactating cows.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Group 1</th>
<th>Control</th>
<th>NA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CaLCFA</th>
<th>NA+</th>
<th>CaLCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>22.50</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>22.50</td>
<td>35.00</td>
<td>35.00</td>
<td>35.00</td>
<td>35.00</td>
<td>35.00</td>
</tr>
<tr>
<td>Ground Shelled Corn</td>
<td>35.55</td>
<td>.00</td>
<td>.00</td>
<td>.00</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>High Moisture Shelled Corn</td>
<td>.00</td>
<td>38.26</td>
<td>38.26</td>
<td>35.49</td>
<td>35.49</td>
<td>35.49</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>16.50</td>
<td>13.50</td>
<td>13.50</td>
<td>14.00</td>
<td>14.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>.90</td>
<td>1.30</td>
<td>1.30</td>
<td>.53</td>
<td>.53</td>
<td>.53</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>.75</td>
<td>.75</td>
<td>.75</td>
<td>.75</td>
<td>.75</td>
<td>.75</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
<td>.52</td>
<td>.52</td>
<td>.52</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>.30</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>.20</td>
<td>.22</td>
<td>.22</td>
<td>.22</td>
<td>.22</td>
<td>.22</td>
</tr>
<tr>
<td>Premix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.15</td>
<td>.15</td>
<td>.15</td>
<td>.15</td>
<td>.15</td>
<td>.15</td>
</tr>
<tr>
<td>CaLCFA</td>
<td>.00</td>
<td>.00</td>
<td>.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nicotinic acid (6 g) with soybean meal (34 g) and dry molasses (10 g) was top dressed onto the total mixed ration twice daily. Cows that did not receive nicotinic acid received the carrier twice daily.

<sup>b</sup>Guaranteed analysis: Mg≥5.0%; K≥7.5%; S≥10.0%; Zn≥3.0%; Mn≥3.0%; Fe≥2.0%; Cu≥.5%; Se≥.015%; vitamin A≥2200 IU/g; vitamin D≥660 IU/g; vitamin E≥7.7 IU/g.

in Group 1 (15 primiparous and 35 multiparous) calved between November 17, 1986 and February 11, 1987 whereas cows in Group 2 (40 multiparous) calved between March 1, 1987 and September 18, 1987. Major dietary ingredients for the two groups were similar (corn silage, alfalfa silage, corn, and soybean meal) but were fed in different proportions (Table 2-1). The diet of cows in Group 2 contained CaLCFA at 0 or 3% of diet DM and
niacin at 0 or 12 g/cow/d, thus dietary treatments were arranged in a 2 x 2 factorial (Erickson, 1989). Group 1 cows remained on their diets from 1 to 77 d after calving, whereas Group 2 cows received a similar diet from 1 to 14 d after calving and experimental diets from 15 to 98 d after calving. Feed ingredients were mixed completely just prior to feeding. Diets were fed ad libitum twice daily. Feeds were sampled weekly and DM (55.5 °C for 48 h) was determined to maintain proper forage-to-concentrate ratios.

Table 2-2. Chemical composition of diets fed to two experimental calving groups of lactating dairy cows.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Group 1</th>
<th>Control</th>
<th>NA</th>
<th>CaLCFA</th>
<th>Na+</th>
<th>CaLCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %DM</td>
<td>17.95</td>
<td>18.60</td>
<td>18.60</td>
<td>18.30</td>
<td>18.30</td>
<td></td>
</tr>
<tr>
<td>ADF&lt;sup&gt;a&lt;/sup&gt;, %DM</td>
<td>15.20</td>
<td>16.00</td>
<td>16.00</td>
<td>15.90</td>
<td>15.90</td>
<td></td>
</tr>
<tr>
<td>Calcium, %DM</td>
<td>.90</td>
<td>1.10</td>
<td>1.10</td>
<td>1.20</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>Phosphorus, %DM</td>
<td>.47</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>NE&lt;sub&gt;L&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;, Mcal/kg</td>
<td>1.67</td>
<td>1.70</td>
<td>1.70</td>
<td>1.80</td>
<td>1.80</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Acid detergent fiber.

<sup>b</sup>Net energy for lactation.

Diet Consumption, Milk Yield, and Energy Balance

Dry matter intake (DMI) was calculated as feed offered minus feed refused. Dry matter content of feeds was determined weekly and feed composition was determined once every 4 weeks on composite samples collected every week (Northeast Dairy
Herd Improvement Association Forage Testing Laboratory, Ithaca NY, Table 2-2). Cows were milked at 0600 and 1700 h and allowed exercise from 0900 to 1100 h from stanchions. Milk yield was measured daily and milk composition (percent solids-non-fat, SNF; and percent milk fat, FAT) was determined on consecutive morning and evening samples collected once weekly. Cows were weighed weekly (body weight, BW) immediately after morning milking. Energy balance was calculated weekly using the equation $EB = \text{net energy of DMI (NEI)} - \text{net energy required for body maintenance (NEM)} - \text{net energy in secreted milk (NEL)}$. Components of the equation were calculated as follows: $\text{NEI (Mcal/d)} = \text{DMI (kg/d)} \times \text{feed net energy of lactation concentration (Mcal/kg)}$; $\text{NEM (Mcal/d)} = \text{BW (kg)}^{0.75} \times 0.08$ (NRC, 1989); and $\text{NEL (Mcal/d)} = \text{milk yield (lb)} \times [\text{41.84 kg of milk fat} + \text{22.29 \% SNF} - \text{25.58}] / 1000$ (Tyrell and Reid, 1965). Four percent fat corrected milk (FCM) yield was calculated using the equation $\text{FCM} = 0.4 \times \text{milk yield (kg)} + 0.15 \times (\text{milk fat yield (kg)})$.

Blood Collection and Analysis

Blood was collected into heparinized tubes three times weekly from calving until subsequent pregnancy up to 200 d postpartum (Group 1) or from day 15 to 112 postpartum (Group 2). Samples were collected into heparinized tubes and centrifuged at 3000 x g for 10 min. Plasma was collected and stored at -20 °C until assayed for concentrations of progesterone (Knickerbocker et al., 1986b). Timing of
ovulations and corpora lutea formation were estimated from sustained elevations in plasma progesterone concentration (at least 7 days above 3 ng/ml). First ovulation was defined as occurring 5 days prior to first elevation in plasma progesterone or was determined exactly for cows observed in estrus.

**Statistical Analysis of Reproductive Data**

Records containing dates of observed estrus, artificial insemination (initiated after 60 days postpartum), and pregnancy were maintained and used along with progesterone data as measures of reproductive efficiency. Cows were separated into preplanned interval to first ovulation and interval to pregnancy groups. Cows were classified as having first ovulation either in the early postpartum period (less than 21 d, ovulation status [ovstat] = 1), or at later postpartum dates (either 22 to 42 d, ovstat = 2, or greater than 42 d, ovstat = 3). In addition, cows were classified as becoming pregnant early in the breeding period (days from calving to conception [days open] was less than 80 d, days open status [dostat] = 1), midway through the breeding period (days open equalled 81 to 140 d, dostat = 2) or later in the breeding period (days open were greater than 140 d, dostat = 3) or nonpregnant (no pregnancy during the lactation, dostat = 4). Four percent fat corrected milk yield, DMI, EB, and BW were analyzed for each group separately to determine if these measures were similar in cows having different intervals to
first ovulation (ovstat) or different intervals from calving to pregnancy (dostat). The statistical model included the effects of status (i.e., ovstat or dostat), cow within status, week postpartum, the status by week interaction, and residual (SAS, 1987). Energy balance curves were tested using homogeneity of regression procedures (Wilcox et al., 1990). The main effects of ovstat or dostat were examined using preplanned single degree of freedom orthogonal contrasts. Contrasts for the main effect of ovstat were 1+2 vs. 3 (ovstat contrast 1 [oC1]; cyclic vs. anestrous cows) and 1 vs. 2 (oC2). Contrasts for the main effect of dostat were 1+2+3 vs. 4 (dC1; pregnant vs. nonpregnant cows), 1+2 vs. 3 (dC2), and 1 vs. 2 (dC3). In addition, DMI, FCM, BW, and EB, as well as measures of reproductive performance (e.g., interval to first ovulation, interval to first estrus, interval from calving to first insemination, first insemination conception rate, pregnancy rate, inseminations per cow, inseminations per conception, and days open) for ovstat or dostat classifications were analyzed using a statistical model including the main effect of status (ovstat or dostat), calving group, the status-by-group interaction (mean square error of this term was used to test main effect of status), and residual. Single degree of freedom contrasts also were performed as described above. Fertility data (i.e., first insemination conception rate) were subjected to Chi-square
analysis. Only multiparous cows from Groups 1 and 2 were used in the ovstat and dostat analyses.

Energy balance, DMI, FCM production, and BW were analyzed for primiparous and multiparous cows (Group 1) in a model that contained the main effects of parity, cow-within-parity, week, parity-by-week interaction, and residual. Reproductive characteristics of cows in Group 1 also were analyzed considering the main effect of parity. The effect of different dietary treatments (Group 2) on reproductive performance (e.g., interval to first insemination, inseminations per conception, etc.) were tested using a model including the main effects of niacin supplementation (0 or 12 g/d; Control + CaLCFA vs. Niacin + CaLCFA/Niacin), CaLCFA supplementation (0 or 3% of diet DM; Control + Niacin vs. CaLCFA + CaLCFA/Niacin), and the niacin-by-CaLCFA interaction (Control + CaLCFA/Niacin vs. Niacin + CaLCFA).

Results

Interval to Ovulation and FCM Yield, DMI, BW, and EB

Dry matter intake, FCM yield, EB, and body weight for multiparous cows having first ovulation during one of three classification periods are presented in Figure 2-1 (Group 1) and Figure 2-2 (Group 2). Number of cows having short (ovstat 1), intermediate (ovstat 2) and long (ovstat 3) intervals to first ovulation were 17, 13, and 4 for Group 1 and 17, 18, and 5 for Group 2 respectively. In both groups, there was a significant effect of week postpartum (P<.001) for DMI, FCM,
Figure 2-1. Average daily dry matter intake (A), daily fat corrected milk yield (B), energy balance (C) and body weight (D) for cows in Group 1 with different intervals to first ovulation.

EB, and BW, while the interaction of week and ovstat was not significant. Cows ovulating before and after 42 d postpartum consumed similar amounts of feed during the experimental period (Table 2-3). However, cows ovulating before d 21 postpartum tended to consumed more feed (oC2, P<.10) than cows ovulating from d 22 to 42 postpartum. Four percent fat corrected milk production during the experimental period tended to be greater (oC1, P<.10) for cows that ovulated before 42 d postpartum compared with cows ovulating after 42
d postpartum. In addition, 4% FCM production was greater (oC2, P<.05) for cows that ovulated early during the postpartum period (ovstat 1) compared with cows ovulating from 22 to 42 d postpartum. Average daily postpartum EB and BW during the experimental period were similar (oC1 and oC2, nonsignificant) in cows ovulating at different intervals after calving. In addition, postpartum EB curves for ovstat 1, 2, and 3 were parallel when tested by homogeneity of regression.

Figure 2-2. Average daily dry matter intake (A), daily fat corrected milk yield (B), energy balance (C) and body weight (D) for cows in Group 2 with different intervals to first ovulation.
Table 2-3. Mean dry matter intake (DMI), 4% fat-corrected milk production (FCM), energy balance (EB), body weight (BW) and statistical contrasts for means (SE=standard error of lsmean, NS=nonsignificant [P>.10]) from cows in Groups 1 and 2 having first postpartum ovulation either ≤ 21 d (ovulation status [ovstat]=1), 22 to 42 days (ovstat=2), or >42 d (ovstat=3).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Ovulation Status</th>
<th>Group 2 Ovulation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>21.5</td>
<td>20.3</td>
</tr>
<tr>
<td>FCM (kg/d)</td>
<td>37.5</td>
<td>34.1</td>
</tr>
<tr>
<td>EB (Mcal/d)</td>
<td>-1.3</td>
<td>-1.4</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>599</td>
<td>584</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ovstat</th>
<th>Ovstat-by-Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+2 vs. 3</td>
<td>1 vs. 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>1+2 vs. 3</th>
<th>1 vs. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI (kg/d)</td>
<td>NS</td>
<td>P&lt;.10</td>
</tr>
<tr>
<td>FCM (kg/d)</td>
<td>P&lt;.10</td>
<td>P&lt;.05</td>
</tr>
<tr>
<td>EB (Mcal/d)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Multiparous cows assigned to different interval to first ovulation classifications had different intervals to first ovulation (as designed, Table 2-4, Groups 1 and 2 combined). Cows having first ovulation before 42 d (ovstat 1 and 2) had a shorter interval from calving to detected estrus (oC1, P<.09), greater conception rate at first insemination (oC1, P<.05), and a longer interval from calving to conception (oC1, P<.07) compared with cows having first ovulation after 42 d postpartum. Other reproductive characteristics (interval from
Table 2-4. Characteristic of reproduction (lsmean ± SE and statistical contrasts, NS = nonsignificant [P>.10]) in 74 multiparous cows (Groups 1 and 2, combined) having first ovulation at either ≤ 21 d (ovulation status = 1), 22 to 42 d (ovulation status = 2), or > 42 d (ovulation status = 3).

<table>
<thead>
<tr>
<th>Item</th>
<th>Ovulation Status</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Number</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>Ovulation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16±1</td>
<td>31±1</td>
</tr>
<tr>
<td>Estrus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52±5</td>
<td>55±5</td>
</tr>
<tr>
<td>Insem&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69±7</td>
<td>72±7</td>
</tr>
<tr>
<td>CRFI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12/34</td>
<td>10/31</td>
</tr>
<tr>
<td>I/cow&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.7±.3</td>
<td>3.3±.3</td>
</tr>
<tr>
<td>Pregnant&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25/34</td>
<td>22/31</td>
</tr>
<tr>
<td>I/C&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.4±.2</td>
<td>2.5±.2</td>
</tr>
<tr>
<td>Days open&lt;sup&gt;h&lt;/sup&gt;</td>
<td>111±2</td>
<td>112±2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean interval (days) to first ovulation.
<sup>b</sup>Mean interval (days) to first detected estrus.
<sup>c</sup>Mean interval (days) to first insemination.
<sup>d</sup>Conception rate at first insemination (number pregnant/number inseminated).
<sup>e</sup>Inseminations per cow (pregnant and nonpregnant cows).
<sup>f</sup>Pregnancy rate (number pregnant/total number of cows).
<sup>g</sup>Inseminations per conception (pregnant cows only).
<sup>h</sup>Mean interval (days) from calving to conception.

calving to first insemination, inseminations per cow (mean includes pregnant and nonpregnant cows), inseminations per conception (mean includes pregnant cows only), and pregnancy rate (pregnant cows/[pregnant + nonpregnant cows]) were similar for cows ovulating before or after 42 d postpartum. There were no differences among cows ovulating before 21 d postpartum or from 22 to 42 d postpartum for any of the reproductive characteristics which were measured.


Interval to Pregnancy and FCM Yield, DMI, BW, and EB

Numbers of multiparous cows becoming pregnant by 80 d, 81 to 140 d, after 140 d, and not becoming pregnant were 9, 10, 5, and 11 for Group 1, and 8, 8, 11, and 13 for Group 2, respectively. Mean DMI, 4% FCM production, body weight, and mean daily EB are presented in Figure 2-3 (Group 1) and Figure 2-4 (Group 2). Mean DMI, 4% FCM production, and BW were similar (dC1, dC2, and dC3, nonsignificant) among cows having different intervals from calving to conception (Table 2-5). However, EB tended to be greater (dC3, P<.10) for pregnant cows compared with nonpregnant cows, while EB was less for cows in dostat 1 compared with dostat 2.

Mean interval to pregnancy or conception (days open) was 62±2 d, 102±2 d, and 174±3 d for cows assigned to ≤ 80 d, 81 to 140 d, and > 140 d pregnancy interval classifications (Table 2-6, Groups 1 and 2 combined). Interval from calving to first ovulation, interval from calving to first detected estrus, and interval from calving to first insemination were shorter (dC1, P<.01, P<.01, P<.04, respectively), for cows becoming pregnant compared with cows not becoming pregnant during the breeding period. Inseminations per cow were similar for pregnant and nonpregnant cows. Furthermore, pregnant cows conceiving before 140 d postpartum (dostat 1+2) had a shorter interval to first estrus (dC2, P<.01), a higher first insemination conception rate (dC2, P<.05), and required fewer inseminations per conception (dC2, P<.06) compared with
Figure 2–3. Average daily dry matter intake (A), daily fat corrected milk yield (B), energy balance (C) and body weight (D) for cows in Group 1 which had different intervals to pregnancy or were not pregnant (not preg).

cows becoming pregnant after 140 d postpartum. Finally, cows conceiving prior to 80 d postpartum had a shorter interval to first ovulation (dC3, P<.02), shorter interval to first estrus (dC3, P<.02), and higher first insemination conception rate (P<.01) compared with cows conceiving between 81 and 140 d postpartum.
Figure 2-4. Average daily dry matter intake (A), daily fat corrected milk yield (B), energy balance (C), and body weight (D) for cows in Group 2 which had different intervals to pregnancy or were not pregnant (not preg).

Effect of Parity on FCM, DMI, EB, BW, and Reproduction

As expected, primiparous cows produced less 4% FCM (P<.001; 25.4±1.5 vs. 35.5±.8 kg/d) and consumed less dry matter (P<.001; 14.3±.5 vs. 20.7±.3 kg/d) during the experimental period compared with multiparous cows (Figure 2-5a and 2-5b, respectively). Furthermore, EB was lower (P<.05) in primiparous cows compared with multiparous cows (-4.2±1.1 vs. -1.5±.5 Mcal/d, respectively; Figure 2-5c). Mean BW of
Table 2-5. Mean dry matter intake (DMI), 4% fat-corrected milk production (FCM), energy balance (EB), body weight (BW) and statistical contrasts for means (SE=standard error of lsmean, NS=nonsignificant [P>.10]) from cows in Groups 1 and 2 having days open either ≤ 80 d (days open status [dostat]=1), 80 to 140 d (dostat=2), >140 d (dostat=3), or not pregnant (dostat=4).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Days Open Status</th>
<th>Group 2 Days Open Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>20.6</td>
<td>21.1</td>
</tr>
<tr>
<td>FCM (kg/d)</td>
<td>35.2</td>
<td>35.3</td>
</tr>
<tr>
<td>EB (Mcal/d)</td>
<td>-1.8</td>
<td>-1.2</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>583</td>
<td>600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Dostat</th>
<th>Dostat-by-Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+2+3 vs.4</td>
<td>1+2 vs.3</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FCM (kg/d)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EB (Mcal/d)</td>
<td>P&lt;.10</td>
<td>NS</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Primiparous cows decreased steadily during the postpartum period from a maximum of 538±5 kg (week 1) to a minimum of 498±5 kg after 11 weeks of lactation. In contrast, mean BW of multiparous cows declined from 610±3 kg (wk 1) to a minimum of 582±3 kg on week 5 of lactation and then remained constant (parity-by-week interaction, P<.05, Figure 2-5).

Ovarian activity was delayed in primiparous cows compared with multiparous cows (Table 2-7). Primiparous cows had a
Table 2-6. Reproductive characteristics (lsmean + SE and statistical contrasts, NS = nonsignificant [P>.10]) for 75 multiparous cows (Groups 1 and 2 combined) classified according to interval to pregnancy (days open status, < 80 d [status = 1], 80 to 140 d [status = 2], >140 d [status=3], and not pregnant [status = 4]).

<table>
<thead>
<tr>
<th>Item</th>
<th>Days Open Status</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Number</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Ovulation$^a$</td>
<td>22±2</td>
<td>32±2</td>
</tr>
<tr>
<td>Estrus$^b$</td>
<td>44±2</td>
<td>55±2</td>
</tr>
<tr>
<td>Insem$^c$</td>
<td>59±5</td>
<td>67±5</td>
</tr>
<tr>
<td>CRFI$^d$</td>
<td>12/17</td>
<td>3/18</td>
</tr>
<tr>
<td>I/cow$^e$</td>
<td>1.3±.8</td>
<td>2.3±.8</td>
</tr>
<tr>
<td>Pregnant$^f$</td>
<td>17/17</td>
<td>18/18</td>
</tr>
<tr>
<td>I/C$^g$</td>
<td>1.3±.4</td>
<td>2.3±.4</td>
</tr>
<tr>
<td>Days Open$^h$</td>
<td>62±2</td>
<td>102±2</td>
</tr>
</tbody>
</table>

$^a$Mean interval (days) to first ovulation.
$^b$Mean interval (days) to first detected estrus.
$^c$Mean interval (days) to first insemination.
$^d$Conception rate at first insemination (number pregnant/number inseminated).
$^e$Inseminations per cow (pregnant and nonpregnant cows).
$^f$Pregnancy rate (Number pregnant/total number of cows).
$^g$Inseminations per conception (pregnant cows).
$^h$Mean interval (days) from calving to conception.

Mean interval to first ovulation of 42 d later than for multiparous cows (P<.01). First detected estrus and interval to first insemination also were delayed in primiparous cows. However, primiparous cows were similar to multiparous cows with respect to first insemination conception rate, inseminations per cow, inseminations per conception, and days open.
Figure 2-5. Average daily dry matter intake (A), daily fat corrected milk yield (B), energy balance (C) and body weight (D) for multiparous (n=35) and primiparous cows (n=15) in Group 1.

Effect of CaLCFA and Niacin on EB and Reproduction

The main effects of CaLCFA and niacin feeding on the reproductive characteristic of cows in Group 2 are summarized in Table 2-8. Mean EB was similar in cows fed different diets in Group 2 (-1.8±.8 vs. -2.2±.8 Mcal/d, +CaLCFA vs. -CaLCFA; and -2.0±.8 vs. -2.0±.8 Mcal/d, +niacin vs. -niacin, respectively, Figure 2-6). Cows fed CaLCFA had similar intervals to first ovulation, intervals to first observed estrus, first insemination conception rates, and pregnancy
Table 2-7. Reproductive characteristics (lsmean ± SE and probability value, NS = nonsignificant \([P>.10]\)) of primiparous and multiparous cows in Group 1.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Ovulation(^a)</td>
<td>67.9±10.3</td>
<td>26.2±6.8</td>
<td>.002</td>
</tr>
<tr>
<td>Estrus(^b)</td>
<td>96.7±9.8</td>
<td>53.3±6.4</td>
<td>.005</td>
</tr>
<tr>
<td>Insem(^c)</td>
<td>99.5±8.8</td>
<td>67.5±5.8</td>
<td>.004</td>
</tr>
<tr>
<td>CRFI(^d)</td>
<td>5/15</td>
<td>8/35</td>
<td>NS</td>
</tr>
<tr>
<td>I/cow(^e)</td>
<td>3.60±5.7</td>
<td>3.51±.38</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnant(^f)</td>
<td>7/15</td>
<td>24/35</td>
<td>NS</td>
</tr>
<tr>
<td>I/C(^g)</td>
<td>1.86±.51</td>
<td>2.58±.28</td>
<td>NS</td>
</tr>
<tr>
<td>Days Open(^h)</td>
<td>88.7±15.9</td>
<td>102.0±8.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\)Mean interval (days) to first ovulation.  
\(^b\)Mean interval (days) to first detected estrus.  
\(^c\)Mean interval (days) to first insemination.  
\(^d\)Conception rate at first insemination (number pregnant/number inseminated).  
\(^e\)Inseminations per cow (pregnant and nonpregnant cows).  
\(^f\)Pregnancy rate (Number pregnant/total number of cows).  
\(^g\)Inseminations per conception (pregnant cows).  
\(^h\)Mean interval (days) from calving to conception.

rates compared with cows not fed CaLCFA. However, CaLCFA fed cows had a longer interval to first insemination (\(P<.05\), 91 vs. 66 d), required more inseminations per cow (\(P<.10\), 2.6 vs. 1.8), had greater days open (\(P<.01\), 147 vs. 90 d) and a longer calving interval (\(P<.01\), 424 vs. 367 d) compared with cows not fed CaLCFA. Niacin-fed cows were similar to cows not fed niacin in interval to first ovulation, interval to first detected estrus, interval to first insemination, first insemination conception rate, and inseminations per cow.
Table 2-8. Reproductive characteristics (lsmean ± SE and statistical contrasts, \[fat = \text{CaLCFA} + \text{Niacin} + \text{CaLCFA} vs. \text{Control} + \text{Niacin}; \text{Nia} = \text{Control} + \text{CaLCFA} vs. \text{Niacin} + \text{CaLCFA}; \text{FxN} = \text{Control} + \text{Niacin} + \text{CaLCFA} vs. \text{Niacin} + \text{CaLCFA}]; \text{Nonsignificant \([P>.10\]) for cows fed a dietary supplement of 12 g Niacin, Calcium salts of long chain fatty acids (CaLCFA) at 3% of the diet DM or the combination.}

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Niacin</th>
<th>CaLCFA</th>
<th>Niacin + CaLCFA</th>
<th>Fat Nia FxN</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Ovulation(^a)</td>
<td>30±6</td>
<td>32±6</td>
<td>33±6</td>
<td>27±6</td>
<td>NS</td>
</tr>
<tr>
<td>Estrus(^b)</td>
<td>46±12</td>
<td>60±12</td>
<td>78±12</td>
<td>62±12</td>
<td>NS</td>
</tr>
<tr>
<td>Insem(^c)</td>
<td>62±12</td>
<td>70±12</td>
<td>98±12</td>
<td>84±12</td>
<td>.05</td>
</tr>
<tr>
<td>CRFI(^d)</td>
<td>4/9</td>
<td>4/10</td>
<td>3/10</td>
<td>1/10</td>
<td>NS</td>
</tr>
<tr>
<td>I/cow(^e)</td>
<td>1.4±.5</td>
<td>2.2±.5</td>
<td>2.7±.5</td>
<td>2.5±.5</td>
<td>.10</td>
</tr>
<tr>
<td>Pregnant(^f)</td>
<td>6/10</td>
<td>8/10</td>
<td>6/10</td>
<td>7/10</td>
<td>NS</td>
</tr>
<tr>
<td>I/C(^g)</td>
<td>1.5±.6</td>
<td>2.3±.5</td>
<td>2.3±.6</td>
<td>2.6±.5</td>
<td>NS</td>
</tr>
<tr>
<td>Days Open(^h)</td>
<td>77±19</td>
<td>102±16</td>
<td>131±19</td>
<td>163±17</td>
<td>.01</td>
</tr>
<tr>
<td>Gestation(^i)</td>
<td>275±2</td>
<td>281±2</td>
<td>275±2</td>
<td>280±2</td>
<td>NS .01</td>
</tr>
<tr>
<td>CI(^j)</td>
<td>352±19</td>
<td>382±16</td>
<td>406±19</td>
<td>443±17</td>
<td>.01 .07</td>
</tr>
</tbody>
</table>

\(^a\)Mean interval (days) to first ovulation.
\(^b\)Mean interval (days) to first detected estrus.
\(^c\)Mean interval (days) to first insemination.
\(^d\)Conception rate at first insemination (number pregnant/number inseminated).
\(^e\)Inseminations per cow (pregnant and nonpregnant cows).
\(^f\)Pregnancy rate (Number pregnant/total number of cows).
\(^g\)Inseminations per conception (pregnant cows).
\(^h\)Mean interval (days) from calving to conception.
\(^i\)Length of gestation (days).
\(^j\)Calving interval (days).

Niacin fed cows had similar days open but a longer calving interval compared with cows not fed niacin (412 vs. 379 d) due to a shorter gestation length (275 vs. 280 d) in cows not fed niacin.
Figure 2-6. Average daily energy balance for cows fed different diets in group two (CO = control ration; CN = control ration plus niacin; FO = control ration plus fat; FN = control ration plus fat and niacin).

Discussion

The purpose of this study was to examine some factors potentially controlling interval to first ovulation and interval to pregnancy in a herd of high producing cows. Interval to first ovulation was shortest in cows that consumed the most DM during the postpartum period. As might be expected, milk production was highest for these cows. This trend was observed consistently for groups of cows calving at
different times of the year (Group 1 and 2) and confirms previous research by Staples et al. (1990) with the same Illinois herd and agrees with previous research showing a negative correlation between nutrient intake and interval to first estrus (Hansen et al., 1982). It is clear that cows that are anestrous during the postpartum period are not necessarily the highest milk producing cows. Low producing cows, and those consuming less DM were anestrous in this study. Our results also confirm the findings of previous researchers; that is, that cows in more negative EB have extended intervals to first ovulation (see primiparous vs. multiparous cows, Figure 2-5; Butler et al., 1981; Butler and Smith, 1989). These same cows in more negative EB produced the least milk. Therefore, the finding of previous researchers that high producing cows ovulated earlier than low producing cows (Stevenson and Britt, 1979) is not contradictory to the effects of EB on interval to first ovulation. Actually, high producing cows can be in a less negative EB and thus ovulate earlier (as found in this study).

The exact stimulus for early ovulation in high producing cows is unclear. Equal arguments can be made for the effects of DMI and EB. Dry matter intake is the major limiting factor in the production of milk in high producing cows (Reid et al., 1966) and it seems plausible that the key element dictating an early ovulation in these cows is the superior nutrient intake in the first few weeks after calving. This has a potential
twofold affect on the reproductive recrudescence of the cow. First, it is likely that the enhanced nutrient flow experienced by the cow may stimulate the ovary or other parts of the hypothalamic/pituitary/ovarian axis (Nett, 1987). In addition, this high nutrient intake prevents a potentially large negative EB, and therefore circumvents possible effects of severe nutrient depletion on the secretion of gonadotropins (Imakawa et al., 1987b; Chapter 5).

Cows ovulating early (before d 42) were superior in reproductive performance in this trial (see Table 2-4). This is consistent with previous work by Thatcher and Wilcox (1973) where the most fertile cows were those that ovulated earliest in the postpartum period. We did not find that cows ovulating between d 22 and 42 were reproductively disadvantaged compared with those cows ovulating very early postpartum (less than 21 d). These two Groups were identical. It is likely, therefore, that slightly delayed intervals to first ovulation are acceptable in high producing herds. Extended intervals to first ovulation, however, clearly lead to lower fertility (as evidenced by first insemination conception rate) and a lower overall pregnancy rate.

Interval to pregnancy was not related to DMI, FCM production, or BW. It appears that these measures are similar among cows having different intervals from calving to conception. Therefore, specific production traits were not related directly to fertility, and high as well as low
producing cows were equally likely to remain open during the breeding period. However, cows not becoming pregnant during the postpartum period tended to have lower EB compared with pregnant cows. It was clear from these results (see Table 2-6) that nonpregnant cows ovulated later during the postpartum period, had a longer interval to first insemination and estrus, and were inseminated more times than their herdmates. Therefore, the successful management of body tissue reserves through feeding practices targeting the early postpartum period may be one method to improve overall herd fertility through reduced intervals to first ovulation and estrus. After all, 12% of the cows in this study did not ovulate until after 42 days postpartum. This increased to 27% with the inclusion of primiparous cows.

The primiparous cows used only in Group 1 produced less milk, ate less feed, and had dramatically lower EB compared with multiparous cows. This lower EB resulted in primiparous cows losing weight through the 11th week, while multiparous cows lost weight for only the first 5 week of the study. The postpartum energetics of the primiparous cows seemed to be translated into less ovarian activity during the early postpartum period with primiparous cows having a longer interval to first ovulation, first estrus and first insemination. However, these events did not delay the mean interval from calving to conception. This may suggest that although the ovaries of the primiparous cows initiated ovarian
cycles later, their eventual fertility was normal or even greater than multiparous cows. Perhaps the reproductive organs of primiparous cows are healthier and more fertile because they have experienced fewer pregnancies.

Additional factors influencing reproduction are the dietary ingredients. Effect of feeding 12 g of niacin per d was minimal. Feeding of CaLCFA, commonly believed to enhance reproduction (Ferguson et al., 1987), had negative effects in this study. Cows fed CaLCFA produced more milk than cows not fed the fat (Erickson, 1989). However, because the energy density of the CaLCFA diet was higher than the control diet, the overall EB of cows on all diets was nearly equivalent. This may suggest that factors other than EB were influencing the fertility of the fat-supplemented cows. It may be that the enhanced milk production itself has effects on reproduction apart from EB (although our analyses of Group 1 and 2 do not suggest this, see Figures 2-3 and 2-4). One alternative may be the effect that fat feeding has on the development and distribution of ovarian follicles. Cows fed CaLCFA developed greater numbers of large follicles (>15 mm; Chapter 5) and these follicles could alter the fertility of cows fed fat through a heightened estrogen environment around the time of maternal recognition of pregnancy (Thatcher et al., 1989a). Certainly, hormonal treatments designed to eliminate such follicles have increased fertility in some, but not in all studies (Macmillan et al., 1986).
In conclusion, cows that ovulated early during the postpartum period produced the most FCM and ate the most DM. Anestrous cows failed to consume large quantities of feed and to produce large amounts of milk. Cows having different intervals from calving to conception were similar in terms of FCM production, DMI, and BW, while EB tended to be lower in nonpregnant cows. Dietary supplements (especially CaLCFA) decreased fertility in this study. This may be related to altered follicular development in CaLCFA fed cows. Continued examination into the effects of EB and diet on overall herd fertility is warranted.
CHAPTER 3
EFFECT OF INTRAVENOUS INFUSION OF A SOYBEAN OIL EMULSION ON PLASMA CONCENTRATION OF 15-KETO-13,14-DIHYDRO-PROSTAGLANDIN F2α AND OVARIAN FUNCTION IN CYCLING HOLSTEIN HEIFERS

Introduction

Uterine secretion of prostaglandin F2α ([PGF] as measured by the plasma concentration of the primary metabolite 15 keto-13,14-dihydro-prostaglandin F2α; PGFM) reaches a maximum 3 days after partuition in cattle and subsequently declines to a nadir in 2 to 3 weeks (Guilbault et al., 1984a). The magnitude of postpartum PGFM secretion is correlated positively with the resumption of ovarian activity (i.e., follicular growth; Madej et al., 1984; Guilbault et al., 1987ab). Villeneuve et al. (1988) and Guilbault et al. (1987b) demonstrated an increased recruitment of ovarian follicles in cattle given exogenous PGF during the first 2 weeks postpartum. In addition, some studies have shown enhanced fertility in early postpartum dairy cattle injected with PGF (days 14 to 24) prior to first service (Young et al., 1984; Benmrad and Stevenson, 1986; Etherington et al., 1988). Consequently, an increase in the concentration of PGF in the plasma may be beneficial to postpartum fertility.

Linoleic acid is an 18 carbon essential fatty acid in mammals and is the precursor of the two series prostaglandins (i.e., PGE₂ and PGF; Oliw et al., 1983). Feeding diets high
in linoleic acid increased the concentration of arachidonic acid in the tissue of rats (Rivers and Frankel, 1981) and stimulated basal secretion of PGF in rats (Dupont et al., 1978) and humans (Mathias and Dupont, 1979; Adam et al., 1982). Dietary linoleic acid may be an ideal feed additive for the early postpartum cow because of its ability to stimulate basal PGF secretion and potentially enhance fertility. In addition, linoleic acid would be suitable as an energy source not unlike other long chain fatty acids (LCFA; Chalupa et al., 1986).

Linoleic acid is efficiently reduced to fatty acids which are not PGF precursors by ruminal biohydrogenation (Tove and Mochrie, 1963). Therefore, in this initial investigation, a soybean oil emulsion (50% linoleic acid) suitable for intravenous infusion was employed as the delivery system for linoleic acid. The objective of this study was to determine if intravenous infusion of linoleic acid would influence the plasma concentration of PGFM and alter ovarian follicular and luteal function. As a model system to test these effects, Holstein heifers were utilized during the estrous cycle.

**Materials and Methods**

**Animals**

Six Holstein heifers (mean body weight = 310 kg) were injected twice at an 11-day interval with 25 mg Lutalyse (PGF Tham salt; UpJohn Co., Kalamazoo, MI) to synchronize estrus. Heifers were kept in a tie stall barn throughout the
experimental period and fed a totally mixed ration ad libitum consisting of corn silage (35%; percent of ration DM), soybean meal (11%), corn grain (15%), corn distillers meal (14%), whole cottonseed (9%), and alfalfa hay (14%). Average feed consumption was 6.7 kg DM/heifer/day which was equivalent to 20.4 MCal calculated DE/heifer/day. Animals were fitted with jugular cannula on day 8 of the estrous cycle. A 12 gauge needle was inserted into the jugular vein and approximately 30 cm of polyvinyl tubing (V-9 cannula; Bolab Inc., Lake Havasu City, AZ) was fed through the needle bore, and an additional 30 cm of tubing exteriorized. The needle was then removed and external tubing secured using an Elasticon (Johnson and Johnson, New Brunswick, NJ) pouch (10 cm²) attached to the neck with adhesive cement over the site of cannula exit. The cannula was then flushed with heparinized saline (200 U/ml) to prevent occlusion.

Infusions

On each of days 9 to 13 of the estrous cycle, three heifers received intravenously (IV), via jugular vein cannula, 1 liter of physiological saline (0.9% NaCl; SAL heifers); and three heifers received 1 liter of a 20% soybean oil emulsion (20% Intralipid; Kabivitrum Inc., Alameda, CA; 50% linoleic acid, 26% oleic acid, 10% palmitic acid, 9% linolenic acid, 3.5% stearic acid; 2 Mcal/l; 10% of dietary digestible energy; IL heifers). Infusions were carried out during a 4 h period beginning at 1300 h of each day.
Blood Collection and Analysis

Thirty ml of jugular blood were collected from each animal twice daily (0600 h and 1700 h or after infusion) on days 8 to 16 and once daily from day 17 to estrus. Blood was collected into heparinized tubes (25 units), put on ice, and centrifuged within 1 h at 3000 x g for 30 minutes. Plasma was aspirated and stored at -20 C until analysis for PGFM, progesterone, and estradiol. Plasma samples were assayed for PGFM (days 8 to 16) by a radioimmunoassay system described previously (Guilbault et al., 1984a). Samples were assayed in duplicate with intra- and interassay coefficients of variation (CV) of 7.0 and 10.1%, respectively. Plasma samples from day 8 to estrus were analyzed for progesterone (Knickerbocker et al., 1986b; intra assay CV = 13.4%; inter assay CV = 24.6%) and estradiol (Guilbault et al., 1987b; days 8 to 16; intra assay CV = 6.6% and inter assay CV = 20.0%) by validated radioimmunoassays. Dilutions of Intralipid (1:10 to 1:2000) did not crossreact with estradiol or PGFM radioimmunoassays.

Estradiol and Oxytocin Injections

On day 15 of the estrous cycle (0900 or 40 h after last infusion), heifers were injected (IV) with 3 mg of estradiol-17β in 6 ml of saline/ethanol (50:50) vehicle. Ten ml of blood were collected every 30 minutes from 2 h before injection to 10 h after injection. Plasma was harvested and assayed for PGFM. On the following day or day 16 of the estrous cycle (i.e., day 16), heifers were injected (IV) with
100 units of oxytocin (5 ml of 20 U/ml, Vedco Company, Arcadia, CA). Blood (10 ml) was collected at -60, -45, -30, -15, 0, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes from injection of oxytocin, and plasma subsequently assayed for PGFM.

Ovarian Ultrasound

On estrous cycle day 16 (after the oxytocin challenge), populations of ovarian follicles were quantitated using transrectal real-time linear-array scanning ultrasound (LS-300 Ultrasound Diagnostic System, Tokyo Reiki Co., Ltd., Tokyo, Japan). Size and number of ovarian follicles greater than 3 mm were recorded.

Statistical Analysis

Experimental responses were analyzed by least squares analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS, 1987). The experimental design was in the nature of a split plot analysis of variance with repeated measurement of each cow over time. Sources of variation included treatment, animal within treatment, day, and treatment-by-day interaction. Time trends were tested for higher order polynomials and the gain in residual variance was analyzed by homogeneity of regression (Wilcox et al., 1990). Differences in residual variances between treatments were examined by performing an F test on the ratio of error variance among treatments.
Figure 3-1. Plasma 13,14 dihydro-15-keto-prostaglandin $F_{2\alpha}$ during cycle days 8 to 14 in heifers intravenously infused with 1 liter saline or 1 liter 20% soybean oil emulsion (Intralipid) on cycle days 9 to 13.

Results

Plasma PGFM During Infusion

The concentrations of PGFM in plasma during the infusion period are presented in Figure 3-1. During the preinfusion samples, concentrations of PGFM in plasma were similar in saline (SAL; 31.5 ± 2.6 pg/ml) and Intralipid- (IL; 32.1 ± 3.9 pg/ml) treated heifers. After infusion, plasma PGFM increased (P < .001) in IL heifers and remained basal (29.6 ± 3.6 pg/ml) in SAL heifers. Concentrations of plasma PGFM after infusion
in IL heifers were 309 ± 53.2, 152 ± 42.3, 78 ± 18.5, 84 ± 18.4, and 97 ± 19.3 pg/ml for days 9 to 13, respectively.

**Plasma Estradiol During Infusion**

The concentrations of estradiol in plasma are presented in Figures 3-2 (SAL heifers) and 3-3 (IL heifers). Plasma estradiol averaged 7.6 ± .5 pg/ml for all samples collected between days 9 and 14 for SAL heifers and concentrations were not different from IL heifers (9.3 ± .9 pg/ml). However, the residual variance associated with plasma estradiol during the infusion period was greater for IL heifers compared with SAL heifers (15.17 > 4.24 pg/ml; P< .01).

**Plasma Progesterone During the Treatment Cycle**

The dynamics of plasma progesterone during the estrous cycle are presented in Figures 3-4 (SAL heifers) and 3-5 (IL heifers). Normal luteolysis occurred on estrous cycle days 19, 20, and 21 (mean = day 20) for SAL heifers and on estrous cycle days 17, 19, and 19 (mean = day 18.3) for IL heifers. Progesterone profiles tended to be different (P=.10) among groups when analyzed by polynomial regression. There was an apparent decline in plasma progesterone during the infusion for two of the IL-treated heifers.

**Plasma PGFM after Estradiol and Oxytocin**

Administration of estradiol caused an increase in plasma PGFM beginning at 4 hours after injection, a maximum was obtained at approximately 7 hours after injection, and return to basal concentrations occurred by 10 hours after injection.
Figure 3-2. Plasma estradiol during cycle days 8 to 15 in heifers intravenously infused with 1 liter of saline on cycle days 9 to 13.

(Figure 3-6). Profiles of PGFM in response to estradiol were not affected by treatment; SAL- and IL-treated heifers had peak PGFM responses at 6.5 h (243.2 ± 34.4 pg/ml) and 7 h (211.3 ± 51.3 pg/ml), respectively.

Injection of oxytocin caused an immediate increase in plasma PGFM with maximum concentrations at 15 minutes and a subsequent return to baseline by 1.5 h after injection (Figure 3-7). Release of PGFM in response to oxytocin was not affected significantly by treatment and averaged 328 ± 118.7
Figure 3-3. Plasma estradiol during cycle days 8 to 15 in heifers intravenously infused with 1 liter of Intralipid on cycle days 9 to 13.

pg/ml for SAL-treated heifers and $193 \pm 27.1$ pg/ml for IL-treated heifers at 15 minutes after injection.

Ovarian Ultrasound

On day 16, heifers treated with IL had a greater number of follicles per ovary ($P<.01$) and a greater accumulative follicular diameter per ovary ($P<.01$; Table 3-1). Size of the largest follicle tended ($P=.10$) to be greater in IL-treated heifers, whereas average follicular diameter was unaffected by treatment (5.35 mm).
Figure 3-4. Plasma progesterone (ng/ml) from cycle days 7 to 23 in heifers intravenously infused with 1 liter of saline on cycle days 9 to 13.

**Discussion**

Infusion of a soybean oil emulsion increased the concentrations of PGFM in plasma during diestrus in heifers. Immediate increases in prostaglandins after soybean oil infusion has been suggested by data from sheep (McKeem et al., 1978). It is not known whether these changes in PGFM are a result of a direct conversion of linoleic acid to PGF, however, this seems unlikely. Human studies suggest that 2 to 3 days are required for linoleic acid to be converted to
Figure 3-5. Plasma progesterone from cycle day 7 to 23 in heifers intravenously infused with 1 liter of Intralipid on cycle days 9 to 13.

Arachidonic acid and subsequently to PGF (Nichaman et al., 1967). The slow conversion of linoleic acid to PGF is supported by work in other species where 4 to 5 days of linoleic acid feeding are required before basal increases were detected in PGF (Adam et al., 1982). Cattle may differ from other species in the time required for conversion of linoleic to PGF, or perhaps the PGFM detected was not derived from the linoleic acid in the Intralipid. This suggests that linoleic acid specifically or fatty acids in general can cause PGF
synthesis and secretion. The release of PGF apparently is not an artifact of oil emulsion infusion because, when 400 ml of safflower oil (70% linoleic) or olive oil (7% linoleic) were infused into the abomasum of cattle, similar increases in PGFM (plasma of jugular vein) and PGF (plasma of posterior vena cava) after about 4 h (data not shown) were detected.

Changes in estradiol during the infusion period were dramatic in two of the IL-treated heifers. This result, which may have been caused by fatty acids in general or specifically
Figure 3-7. Plasma PGFM on cycle day 16 after intravenous injection of 100 units of oxytocin (oxy) in heifers infused with 1 liter of saline or Intralipid on cycle days 9 to 13.

by PGF, suggests enhanced follicular recruitment. Large increases in estradiol concentrations in response to fatty acids and PGF are not without precedence. Shemesh and Hansel (1975a) detected large increases in plasma estradiol when they injected arachidonic acid directly into the bovine corpus luteum (CL) in vivo. In addition, Carlson et al. (1973) detected increases in both estradiol and LH when they infused PGF into the ewe. One IL-treated heifer had no apparent fluctuations in estradiol concentrations in response to
Table 3-1. Ovarian follicular response (estrous cycle day 16) of heifers infused with intralipid or saline.

<table>
<thead>
<tr>
<th>Response (per ovary)</th>
<th>Intralipid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SE</td>
<td>Mean  SE</td>
</tr>
<tr>
<td>Number of Follicles</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt; 1.4</td>
<td>2.0 0.3</td>
</tr>
<tr>
<td>Largest Follicle Diameter</td>
<td>10.2&lt;sup&gt;b&lt;/sup&gt; 1.7</td>
<td>7.0 0.7</td>
</tr>
<tr>
<td>Mean Follicle Diameter</td>
<td>5.3 1.3</td>
<td>5.4 0.6</td>
</tr>
<tr>
<td>Accumulated Follicle Diam.</td>
<td>31.0&lt;sup&gt;a&lt;/sup&gt; 4.0</td>
<td>10.8 1.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Intralipid > Saline, P<.01.
<sup>b</sup>Intralipid > Saline, P<.10.

treatment; likewise, this may have been due to a lack of estrogen-active follicles on her ovary during infusion.

Estradiol and oxytocin challenges were designed to detect differences in the uterine PGF synthetic and secretory capacities of the heifers (Knickerbocker et al., 1986a). Injection of estradiol caused a delayed release of PGFM in this study as has been seen by others (Knickerbocker et al., 1986a; Thatcher et al., 1986). The immediate release of PGF in response to oxytocin is also characteristic (LaFrance and Goff, 1985). Treatment with IL did not influence these responses. The unexpected active secretion of PGF (associated with infusion on estrous cycle days 9 to 13) in the IL-treated heifers, as well as their apparently hastened luteolysis
suggests that the estradiol and oxytocin challenges may have been administered too late. A reduction in arachidonic acid reserves and/or availability, and the physiological advancement of the estrous cycle of the IL-treated heifers may have eliminated detection of treatment effects when estradiol and oxytocin were administered on days 15 and 16.

The effect of IL treatment on ovarian follicular recruitment was dramatic. It is possible that the increased dietary energy experienced by IL-treated heifers may have had a direct effect on the ovary or on hypothalamic regulation of pituitary gonadotropin secretion. This could have resulted in active follicular growth. We chose saline as our control substance because: 1) if the response was a result of LCFA in general (not linoleic acid specific) then other fatty acid emulsions (e.g., an oleic acid rich emulsion) would not be a negative control, and 2) infusion of alternate energy substrates (e.g., glucose), while equal in energy, may have initiated undesirable metabolic effects in the heifers. These concerns suggest that saline was a reasonable choice for a control substance. Alternatively, previously elevated concentrations of PGF during the infusion period may have stimulated follicular development. The response of follicles to PGF apparently is positive. Villeneuve et al. (1988) and Guilbault et al. (1987b) have demonstrated increased follicular recruitment in early postpartum PGF treated cattle. These latter results and those of the present study
suggest that PGF either directly and/or indirectly causes follicular growth. Apparent mitogenic affects of PGE$_2$ have been demonstrated in rat intestine (Johnson and Guthrie, 1976) and a similar effect may be occurring in follicular granulosa and theca cells. Alternatively, PGF may be increasing pituitary gonadotropin release as demonstrated in the cow (Hafs et al., 1974). Increased gonadotropin secretion may enhance concentrations of estradiol in follicular fluid (promoting growth) or estradiol may influence follicular FSH and LH receptor populations in a manner similar to follicular growth factors. Platelet derived growth factor, known to potentiate the FSH effect on LH receptors on rat granulosa cells (Mondschein and Schomberg, 1984), has been shown to initially activate cyclooxygenase and cause PGF secretion when added to fibroblasts (Stiles, 1983). This may suggest a relationship between PGF and LH receptor numbers.

Progesterone profiles were not similar between treatments. Two IL-treated heifers had an apparent decline in plasma progesterone during the infusion period. This may have been caused by increased exposure of the CL to PGF which decreased progesterone secretion. In fact, time of completed luteolysis occurred on the average 1.3 days earlier in the IL-treated heifers. An earlier luteolysis may have resulted from increased follicular development during late diestrus that hastened uterine PGF secretion and luteolysis.
In conclusion, fatty acids, and perhaps linoleic acid specifically, can increase basal PGFM secretion in the cow, alter ovarian function, and stimulate follicular recruitment. These data suggest that development and feeding of a protected form of linoleic acid or fatty acids in general may be beneficial for the early postpartum cow where follicular growth is a prerequisite for restoration of estrous cycles. Additional research in this area is warranted.
CHAPTER 4
EFFECT OF ENERGY BALANCE ON THE SIZE AND NUMBER
OF OVARIAN FOLLICLES DETECTED BY ULTRASONOGRAPHY
IN EARLY POSTPARTUM DAIRY COWS

Introduction

The importance of postpartum energy balance (EB) to the recrudescence of normal ovarian cycles in high producing dairy cattle has been recognized in several studies (Butler and Smith, 1989). Energy balance has been defined as the difference between the net energy (NE) intake of the animal minus the NE required for maintenance and the NE content of the secreted milk. Dairy cattle undergo an energy deficit in early lactation because maximum milk production is attained prior to gastrointestinal growth and expansion allowing for greater feed consumption (Bauman and Currie, 1980; Coppock, 1985). This situation leads to a compensatory response (homeorhesis; Bauman and Currie, 1980) involving adipose tissue (increased lipolysis), liver (increased gluconeogenesis and glycogenolysis), muscle (mobilization of protein reserves) and bone (mineral mobilization). Eventually, increased gastrointestinal capacity and activity (turnover) lead to increased energy intake and a positive energy balance. Calculated energy balance may reach -15 Mcal/day in some postpartum cows and over 50 days (d) of lactation may be required to achieve an energy intake which allows for energy
neutrality (Beghorn et al., 1988; Villa-Godoy et al., 1988). Deficiencies in energy intake will likely become increasingly commonplace as genetic selection and exogenous hormonal treatments (i.e., bovine somatotropin) act to increase average lactational milk yield within dairy herds (Peel and Bauman, 1987).

During the period of energy deficiency, the hypothalamic-pituitary-ovarian axis recovers from the influence of previous pregnancy and undergoes active changes leading to the reinitiation of estrous cycles (Nett, 1987; 1990). In most cows, pituitary secretion of luteinizing hormone increases during the first two to three weeks after parturition (Echternkamp and Hansel, 1973; Edgerton and Hafs, 1973; Fernandes et al., 1978). Luteinizing hormone (LH), acting on ovarian follicles, induces waves of follicular growth which lead to the selection and ovulation of a follicle between 15 and 25 d after calving (Stevenson and Britt, 1979; Lamming et al., 1982). This ideal scenario is apparently affected by the postpartum energy balance of the animal. An extremely negative energy balance will dampen pulsatile secretion of LH (Imakawa et al., 1987b) and delay ovulation. This contributes to on-farm inefficiency through economic losses associated with postpartum anestrus. Possible modulators of these events are opioide peptides acting at the level of the hypothalamus to decrease the release of gonadotropin-releasing hormone (GnRH) and therefore LH (Butler and Smith, 1989). Alternatively,
metabolically-responsive hormones like insulin may act directly on the ovary to influence follicle growth or be transported to the cerebral spinal fluid to modulate hypothalamic function in accordance with energy status (Baskin et al., 1987; Poretsky and Kalin, 1987). Isolation of insulin receptors within the hypothalamus (Havrankova et al., 1983) supports the concept of a possible role for insulin in the coordination of energy balance and reproductive function.

Ultrasonic examination of the ovary has been shown to be an accurate and reliable method of quantitating ovarian follicle populations (Pierson and Ginther, 1984). The objective of this experiment was to evaluate the recrudescence of ovarian follicular growth using ultrasonography, and to determine how energy balance and diet influence changes in ovarian follicular populations in postpartum dairy cattle whose reproductive cycles had been programmed for scientific study.

Materials and Methods

Animals and Diet

Fifty-two multiparous Holstein cows calving between August 15 and November 17, 1988 at the University of Florida Dairy Research Unit, Hague, Florida, were used. The cows were housed in open-air free stall barns with free access to dirt exercise lots from 10 d prior to expected calving until 77 d postpartum. At calving, cows were assigned randomly to one of six diets (treatment), which were fed for the entire
Table 4-1. Composition (%) DM and formulated energy content of the six diets fed to cows from 0 to 77 d postpartum.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermudagrass hay</td>
<td>14.7</td>
<td>14.7</td>
<td>14.7</td>
<td>14.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.7</td>
<td>-</td>
</tr>
<tr>
<td>Alfalfa cubes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.4</td>
</tr>
<tr>
<td>Lipid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn silage</td>
<td>27.8</td>
<td>27.8</td>
<td>27.8</td>
<td>27.2</td>
<td>31.6</td>
<td>37.9</td>
</tr>
<tr>
<td>Ground corn</td>
<td>17.4</td>
<td>17.4</td>
<td>17.4</td>
<td>17.1</td>
<td>15.8</td>
<td>12.1</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
<td>7.1</td>
<td>6.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Distillers' grains</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.2</td>
<td>13.4</td>
<td>13.6</td>
</tr>
<tr>
<td>Whole cottonseed</td>
<td>14.7</td>
<td>14.7</td>
<td>14.7</td>
<td>14.4</td>
<td>14.7</td>
<td>15.3</td>
</tr>
<tr>
<td>Minerals/vitamins</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>3.4</td>
<td>3.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>

NEL (Mcal/Kg) | 1.74 | 1.70 | 1.70 | 1.75 | 1.68 | 1.70

<sup>a</sup>Unbalanced basal diet formulated to be deficient in nutrients supplied by 2.9 kg DM of long bermudagrass hay offered separately.

<sup>b</sup>Balanced basal diet with long bermudagrass hay offered separately.

<sup>c</sup>Calcium salt of palm oil (Megalac).

Experimental period. Diets included a totally mixed ration (TMR) consisting of corn grain, corn silage, soybean meal, distillers grains, and whole cottonseed, supplemented with different species and forms of hay (Table 4-1). Bermudagrass was fed as long hay (diets 1 and 2) or chopped and mixed with the TMR (diets 3 and 4). Alfalfa was mechanically cubed and mixed with the TMR (diet 6), or chopped and mixed with the TMR.
(diet 5). Diet 4 was supplemented with Ca salts of long chain fatty acid (2.2% of DM; Megalac, Church and Dwight Co., Inc., Princeton, NJ). Ration ingredients were mixed daily and offered in two feedings at 0830 and 1430 h in amounts that allowed 5 to 10% refusals. The Calan electronic door feeding system (American Calan, Inc., Northwood, NH) was used to monitor feed intake and refusals of individual cows on a daily basis.

Calculation of Energy Balance

Feed intake was determined daily for each individual cow (feed offered minus orts). Dry matter content of the feed and orts were determined by weekly analysis. Nutrient content of ration components was estimated by weekly sampling and ration analysis (New York State Forage Testing Laboratory, Ithaca, NY). Calculation of the net energy intake (NEI) was made by multiplying the DM consumption (TMR and hay) by the calculated Net Energy of Lactation (NEL) of the ration.

Daily milk production (MP) was recorded electronically and body weight (BW) measurements made once weekly. Milk fat composition was determined by the DHIA testing laboratory (Raleigh, NC) for weekly samples of four consecutive (am and pm) milkings. Net energy required for body maintenance (NEM) was calculated using the equation \( \text{NEM} = (\text{kgBW}^{0.75}) \times 0.08 \) (NRC, 1989). Daily BW of cows between measurements was estimated by linear interpolations. Net energy in the secreted milk (NEL) was calculated using the equation \( \text{NEL} = \text{MP} \times 0.312 + \)
[.0962*fat%]) (NRC, 1989). Net energy balance (NEB) was derived on a daily basis using the equation $\text{NEB} = \text{NEI} - \text{NEM} - \text{NEL}$. Values were then subjected to a third order regression analysis for each cow to achieve a smoothed energy balance curve. Predicted values (PEB) from these regressions for each d (0 - 77) were then used in statistical analyses.

**Reproductive Management**

Cows were treated for uterine or metabolic disorders for the first 25 d postpartum. Ovarian disorders (i.e., cystic structures), if diagnosed, were not treated. On d 25, all cows were injected with 25 mg of prostaglandin $F_2\alpha$ (PGF; Lutalyse; UpJohn Co., Kalamazoo, MI) and fitted with an intravaginal progesterone releasing device (Controlled Internal Drug Release-Bovine, CIDR-B; Eazi-breed, AHI Plastic Co., New Zealand) containing a total of 1.9 g of progesterone for 15 d. This was done to standardize the ovarian follicular environment and prevent ovulation during a period of reported negative energy balance (i.e., 3 to 5 weeks of lactation). On d 40 postpartum, the CIDR-B was removed and cows were observed for estrus for 14 d. Any cow not expressing estrus after 14 d was subjected to ovarian ultrasound examination and those cows not having a corpus luteum at that time were diagnosed as having no ovulation (or estrus) after CIDR removal. The cows were first inseminated at the first observed estrus following the CIDR-induced estrous cycle (about 60 d postpartum).
Ovarian Ultrasonic Examination

Ovaries were examined using an Equisonic model 300A linear array ultrasound scanner equipped with a 7.5 MHz transducer (Equisonics 300A, Tokyo, Keiki, Tokyo, Japan). Briefly, fecal material was removed from the rectum, and the transducer inserted into the rectum and held adjacent to the ovary. Serial sections were examined; size (diameter) and number of ovarian follicles (≥ 3 mm) were recorded onto follicular maps. Ovarian follicles were organized into 4 classes based on diameter. They were: class 1 (3 to 5 mm); class 2 (6 to 9 mm); class 3 (10 to 15 mm) and class 4 (> 15 mm). In addition, presence and diameter of corpora lutea (CL) were recorded. The relationship between calculated follicular volume (CV; mm³; based on diameter determination by ultrasound) and aspirated follicular fluid volume (AV; cm³) for follicles greater than 5 mm in diameter was $CV = -73 + 1482*AV$ \ ($R^2 = .73$; K.L. Macmillan and W.W. Thatcher, unpublished observations).

Ultrasound examinations occurred before insertion of the CIDR (d 7, 16, 18, 20, 22, 25), during the period of CIDR insertion (d 30, 35, and 40), and after removal of the CIDR (every other day until estrus, and then on d 6, 12, and 18 of the subsequent estrous cycle).

Blood Collection and Analysis

Ten milliliters of blood were collected by coccygeal venipuncture on each day that a cow was examined by
ultrasonography. Blood was collected into evacuated heparinized (143 units) tubes (Vacutainer, Becton Dickinson, East Rutherford, NJ) and put on ice and centrifuged at 3,000 x g for 20 minutes within 30 minutes of collection. Plasma nonesterified fatty acids (NEFA), glucose, and insulin were measured on d 7, 16, 20, 25, 30, 35, and 40 postpartum. Plasma NEFA were measured by an enzymatic colorimetric method using a prepared kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma glucose was measured in duplicate samples (25 ul) using an oxidase/peroxidase colorimetric method (Glucose No. 510; Sigma Chemical Co., St. Louis, MO). Plasma insulin was measured in duplicate samples (150 ul) by radioimmunoassay described previously (Collier et al., 1982). Intra- and interassay coefficients of variation for insulin analysis were 2% and 12%, respectively.

Statistical Analyses

Total number of follicles within each size class was analyzed using the General Linear Models procedure of the Statistical Analysis System (SAS, 1987). Numerous models were employed including treatment (diet), animal nested within treatment, day postpartum, follicle size class, as well as second- and third-order interactions. Predicted energy balance (PEB) for the specific day in which follicular data were recorded was included as a covariate in the analyses. Interactions of PEB with class variables were also tested. During the CIDR and post-CIDR period, the main effect of day
was not significant so models included treatment, animal-
within-treatment, class, and treatment-by-class interaction. In addition, the relationship between NEFA and energy balance, as well as interval to first ovulation and energy balance was tested by linear regression. Plasma insulin and glucose were analyzed using a model that included treatment, animal-within-treatment, day postpartum, the treatment-by-day interaction and residual.

Results

Follicular Development Before Day 25

Average number of follicles in each size class on successive days postpartum is presented in Figure 4-1. Before day 25, the average number of small (class 1) follicles decreased while the number of large (class 3 and class 4) follicles increased with increasing days postpartum (follicular class-by-day interaction, P<.01). Dietary treatment did not influence the total number of follicles recorded. However, a treatment-by-class interaction existed (P<.06), suggesting that the number of follicles within each follicular size class was not similar among diets (Table 4-2). Including the PEB-by-class interaction in the statistical model increased the probability associated with the treatment-by-class interaction to 0.46 (day-by-class interaction remained significant, P<.06) suggesting treatment-by-class differences were associated with differences in PEB among diets (see below).
Figure 4-1. Average number of follicles within different size classes (class 1, 3 to 5 mm; class 2, 6 to 9 mm; class 3, 10 to 15 mm; class 4, >15 mm) in cattle (n=52) from d 7 to 25 after calving.

Development of Individual Follicles Before Day 25

Graphs of the growth and decline of individual follicles in early postpartum cows from d 16 to 25 after calving are presented in Figures 4-2 and 4-3. Two of thirty-seven cows that ovulated before CIDR insertion are presented in panels 4-2a and 4-2b. In these cows, ovulation occurred after 4 to 6 d of follicular growth, ending in the rapid disappearance of the follicle and subsequent development of a CL. For the cow
Table 4-2. Average number of follicles (per cow-d) in different follicular size classes (Cl; Class 1, 3 to 5 mm; class 2, 6 to 10 mm; class 3, 10 to 15 mm; and class 4, >15 mm) in cows (N=52) on six different diets before the insertion of the CIDR (Pre-CIDR, d 7 - 25 postpartum; Period 1), during the insertion of the CIDR (d 25 - 40; Period 2), and during an estrous cycle after CIDR insertion (post-CIDR; Period 3).

<table>
<thead>
<tr>
<th>Dietary Treatment(^{a,b,c})</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period Cl</td>
<td>X</td>
<td>SE</td>
<td>X</td>
<td>SE</td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>1 1</td>
<td>3.0</td>
<td>.4</td>
<td>2.6</td>
<td>.4</td>
<td>4.6</td>
<td>.6</td>
</tr>
<tr>
<td>1 2</td>
<td>1.2</td>
<td>.2</td>
<td>1.3</td>
<td>.2</td>
<td>1.3</td>
<td>.2</td>
</tr>
<tr>
<td>1 3</td>
<td>0.8</td>
<td>.1</td>
<td>0.9</td>
<td>.1</td>
<td>0.9</td>
<td>.1</td>
</tr>
<tr>
<td>1 4</td>
<td>0.2</td>
<td>.1</td>
<td>0.2</td>
<td>.1</td>
<td>0.1</td>
<td>.1</td>
</tr>
<tr>
<td>2 1</td>
<td>2.5</td>
<td>.5</td>
<td>3.4</td>
<td>.7</td>
<td>2.6</td>
<td>.5</td>
</tr>
<tr>
<td>2 2</td>
<td>0.8</td>
<td>.3</td>
<td>1.3</td>
<td>.3</td>
<td>0.6</td>
<td>.2</td>
</tr>
<tr>
<td>2 3</td>
<td>0.9</td>
<td>.2</td>
<td>0.7</td>
<td>.2</td>
<td>0.7</td>
<td>.1</td>
</tr>
<tr>
<td>2 4</td>
<td>0.8</td>
<td>.1</td>
<td>0.7</td>
<td>.1</td>
<td>0.7</td>
<td>.1</td>
</tr>
<tr>
<td>3 1</td>
<td>2.9</td>
<td>.6</td>
<td>2.5</td>
<td>.9</td>
<td>1.6</td>
<td>.5</td>
</tr>
<tr>
<td>3 2</td>
<td>1.0</td>
<td>.2</td>
<td>1.3</td>
<td>.4</td>
<td>1.1</td>
<td>.4</td>
</tr>
<tr>
<td>3 3</td>
<td>1.4</td>
<td>.3</td>
<td>1.4</td>
<td>.3</td>
<td>1.1</td>
<td>.2</td>
</tr>
<tr>
<td>3 4</td>
<td>0.3</td>
<td>.1</td>
<td>0.3</td>
<td>.1</td>
<td>0.2</td>
<td>.1</td>
</tr>
</tbody>
</table>

\(^a\) Period 1, Treatment-by-class, P<.06.
\(^b\) Period 2, Treatment-by-class, P<.01.
\(^c\) Period 3, Treatment-by-class, nonsignificant.
Figure 4-2. Patterns of follicular growth in two cows (A and B) that ovulated before d 25 postpartum. Individual lines represent changes in diameter for large follicles (FOL) on either ovary detected by ultrasound (*=ovulation).

depicted in Figure 4-2a, a large follicle existed (FOL 1) and finally ovulated, while a second follicle (FOL 2) grew in size but did not subsequently ovulate. The cow depicted in Figure 4-2b had a large follicle (14 mm; FOL 2) on d 16 which decreased in size and was followed by the growth of three other follicles (FOL 1, 3, and 4), one of which eventually ovulated (FOL 1). Fifteen cows did not ovulate before CIDR insertion. Follicular growth patterns are presented in Figures 4-3a and 4-3b for two of these cows. Cows not ovulating before d 25 were not necessarily devoid of class 3 or 4 follicles (i.e., > 10 mm in diameter). The cow presented in Figure 4-3a had a large follicle (19 mm; FOL 1) on d 16 which decreased in size through d 25 postpartum. Additional follicles (FOL 2 to 5) began growing on d 22 postpartum, but were possibly prevented from ovulation because of the insertion of the CIDR. The cow presented in Figure 4-3b had
five follicles growing from d 16 to 18, with two follicles (FOL 1 and 2) continuing to increase in size until the insertion of the CIDR (d 25).

Figure 4-3. Patterns of follicular growth in two cows (A and B) that did not ovulate before d 25 postpartum. Individual lines represent changes in diameter for large follicles (FOL) on either ovary detected by ultrasound.

Effect of Diet and PEB on Plasma Metabolites and Insulin

A representative energy balance curve with actual EB and PEB (based on third order fitted curve) for one cow in the trial is presented in Figure 4-4. Average PEB for all cows ranged from a minimum on d 3 of + .9 ± .9 Mcal/d to a maximum on d 55 of 7.5 ± .9 Mcal/d. Minimum predicted energy balance among cows during the first 25 d postpartum ranged from −15.9 Mcal/d to +9.5 Mcal/d (mean = −1.9 ± .9 Mcal/d) with 4, 5, 19, 15, 7, and 2 cows having minimum PEB falling into the range of < −10, −9.99 to −5, −4.99 to 0, 0.01 to 5, 5 to 10, and >10 Mcal/d, respectively. Predicted energy balances for the six diets differed (P<.001) from d 0 to 77 postpartum (Figure 4-5) and indicated that cows on different diets in this trial were
not at equivalent energy statuses. Concentrations of NEFA in plasma were associated negatively with PEB \((P<.01; \text{NEFA} = 295 - 11.5\times\text{PEB}; R^2 = .146)\).

![Graph showing actual and predicted energy balance over postpartum days.](image)

Figure 4-4. Daily actual energy balance and predicted energy balance calculated for a cow during the first 77 d after calving. Predicted energy balance was determined from EB by third order regression analysis.

Concentrations of glucose in plasma increased during the postpartum period (day effect; \(P<.001\); Table 4-3). Glucose was similar for cows on different diets but was not dependent on PEB (covariate nonsignificant). Plasma insulin also increased during the postpartum period (day effect; \(P<.08\); Table 4-3) and was not similar among diets \((P<.01)\). Plasma
Figure 4-5. Average daily predicted energy balance for cows (n=52) on six different diets from 0 to 11 weeks of lactation.

Table 4-3. Least square means (mean) and SE for plasma insulin (ng/ml) and glucose (mg %) in cows (n=52) from d 7 to 40 postpartum.

<table>
<thead>
<tr>
<th>Day</th>
<th>Insulin&lt;sup&gt;a&lt;/sup&gt; (ng/ml)</th>
<th>Glucose&lt;sup&gt;b&lt;/sup&gt; (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>7</td>
<td>0.89</td>
<td>0.06</td>
</tr>
<tr>
<td>16</td>
<td>0.91</td>
<td>0.05</td>
</tr>
<tr>
<td>20</td>
<td>0.94</td>
<td>0.04</td>
</tr>
<tr>
<td>25</td>
<td>0.99</td>
<td>0.05</td>
</tr>
<tr>
<td>30</td>
<td>1.02</td>
<td>0.05</td>
</tr>
<tr>
<td>35</td>
<td>0.97</td>
<td>0.05</td>
</tr>
<tr>
<td>40</td>
<td>1.08</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Day, P < .08;
<sup>b</sup> Day, P < .001;
insulin averaged 1.26 ± .10, 1.14 ± .09, .81 ± .12, .86 ± .10, .96 ± .10, .80 ± .10 ng/ml for diets 1 to 6, respectively. Plasma insulin was dependent on PEB (P<.0001) with higher PEB associated with greater plasma insulin (Y = .77 + .043*PEB; r² = .13). Including PEB as a covariate in the statistical model eliminated dietary treatment and day as significant sources of variation suggesting that differences in insulin concentration among diets were caused by differences in overall energy balance between diets and days.

**Influence of Energy Balance on Follicle Populations**

Effect of PEB on number of follicles in each size class before d 25 is presented in Figure 4-6. Regression lines were estimated using the General Linear Models procedure of SAS based on statistical models including all main effects described previously (including treatment). Effect of PEB on the average number of follicles per cow differed among follicular size classes (PEB-by-class P<.01). Number of class 1 and 2 follicles decreased with increasing PEB while numbers of class 3 follicles increased. Frequency of class 4 follicles (> 15 mm) showed no change with increasing PEB. Careful interpretation of these data is necessary because the PEB-by-class interaction is not significant if diet 4 (lipid supplemented) is removed from the analyses (see Chapter 5).

Multiple ovulation (i.e. the presence of 2 or more functional CL during a single estrous cycle) occurred in 12 cows before insertion of the CIDR. Cows experiencing multiple
Figure 4-6. Predicted regression lines for the interaction between PEB and follicular class determined from analysis of follicular class data.

Ovulations (preCIDR) had higher average PEB (P<.05; 7.58 ± 1.30 Mcal/d) than cows having single (n=23; 3.89 ± .94 Mcal/d) or no ovulations (n=15; 3.82 ± 1.16 Mcal/d). The day when the first postpartum CL was detected by ultrasound was dependent on PEB. Interval (days) to CL detection occurred earlier in those individuals with a more positive mean PEB. (P < .06; Interval = 18.53 - .18*PEB mean; r² = .14).

After insertion of the CIDR, total number of follicles in all size classes were not dependent on PEB. However, numbers
of follicles within each size class were different for cows on different diets (treatment-by-class, P<.01; Table 4-2). When the CIDR was removed, 34 cows ovulated within 1 to 8 d after CIDR removal (mean = 3.6 d), eight cows did not ovulate within 14 d, and eight cows developed ovarian follicular cysts. Subsequent reproductive status after removal of the CIDR was similar in cows ovulating or not ovulating before insertion of the CIDR (Table 4-4). Overall, three cows (6%) on the experiment did not ovulate before or after the CIDR and were considered physiologically anestrus. Considering diets individually, the number of cows ovulating before CIDR insertion was nearly equivalent (Table 4-5). However, after removal of the CIDR, there appeared to be dietary differences in terms of the number of cows eventually ovulating. Sixty-two percent (21/34) of cows consuming bermudagrass (diets 1 to 4) ovulated after CIDR removal compared with 81% (13/17) of cows consuming alfalfa. Although not significant statistically, incidence of cysts or nonovulation in cows fed bermudagrass was twice (on percentage basis) that of cows fed alfalfa.

Energy balance profiles (d 0 to 50) for cows having ovulation and forming a CL, developing ovarian cysts, or having no ovulation after the CIDR were not alike (P<.001; Figure 4-7). Those cows developing follicular cysts had an average PEB lower than other cattle. In contrast, those cows not ovulating after CIDR removal had an average PEB that was
Table 4-4. Number and percentage (%) of cows having estrus, ovarian follicular cysts, or no estrus after CIDR treatment from d 25 to 40 postpartum separated according to ovulatory status before insertion of the CIDR.

<table>
<thead>
<tr>
<th>Pre-CIDR Status</th>
<th>n</th>
<th>Estrus</th>
<th>Cystic</th>
<th>No Estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulated</td>
<td>37</td>
<td>26 (70)</td>
<td>6 (16)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Anestrus</td>
<td>13</td>
<td>8 (62)</td>
<td>2 (15)</td>
<td>3 (23)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>50</td>
<td>34</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 4-5. Number of cows on each diet having (Ovul) or not having ovulation (Nonovul) before d 25 postpartum (CIDR insertion) and subsequently having estrus (ovulation), follicular cysts, or no estrus (anestrus) after CIDR treatment.

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Ovul</th>
<th>NonOvul</th>
<th>Estrus</th>
<th>Cystic</th>
<th>Anestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1</td>
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<td>4</td>
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<tr>
<td>6</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>52</td>
<td>37</td>
<td>15</td>
<td>34</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
initially lower and subsequently higher than other cattle. Cows ovulating and forming a CL were intermediate in their postpartum PEB changes. Considering those cows having an ovulation after CIDR removal, previous energy balance profiles were different between cows having short (1 to 2 d), intermediate (3 to 4 d), and long (5 to 8 d) intervals to estrus (P<.001; Figure 4-8). Cows having short and intermediate intervals to estrus had apparently different (short EB > intermediate EB) but parallel curves, while cows
with long intervals (5 to 8 d) had previous PEB profiles initially lower then higher than other cattle. There was no effect of day (d 6, 12, and 18 of the estrous cycle), PEB or diet on the number of follicles within size classes during the ensuing estrous cycle (Table 4-2).

Figure 4-8. Average daily energy balance for cows having different intervals to estrus after removal of the CIDR device. Cows either had short (1 to 2 d;[___]), intermediate, (3 to 4 d; [----]), or long (5 or > d [....]) intervals to estrus.
Subsequent reproductive performance of cattle fed each diet is presented in Table 4-6. Interval to first service averaged 86 d for all cows and ranged from 78 to 95 d (diets 3 and 6). Percentage of cows eventually becoming pregnant during the lactation was quite variable, ranging from 33 (diet 1, 3, and 6) to 100% (diet 5). Services per conception and services per cow was 1.92 and 2.34, respectively, for all cattle.

Table 4-6. Interval to first service (days), number of cows pregnant (Preg), services per conception (S/C) and services per cow (S/COW) for animals fed different diets from d 0 to 77 postpartum.

<table>
<thead>
<tr>
<th>Diet N</th>
<th>Mean</th>
<th>SE</th>
<th>Preg (%)</th>
<th>S/C</th>
<th>S/COW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 9</td>
<td>94.1</td>
<td>9.5</td>
<td>3 33</td>
<td>2.14</td>
<td>2.33</td>
</tr>
<tr>
<td>2 8</td>
<td>87.3</td>
<td>7.3</td>
<td>4 50</td>
<td>2.75</td>
<td>2.25</td>
</tr>
<tr>
<td>3 9</td>
<td>77.7</td>
<td>7.9</td>
<td>3 33</td>
<td>2.42</td>
<td>1.67</td>
</tr>
<tr>
<td>4 9</td>
<td>80.6</td>
<td>5.9</td>
<td>6 67</td>
<td>1.67</td>
<td>1.88</td>
</tr>
<tr>
<td>5 8</td>
<td>82.6</td>
<td>7.3</td>
<td>8 100</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>6 9</td>
<td>95.3</td>
<td>8.4</td>
<td>3 33</td>
<td>1.67</td>
<td>3.00</td>
</tr>
<tr>
<td>52 86.1</td>
<td>3.1</td>
<td>27 52</td>
<td>1.92</td>
<td>2.34</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The follicular events leading up to first ovulation in this group of dairy cows were characterized by decreasing numbers of smaller (class 1 and 2) follicles and increasing numbers of larger (class 3 and 4) follicles as day postpartum
increased. This pattern is consistent with the concept of follicular recruitment and selection leading to terminal follicular growth and dominance (Ireland and Roche, 1987). Apparently, smaller class follicles move into larger classes with increasing day postpartum and are not replenished during the first 25 d postpartum. The number of medium (class 2) follicles remains unchanged, likely because this represents a transitory size class with follicles moving into and then out of this diameter range. These patterns of growth cause an interaction between follicular frequency among classes and day postpartum.

Energy balance seems to modify these follicular population changes and affects the average number of follicles per cow for the first 25 d postpartum. As PEB increases, average number of small (class 1 and 2) follicles decrease, whereas the average number of class 3 follicles increase. This suggests that as the overall PEB of the cow increases there is a movement of small follicles into larger size classes. This theoretically leads to an earlier ovulation in cows with higher PEB. These data supports this concept since an earlier day to first ovulation was detected for those cows having higher PEB. However, the efficiency for the relationship between EB and first ovulation was fairly low ($R^2 = .10$), suggesting other factors are important when predicting the interval to first ovulation. Differences between diets in number of follicles with different size classes before d 25
was accounted for by a PEB-by-class interaction. This indicates that, when considering effects of different diets on follicular number, PEB should be considered as an important factor which can modulate follicle numbers across diets varying in composition. A cautious interpretation of the PEB-by-class interaction is needed, however, because one of the six diets contained CaLCFA (a dietary ingredient which affects follicle populations [see chapter 4]). Removal of diet 4 shifted the probability associated with the PEB-by-class interaction to above .10.

An interesting finding in this study was a higher ovulation rate associated with greater postpartum PEB. It has been established by others (Maurasse et al., 1985) that nutritional flushing will increase ovulation rate in sheep (Dufour and Matton, 1977; McNeilly et al., 1987) and pigs (Aherne and Krikwood, 1985) but has no consistent effect in cattle. Our findings suggest that, under certain physiological conditions, ovulation rate is influenced by energy balance in cows. This phenomena may be unique to postpartum cattle where patterns of follicular growth and development may be altered by previous pregnancy, metabolic hormones or metabolites, or homeorhetic responses. These data suggest that ovarian regulation of follicle growth in postpartum dairy cattle may not be physiologically normal in terms of their ability to control the growth of other
follicles (dominance) and this leads to errors in ovulation rate (i.e., > 1).

Energy balance did not influence number of follicles after d 25 postpartum (i.e., during the CIDR or post CIDR period). The CIDR device and PGF were used to program ovarian follicular environment at a time when cows are normally found at very different reproductive stages (i.e., anestrus or various phases of the estrous cycle). The lack of an effect of PEB during or after CIDR removal suggests that follicles did not respond to changes in PEB after d 25. This is consistent with our understanding of the single ovulatory nature of the species. These results lead to the implication that once a cow starts having regular estrous cycles, PEB does not influence her follicular dynamics. This is only partially correct, however, since we found that estrous cycling cows placed into energy deficit during final follicular maturation had retarded follicular growth (See Chapter 6). Changes in daily follicular growth rates were not measured in this trial and may represent an additional level of modulation of ovarian follicular dynamics by energy balance.

The reproductive response of cows after CIDR removal was influenced apparently by changes in PEB prior to and during the CIDR period. Those cows having an ovulation were intermediate in their profiles of PEB while those cows forming follicular cysts had lower PEB (Figure 4-7). A putative association between ovarian cysts and negative energy balance
has been reviewed previously (Kesler and Garverick, 1982). Our findings support the concept that events associated with negative energy balance can directly or indirectly influence the ovary and initiate physiological events culminating in a follicular cyst. Cystic follicles are believed to be a result of aberrations in ovulatory physiology and these data provide evidence that EB will alter events leading to normal ovulation in postpartum cows. In contrast, cows having no estrus (or ovulation) had rapidly changing profiles of PEB, initially lower and then higher than other cattle. Theoretically, the subsequent anestrus could be related to the shift away from body tissue mobilization toward nutrient deposition at the time of CIDR treatment. Considering cows having ovulation after CIDR removal, long intervals to estrus also were associated with similar PEB profiles as anestrous cows (i.e., relatively lower and then a transition to higher EB compared with other groups; Figure 4-8). Therefore, high PEB is associated not only with anovulation but delayed estrus after long term progesterone treatment.

Plasma glucose remained low during the early postpartum period (< 30 d) and increased thereafter. These changes were not dependent on PEB, supporting the general concept that glucose concentration in postpartum cattle is low and not perturbable by changes in overall energy status. Alternatively, plasma insulin was dependent on PEB with higher PEB being associated with greater plasma insulin. This was
anticipated given the importance of insulin to the coordination of metabolic processes surrounding nutrient partitioning and utilization. Dietary differences in insulin concentrations were explained by dietary differences in PEB. These results may implicate insulin as one potential modulator of the actions of PEB on follicular function. Two potential sites of action of insulin include the ovarian follicle and the hypothalamus (via the cerebral spinal fluid). Localization of insulin receptors in the brain of other species (Havrancova et al., 1983) suggests potential direct effects of insulin on brain function. In addition, the mitogenic effect of insulin on follicular proliferation via insulin receptors themselves, or cross reactions between insulin and IGF receptors at this time cannot by discounted (Poretsky and Kalin, 1987).

Diets seemed to influence the outcome of CIDR treatment and subsequent incidence of pregnancy in these cows. Cows consuming bermudagrass were twice as likely (on a percentage basis) to develop follicular cysts or become anestrus compared with cows fed alfalfa. Although the number of cows assigned to each diet prevented the detection of possible treatment differences, results of this study suggest that forage types, interacting with rumen function, can secondarily influence ovarian function. In addition to its greater nutrient content, alfalfa hay has greater buffering capacity than bermudagrass because of greater cation exchange capacity (Van
Soest, 1987). A less acidic rumen in cows fed alfalfa could potentially interact with ovarian responsiveness to treatments. While the present experiment was not a sensitive test of fertility, cows fed alfalfa hay appeared to be more fertile after the trial was completed. Interestingly, feeding regimens during the early postpartum period affected fertility later. It is possible that feeding strategies during the very early postpartum period may have direct effects on developing oocytes which initiate growth 40 to 60 d prior to ovulation (Lussier et al., 1987).

Mean daily PEB for cows in this study was higher than what is classically reported (NRC, 1989). The PEB of cows in this study was distributed uniformly (negatively and positively) around the mean PEB profiles presented in the results section and Figure 4-2. Estimates of PEB were considered accurate because PEB was correlated negatively with plasma NEFA and the regression equation generated from cows in this study predicts NEFA from EB reported for other herds (see wk 3 to 7 from Staples et al., 1990). The higher than expected PEB for cows in the present study relates, in part, to the production characteristics of Florida dairy cattle. Indeed, average milk production for the first 60 days for cows on this study was 26.3 kg (results reported elsewhere by Staples et al., 1989). Including cows with positive and negative PEB may have increased detection of effects of PEB on follicular populations. Similar investigations with higher
producing cows in greater negative energy balance would add to the knowledge gained from this study.

Finally, anestrous postpartum cows did not lack follicle growth and development (see Figure 4-3). This suggests that growth of follicles is not the sole cause of anestrus. Additional factors leading to ovulation also seem to be important. These may include absence of follicular estrogen secretion or the inability of the hypothalamus to respond to increasing estrogen with an ovulatory surge of LH. These other factors likely account for the generally imperfect relationship between EB and day to first ovulation.

In conclusion, PEB does influence follicular growth in early postpartum cows. These effects of PEB seem to influence day to first ovulation by influencing the movement of follicles into larger size classes. After estrous cycles have been initiated, PEB does not influence follicular growth or numbers of follicles within size classes, but dynamic changes in PEB influence ovulation or development of cystic ovaries. It is not always true that postpartum cows do not ovulate because of a lack of follicular growth. Other factors can modulate this system. Given the impetus for more consistent reproductive events in postpartum cattle, these factors need to be addressed in the future.
CHAPTER 5
EFFECT OF FEEDING CALCIUM SALTS OF LONG-CHAIN FATTY ACIDS TO EARLY POSTPARTUM DAIRY COWS ON PLASMA CONCENTRATIONS OF PROSTAGLANDIN F\(_{2\alpha}\) METABOLITE AND FOLLICULAR GROWTH

Introduction

Strategic feeding regimens (Kent and Arambel, 1988; Schneider et al., 1988), including use of Ca salts of long chain fatty acids (CaLCFA), have been employed as a method to alleviate a portion of the dietary energy deficit experienced by early postpartum dairy cows. The energetic requirements for milk production exceeds the cow's ability to consume feed during early lactation (Bauman and Currie, 1980; Coppock, 1985). Calcium salts of long-chain fatty acids provide the energy density of dietary fat while not depressing ruminal microbial function (Chalupa et al., 1984; Jenkins and Palquist, 1984; Chalupa et al., 1985; Grummer, 1988). This is possible because CaLCFA are insoluble at the neutral or slightly acidic pH's normally found within the rumen, but are highly soluble in the more acidic environment of the ruminant abomasum. The dissociation of Ca from LCFA in the abomasum leads to intestinal absorption of Ca and LCFA for utilization for milk components and energy substrates (Story, 1981; Jenkins and Palquist, 1984).

Dietary ingredients aimed at the alleviation of negative energy balance may yield important benefits to the
reproductive health of the cow. Postpartum anestrus, associated with extreme negative energy balance, (Butler and Smith, 1989; Staples et al., 1990), may be alleviated partially by feeding of energy-rich additives similar to CaLCFA. These may modulate the recrudescence of hypothalamic and pituitary function (and therefore ovarian activity) through effects on the overall energy status of the cow. Furthermore, greater fat ingestion may have direct effects on ovarian structures (i.e., follicles and/or corpora lutea [CL]). Recently, Williams (1989) found that feeding high-lipid diets containing whole cottonseed to postpartum beef cattle increased the length of luteal phases during first postpartum estrous cycles. Rhodes et al. (1978) found that feeding a casein-protected-fat diet to heifers delayed onset of puberty and therefore linked dietary fats to ovarian function.

In addition to their more obvious effects on energy balance, LCFA may increase postpartum release of uterine prostaglandin F$_{2\alpha}$ (PGF) which has been implicated as an important modulator in the initiation of estrous cycles after calving (Madej et al., 1984; Guilbault et al., 1987ab; Villeneuve et al., 1988). One practical method to increase PGF (tested in nonfarm species) is feeding linoleic acid, a dietary precursor molecule for PGF (Dupont et al., 1978; Mathias and Dupont, 1979; Adams et al., 1982). It may therefore be possible to augment postpartum PGF concentrations and enhance ovarian function by feeding various forms of LCFA.
to cows. Objectives of this study were to 1) measure concentrations of PGFM and triglyceride in plasma in lactating cows fed diets with or without CaLCFA, 2) relate postpartum energy balance (EB) and CaLCFA ingestion to luteinizing hormone secretion on d 10 postpartum, and 3) monitor follicular populations in these same cows from d 7 to d 60 postpartum.

Materials and Methods

Animals

Eighteen multiparous Holstein cows calving between August 15, and November 17, 1988 at the University of Florida Dairy Research Unit (Hague, Florida) were assigned to one of two diets. These are the same cows fed diets 3 and 4 in Chapter 4. The control (C) group was fed a totally mixed diet consisting of corn silage, corn grain, soybean meal, whole cottonseed, dried distillers grains, and chopped bermudagrass hay (Table 5-1). The diet contained 1.70 Mcal NEL/kg of DM. The experimental cows were fed the exact same diet as C cows, except that Ca salts of palm oils (CaLCFA; Megalac; Church and Dwight Co., Inc., Princeton, NJ) were included at 2.2% of diet DM. This lipid supplement contained approximately 56% palmitic acid, 4% stearic acid, 33% oleic acid and 6% linoleic acid and the corresponding NEL concentration of the diet was 1.75 Mcal/kg DM. Feeding, reproductive management, ultrasound, and energy balance calculations for these cows have been described in Chapter 4.
Table 5-1. Ingredient composition (% diet DM) of two experimental diets with and without calcium salts of long chain fatty acids (CaLCFA) fed to lactating dairy cows.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>CaLCFA</th>
</tr>
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<tbody>
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</tr>
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<tr>
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<td>Ground Corn</td>
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</tr>
<tr>
<td>Soybean meal</td>
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</tr>
<tr>
<td>Distillers grains</td>
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<td>14.2</td>
</tr>
<tr>
<td>Whole Cottonseed</td>
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<td>14.4</td>
</tr>
<tr>
<td>Minerals-Vitamins</td>
<td>3.6</td>
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</tr>
</tbody>
</table>

Blood Collection and Analyses

Ten milliliters of blood were collected by coccygeal venipuncture into evacuated, heparinized (143 USP units) tubes (Vacutainer; Becton Dickinson, Rutherford, NJ) from all cows on d 1 postpartum (calving = d 0) and every other day until d 21 postpartum. In addition, blood was collected prior to each ultrasound examination. After collection, blood was put immediately on ice and centrifuged at 3,000 x g at 4 C for 15 min. Plasma was stored at -20 C until assayed.

On the morning of d 10 postpartum, sixteen cows were fitted with a jugular cannula (16 gauge, 13.3 cm Angiocath, Becton Dickinson, Rutherford, NJ) and 10 ml of blood collected every 10 min for 8 h. Individual samples were mixed with 50
units of sodium heparin (Sigma Chemical Co., St Louis, MO) and plasma processed as described above.

Plasma 13,14 dihydro,15-keto prostaglandin F$_{2\alpha}$ (prostaglandin F$_{2\alpha}$ metabolite, PGFM) was measured on d 1, every other day until d 21, and on d 25, 30, 35, and 40. Competitive binding radioimmunoassay was performed on nonextracted samples as described previously (Guilbault et al., 1984a). Intra- and interassay coefficients of variation (CV) for PGFM were 7.9 and 10.9%, respectively. Plasma estradiol-17β was measured on d 7, 16, 20, 25, 30, 35, and 40 postpartum by radioimmunoassay (Guilbault et al., 1987b), intra- and interassay CVs were 2.4 and 8.2%, respectively.

Plasma LH was measured by radioimmunoassay in samples collected during the frequent bleeding on d 10 postpartum. Two-hundred µl of plasma were diluted in phosphate buffered saline (PBS) and incubated with 100 µl anti-LH (USDA-309-684p; 1:75,000 dilution in phosphate buffered saline (PBS) for 24 h at 23 C. On the second day, 100 µl of PBS containing approximately 20,000 cpm 125 I-LH (USDA-bLH-I-1) were added and the incubation continued for an additional 24 h at 23 C. Precipitation of the antibody complexes was performed on the third day with addition of 200 µl of sheep anti-rabbit IgG plasma (1:5 dilution) and one ml of 6% (w/v) polyethylene glycol-6000. After 15 min incubation, assay tubes were centrifuged for 30 min at 3000 x g. Supernatant was decanted and gamma emissions from the precipitate counted for 1 min.
Concentrations of LH in unknown samples was estimated from a standard curve (.02, .03, .06, .13, .25, .5, 1, 2, and 4 ng/tube) using bLH (USDA-bLH-55). Crossreactivities were: 1.0% for USDA-bFSH-B1; 1.1% for USDA-bGH-B1; .002% for USDA-bPRL-B1; and .5% for USDA-bTSH-I1. Increasing volume of cow plasma (50 µl, 100 µl, 200 µl, and 300 µl) resulted in a displacement curve which was parallel to the standard curve (tested by heterogeneity of regression, P > .10). Addition of different masses (y = 31.2, 125, 500, 1000, and 2000 pg) of bLH resulted in linear recovery of mass (Y;R² = .98) at assay volumes of 50 µl (Y = .20 + 1.08X), 100 µl (Y = .29 + 0.92X), 200 µl (Y = .33 + 0.93X), and 300 µl (Y = .44 + 0.92X). Concentrations of LH in unknown samples represented the average of two replicates. Intra- and interassay coefficients of variation were 10.9 and 13.4%, respectively.

Concentrations of triglyceride in plasma were measured by a colorimetric Hantzsch condensation reaction as described initially by Foster and Dunn (1973). Amount of triglycerides in plasma were estimated by comparing light absorbance at 405 nm with a triolein standard curve (Sigma Chemical Co., St. Louis, MO).

Statistical Analysis

Concentrations of PGFM (d 25, 30, 35, 40), estradiol-17B, and triglyceride in plasma were analyzed using the General Linear Models procedure (PROC GLM) of the Statistical Analysis System (SAS, 1987). The model included treatment, animal-
within-treatment, day postpartum, treatment-by-day interaction, and residual. Plasma PGFM values during the first 21 d postpartum were analyzed by tests for homogeneity of regression for third order fitted curves.

Plasma LH on d 10 was subjected to the PULSAR algorithm (Meriam and Wachter, 1982) for determination of number of LH peaks, magnitude of peaks, and smoothed basal concentration. These responses were analyzed using PROC GLM in models that included treatment and energy balance on d 10 postpartum.

Ovarian follicles were grouped into four follicular size (diameter) classes. Total number of follicles in class 1 (3 to 5 mm), class 2 (6 to 9 mm), class 3 (10 to 15 mm), and class 4 (>15 mm) were analyzed in models which included treatment, cow-within-treatment, class, day postpartum, predicted energy balance (covariate), as well as all interactions of main effects. Data were analyzed for measurements made before CIDR insertion (< d 25), during CIDR usage (d 25 to 40 postpartum), and during the post-CIDR estrous cycle on d 6, 12, and 18. Size of the largest ovarian follicle, second largest ovarian follicle, and difference between the largest and second largest ovarian follicles were analyzed using a model containing the main effects of treatment, animal-within-treatment, day postpartum, the treatment-by-day interaction, and residual.
Results

Net Energy Balance

Predicted EB increased from a minimum of -1.17 Mcal on d 2 postpartum to a maximum of 8.3 Mcal on d 55 for C cows. Cows fed CaLCFA had a minimum PEB of 2.05 Mcal on d 1 which increased to a maximum of 9.5 Mcal on d 65 (see Chapter 4). Cows fed CaLCFA had a higher (P<.001) PEB compared with C cows. Considering individual cows, minimum postpartum PEB ranged from -11.60 to +2.4 Mcal/d for the C group and -6.5 to +7.5 Mcal/d for the CaLCFA group.

Plasma Hormones and Metabolites

Concentration of plasma triglycerides across all sampling days was not different between cows fed CaLCFA (24.2 mg%) compared with C (20.6 mg%) cows. Considering all cows, plasma triglyceride increased with day postpartum (P<.001) from a minimum of 14.77 ± 2.4 mg% on d 16 to a maximum of 28.4 ± 3.1 mg% on d 60 (Table 5-2).

Mean concentrations of plasma PGFM during the first 21 d postpartum in C and CaLCFA fed cows are presented in Table 5-3. Mean plasma PGFM concentrations were highest on d 3 postpartum (2209.5 pg/ml) and declined thereafter (Day P<.001) to 78.4 pg/ml on d 21 and were not different between CaLCFA and C cows. Mean concentrations of PGFM from d 25 to 40 were similar between CaLCFA (298.3 pg/ml) and C (102.6 pg/ml) cows. A treatment-by-day interaction was detected (P<.07) because of an apparent rise in PGFM concentrations in CaLCFA cows on d
Table 5-2. Average daily plasma triglyceride (least square mean [mean] and SE; mg %) from d 7 to 16 (pre-CIDR), d 25 to 40 (CIDR period) and d 42 to 60 (post-CIDR) in lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
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<th>CaLCFA</th>
</tr>
</thead>
<tbody>
<tr>
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<td>SE</td>
</tr>
<tr>
<td><strong>Period</strong></td>
<td><strong>Day</strong></td>
<td></td>
</tr>
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</tr>
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<td>13.2</td>
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<td>44</td>
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<td>3.3</td>
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<tr>
<td>48</td>
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<td>54</td>
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<tr>
<td>60</td>
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<tr>
<td><strong>Total</strong></td>
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</table>

* Day, P < .001.

35. This was caused by high PGFM values in two of nine CaLCFA cows.

Characteristics of plasma LH release on d 10 for cows on this trial are presented in Table 5-4. As determined by the PULSAR algorithm, the average number of LH peaks within 8 h on d 10 was 8.94 ± .63 for all cows. Average smoothed mean
Table 5-3. Average daily plasma PGFM (least square mean [mean] and SE; pg/ml) from d 1 to 21 (pre-CIDR) and d 25 to 40 (CIDR period) in lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

<table>
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<tr>
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<th>Ca LCFA Mean</th>
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<td>2019.9</td>
<td>267.8</td>
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<td></td>
<td>3</td>
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<td>2557.0</td>
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<td>1427.7</td>
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<td>12.8</td>
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<sup>a</sup> Day, P<.001.
<sup>b</sup> Treatment-by-day, P < .07.
Table 5-4. Characteristics of the plasma LH profiles (estimated by PULSAR algorithm; mean = average LH concentration; smoothed mean = average LH baseline; number = number of LH peaks per cow; amplitude = average magnitude of LH peaks) during the 8 hour sampling period on d 10 postpartum for lactating dairy cows fed diets with or without calcium salts of long chain fatty acids (CaLCFA).

<table>
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<tr>
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<th>Amplitude (pg/ml)</th>
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<td>355</td>
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<td>489</td>
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<td>9893</td>
<td>510</td>
<td>435</td>
<td>7</td>
<td>403</td>
</tr>
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<td>807</td>
<td>660</td>
<td>8</td>
<td>485</td>
</tr>
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<td>1237</td>
<td>1005</td>
<td>957</td>
<td>4</td>
<td>369</td>
</tr>
<tr>
<td>Mean</td>
<td>576 ± 109</td>
<td>443 ± 104</td>
<td>8.4 ± 0.8</td>
<td>448 ± 78</td>
</tr>
</tbody>
</table>
concentration of LH was 353.6 ± 65 pg/ml. There were no differences between CaLCFA cows and C cows in terms of the number of LH peaks, basal LH secretion, or residual variance for LH secretion. Considering all cows, extreme variability existed in LH secretion on d 10 postpartum. Profiles of LH secretion for three cows on d 10 are presented in Figure 5-1. Luteinizing hormone secretion pattern ranged from very flat (panel A) to extremely pulsatile with high amplitude peaks (panel B). Relationships existed between the amplitude of LH pulses, EB, and diameter of the largest follicle as determined by ultrasound on d 10. Actual data as well as regression lines are given in Figure 5-2, Figure 5-3, and Figure 5-4. Across treatments, increasing PEB was associated with an increase in the average pulse amplitude on d 10 (ampl = 339.9 + 25.3*PEB, R^2 = .23; P<.06; Figure 5-2). In addition, diameter of the largest follicle increased with LH pulse amplitude on d 10 (diameter = 7.1 + .0085xampl, R^2 = .34; P<.02; Figure 5-3) and increasing EB on d 10 was associated with an increase in the diameter of the largest follicle (diameter = 9.24 + .56*PEB, R^2 = .48; P<.004; Figure 5-4). Furthermore, day of detection of the first CL occurred earlier as average PEB (before d 25) increased (CL day = 19.2 - 0.30*PEB, R^2 = .31, P<.06).

Plasma estradiol-17β before d 40 postpartum increased with day postpartum (day, P<.001) but was not different between control cows or cows fed CaLCFA (Table 5-5). For all
cows, plasma estradiol increased from a minimum of 7.4 pg/ml on d 7 to 11.1 pg/ml on d 35. Maximum estrogen secretion on d 35 was related apparently with follicular dynamics associated with the presence of the CIDR which have been described previously (Lucy et al., 1990).

Figure 5-1. Concentrations of plasma LH for two cows on d 10 postpartum during frequent blood sampling (once every 10 min) for 8 hours. Plasma LH release varied from low (Panel A) to high (Panel B).

Follicle Development

Daily average number of class 1 to 4 follicles per cow from d 7 to 25 are given in Figure 5-5. Prior to d 25, there tended to be a diet-by-follicular class interaction (P=.13). Control cows had more class 1 follicles and fewer class 2 and 4 follicles. Including the PEB-by-class interaction (P<.001) in the statistical model removed the diet-by-class interaction (P = .68), suggesting that dietary treatment effects were explained by the effect of PEB on number of follicles among classes. The number of cows ovulating before insertion of the CIDR was equivalent (7 out of 9) for each treatment group. The
Figure 5-2. Estimated regression line and actual data for individual cattle (+; control and CaLCFA) for the relationship between LH amplitude and predicted energy balance on d 10 postpartum.

...relationship among average number of follicles with size classes was reversed partially during the CIDR period (d 25 to 40; Figure 5-6) with CaLCFA cows having more class 1 and class 4 follicles (diet-by-class P=.03). There was no effect of PEB on the number or distribution of follicles among classes during the CIDR period (d 25 to 40). After removal of the CIDR, the number of cows having ovulation and CL formation, cystic follicles, and no ovulation was 7, 2, and 0 for the CaLCFA group and 5, 3, and 1 for the C group. For those cows
Figure 5-3. Estimated regression line and actual data for individual cattle (+; control and CaLCFA) for the relationship between LH amplitude and diameter of the largest follicle on d 10 postpartum.

having ovulation and CL formation, frequency of follicles in individual size classes is presented in Figure 5-7. Number of follicles in all size classes was greater with increasing PEB during this period (P<.05) and, in addition, there existed a diet-by-class interaction (P<.03) with CaLCFA cows having more class 1 and class 4 follicles and fewer class 2 follicles. Average number of class 4 follicles was 238% higher (.71 vs .21) in CaLCFA cows during the estrous cycle after CIDR removal.
Figure 5-4. Estimated regression line and actual data for individual cattle (+; control and CaLCFA) for the relationship between predicted energy balance and the diameter of the largest follicle on day 10 postpartum.

The average diameter of the largest follicle, second largest follicle, and size difference between the largest and second largest follicles before, during, and after the CIDR is presented in Table 5-6. During the period before the CIDR, the average size of the largest and second largest follicle increased with day postpartum (P<.01) and was not different between cows fed C or CaLCFA diets. Size difference was not influenced by day postpartum. For all days prior to CIDR insertion, the largest, second largest, and size difference
Table 5-5. Average daily plasma estradiol-17 B (least square mean [mean] and SE; pg/ml) on certain days postpartum in lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

<table>
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<th>Dietary Treatment</th>
<th>Control</th>
<th>CaLCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Pre-CIDR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.9</td>
<td>1.1</td>
</tr>
<tr>
<td>16</td>
<td>7.4</td>
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</tr>
<tr>
<td>25</td>
<td>8.2</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>CIDR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8.8</td>
<td>1.0</td>
</tr>
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<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

was 11.0 ± .8, 6.5 ± .6, and 4.6 ± .8 mm, respectively for control cows and 12.5 ± .8, 7.3 ± .6, and 5.3 ± .8 mm, respectively for CaLCFA fed cows.

During the CIDR period, there was no effect of day or dietary treatment on the diameter of the largest follicle, or second largest follicle, or the size difference. These measures averaged 16.9 ± 1.2, 8.4 ± 1.6, and 8.6 ± 1.6 mm, respectively for C cows and 19.1 ± 1.2, 9.9 ± 1.6, and 9.3 ± 1.6 mm, respectively for CaLCFA cows. In contrast, during the
Figure 5-5. The average number of follicles within different follicle size classes before d 25 postpartum for lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

First estrous cycle after CIDR removal, the average size of the largest (18.2 ± 1.5 vs 12.4 ± 1.9 mm) and second largest (10.9 ± 1.1 vs 7.4 ± 1.4 mm) follicles were greater (P < .04, P < .07, respectively) in cows fed CaLCFA compared with C cows. Size difference was not influenced by treatment during this period (5.0 ± 1.6 vs 7.3 ± 1.3 mm; control vs CaLCFA).
Figure 5-6. The average number of follicles within different follicle size classes during the CIDR period (d 25 to 40 postpartum) for lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

**Discussion**

Feeding CaLCFA successfully increased postpartum EB. Predicted EB was approximately 3 Mcal per d higher within the CaLCFA group at the beginning of the experiment and this difference was reduced to .5 Mcal/d by d 45 postpartum. Plasma concentrations of triglycerides for the CaLCFA cows were not higher than for C cows. This has been observed by others (Schneider et al., 1988) and has been attributed to the rapid removal of LCFA by the mammary gland for milk fat
Figure 5-7. The average number of follicles within different follicle size classes during an estrous cycle (d 6, 12, and 18) after removal of the CIDR for lactating dairy cows fed diets with and without calcium salts of long chain fatty acids (CaLCFA).

synthesis. Although concentrations of circulating triglycerides were not higher in CaLCFA cows, metabolic and endocrine events in these cows may have been altered by higher fat ingestion. Indeed, in a recent study, release of insulin after a glucose challenge was greater in cows fed whole cottonseed than in control cows (Umphrey, 1988).

Profiles of plasma PGFM were not different between the two groups. This suggests that LCFA in the proportions given
Table 5-6. Average diameter (mm) of the largest follicle (Largest), second largest follicle (Second), difference between the largest and second largest follicle (Difference) determined by ultrasound in lactating dairy cows fed diets without (control; C) or with calcium salts of long chain fatty acids (CaLCFA).

<table>
<thead>
<tr>
<th>Period Day</th>
<th>Largest C</th>
<th>CaLCFA</th>
<th>Second C</th>
<th>CaLCFA</th>
<th>Difference C</th>
<th>CaLCFA</th>
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<tr>
<td>7</td>
<td>6.1</td>
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<td>5.1</td>
</tr>
</tbody>
</table>


did not influence PGF production or metabolism. This could be a result of the relatively low concentration of linoleic acid (6%; arachidonic acid precursor) in the CaLCFA compound. Possibly, PGF secretion in early postpartum cattle is at a maximum for each individual cow, and not subject to changes in dietary precursors at this time. However, an interaction
(diet-by-day) for plasma concentrations of PGFM in the CIDR period (d 25 to 40) was detected (Table 5-3). This could indicate, that when basal intake of LCFA is increased, prostaglandin secretion will increase at certain times in certain cows, perhaps through enhanced production. This increased concentration in PGFM did correspond to a period of maximal estradiol secretion in these cows (see Table 5-5) and estradiol is a potent stimulator of PGF release (Thatcher et al., 1986). Lower overall requirements for turnover of body LCFA stores found in CalCFA fed cows (Schneider et al., 1988) potentially could conserve precursors for prostaglandin synthesis leading to higher PGF between 25 to 40 d postpartum.

Plasma concentrations of LH on d 10 were not influenced by diet, but were influenced apparently by overall EB among cows. This finding supports results of others (Stevenson and Britt, 1979) who related plasma concentrations of LH in early postpartum cows to differences in milk production (a less accurate indicator of energy status). We found that although feeding CalCFA will alter EB, its effect on LH secretion was not detectable by d 10 postpartum. However, since basal LH secretion seems to be dependent on EB, feeding regimens aimed at increasing early postpartum EB should be beneficial in terms of restoring ovarian function. Indeed, when data in Table 5-4 are grossly examined, several control cows represented the lowest mean LH concentrations observed while the two highest concentrations of LH on d 10 were for cows fed
CaLCFA. Examination of LH profiles for a greater number of cattle and later postpartum (e.g., d 15), may clarify the relationship between LH, EB, and CaLCFA.

Feeding CaLCFA influenced the number of follicles within each size class at all times during the experiment. During the early postpartum period (< d 25), changes in follicle numbers were consistent with the theory that a more positive EB causes movement of follicles from smaller to larger size classes (Chapter 4) Indeed, prior to d 25, cows fed CaLCFA had more class 2 and fewer class 1 follicles. This movement is probably responsible for earlier ovulation in cows having more positive energy status. After d 25 (CIDR and post-CIDR), effects of CaLCFA feeding were consistent in that cows fed CaLCFA had more class 1 and 4 follicles. This suggests that once estrous cycles have been initiated, effects of CaLCFA are quite different in that it influences the number of follicles in all classes. Of interest was the higher proportion of class 4 follicles and the greater size of largest and second largest follicles found in cows fed CaLCFA during the post-CIDR period. This was detected even when comparing cows fed CaLCFA to a much larger group of cows (n=50; Chapter 4). Increased incidence of follicles greater than 15 mm (class 4) may be a result of slower follicular turnover or enhanced follicular growth. Cholesterol-LCFA esters are a critical component of follicular steroidogenesis associated with the maturation of dominant follicles (Gwynne and Strauss, 1982).
Therefore, LCFA may influence these events. Alternatively, insulin, growth hormone, or other metabolic hormones, possibly altered by CaLCFA feeding, may be involved. Recent evidence relating circulating and intrafollicular growth factors and insulin to changes in ovarian dynamics suggests the importance of these peripheral signals (Poretsky and Kalin, 1987; Hammond et al., 1988). One additional factor not thoroughly investigated is the influence that additional Ca load associated with CaLCFA feeding may have on the animal. Ingestion of the amount of CaLCFA fed provided nearly 50% of the Ca requirements of the cow. This rate of Ca ingestion may influence ovarian function in some unknown manner.

Finally, it appears that class 4 follicles in these cows were physiologically active based on the fact that class 2 follicle frequency was reduced within the CaLCFA group (see d 48 to 60; d 6, 12, and 18 of the estrous cycle; Figure 5-7). The ability of larger follicles to influence the growth of smaller follicles is a phenomenon known as follicular dominance and is characteristic of physiologically functional follicles (Ireland and Roche, 1987). This phenomenon seems to be operational in large dominant follicles of cows fed CaLCFA. This causes a reduction in the number of medium sized follicles.

In conclusion, feeding CaLCFA did not alter concentrations of PGFM in plasma of early postpartum cows. The LH pulse amplitude on d 10 was not higher in cows fed
CaLCFA but was related positively with EB and size of the largest follicle. Feeding of CaLCFA increased EB, altered number of follicles within size classes, and increased the average size of largest and second largest ovarian follicles later postpartum. These large follicles apparently were active based on their ability to influence the average number of class 2 follicles during the estrous cycle after the CIDR. At this time it is not clear how the CaLCFA-associated changes in follicular populations affect fertility. More research is warranted to clarify the relationship between dietary LCFA, the ovary, and the return to ovarian function in early postpartum dairy cattle.
CHAPTER 6
FOLLICULAR DYNAMICS, PLASMA METABOLITES, GROWTH FACTORS, HORMONES, AND IGF BINDING PROTEINS IN LACTATING COWS WITH POSITIVE OR NEGATIVE ENERGY BALANCE PRIOR TO ESTRUS

Introduction

Nutritional reserves of the postpartum dairy cow, which are stored during the later period of the previous lactation and dry period, are mobilized for the energetic demands associated with the synthesis of milk in the early postpartum period (Bauman and Currie, 1980). It is during the postpartum period that the ovary recovers from the influence of the previous pregnancy and undergoes active changes leading to first ovulation (Nett, 1987; 1990). Ovarian recrudescence depends on initiation of gonadotropin secretion which stimulates follicular development (Stevenson and Britt, 1979; Butler and Smith, 1989). One hypothesis is that negative energy balance, caused by milk energy production in excess of dietary energy intake, can depress hypothalamic function resulting in low gonadotropin secretion and an absence of follicular development (Butler and Smith, 1989; Richards et al., 1989a). As an alternative, the reinitiation of follicular growth on the ovary may be directly affected by numerous other factors associated with postpartum negative energy balance and nutrient mobilization. These would include other hormones such as insulin [Poretsky and Kalin, 1987],
glucagon, or insulin-like growth factors [IGF-I and IGF-II; Hammond et al., 1988] or energy metabolites such as nonesterified fatty acids (NEFA) or glucose. Interestingly, during periods of dietary energy restriction and negative energy balance, concentrations of IGF-I in the blood decline (see review by Gluckman et al., 1987). Since growth factors in follicular fluid are critically important to the growth and differentiation of the follicle (Adashi et al., 1985a; Hammond et al., 1988), changes in peripheral concentrations of IGF-I in the blood associated with negative energy balance could be one factor altering follicular development in lactating cattle. Although direct effects of low concentrations of IGF-I on the ovary are not known, IGF-I is higher in the blood and follicular fluid of cattle selected for enhanced follicular growth and development (i.e., multiple ovulation; Echternkamp et al., 1990). Therefore, the ovary seems to be responsive to changes in IGF-I in the blood.

One factor which complicates estimation of the biological actions of IGFs are a series of low molecular weight binding proteins (Hossner et al., 1988). The exact function of these binding proteins has not been elucidated. However, certain variants of these proteins, found in animals undergoing metabolic depletion of body nutrients (Phillips et al., 1983; Kuffer and Herington, 1986), are inhibitory to the action of IGFs (Salmon, 1975; Avasthy et al., 1986). Objectives of this study were to examine changes in hormones, growth factors, and
growth factor binding proteins in cows undergoing rapid changes in nutrient partitioning and to relate these to changes in spontaneous preovulatory follicle development observed on the ovary. This information may elucidate additional factors controlling growth and development of follicles in postpartum cows in negative energy balance.

Materials and Methods

Animals

Ten lactating Holstein cows (> 150 d of lactation) at the University of Florida, Dairy Research Unit (Hague, Florida) were used. Cows were milked and fed twice daily. Individual feed consumption was monitored using self-activated feeding stations (American Calan, Inc., Northwood, NH). Daily energy balances (difference between dietary energy consumed and the amount of energy utilized for body maintenance and milk production) were calculated from milk production and composition, individual feed energy consumption, feed energy content (determined by Northeast DHIA Forage Testing Laboratory, Ithaca, NY), and body weight using formulae described previously (Chapter 4).

Experimental Design

Cows were injected with 8 ug Buserelin (Receptal, Hoescht-Roussel, Agrivet, Somerville, NJ) and given a controlled internal drug release device (CIDR, 1.9 g progesterone) to initiate and synchronize growth of follicles leading to eventual estrus (Figure 6-1). Seven days later, all
Figure 6-1. Experimental design for the synchronization of follicular growth and the implementation of dietary treatments (OVX = ovariectomy, LH=frequent blood sampling).

cows were injected with 25 mg of PGF$_{2\alpha}$ (Lutalyse, UpJohn Co., Kalamazoo, MI). The CIDR was removed 48 h later (day 9) to allow for final follicular growth leading to ovulation. Ovaries of all cows were removed by flank incision under local anesthesia at 36 h after CIDR removal (prior to the expression of estrus).

On d 6 of the experiment, five cows were maintained on their previous ration (high energy diet) while five cows were switched to a low energy ration consisting of corn silage and minerals (low energy diet; 6.4 kg of dry matter offered daily; Table 6-1). This amount of energy intake was designed to mimic the energy deficit experienced by cows in early lactation. Cows were fed these diets for 4 d (until the time of ovariectomy). Ten milliliters of blood were collected by
Table 6-1. Ingredient composition of experimental diets (percentage of dry matter).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
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<td>96.6</td>
</tr>
<tr>
<td>Corn</td>
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<td>.0</td>
</tr>
<tr>
<td>Whole cottonseed</td>
<td>12.2</td>
<td>.0</td>
</tr>
<tr>
<td>Distillers grains</td>
<td>15.4</td>
<td>.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.3</td>
<td>.0</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>12.2</td>
<td>.0</td>
</tr>
<tr>
<td>Minerals/vitamins</td>
<td>3.1</td>
<td>.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Trace mineral salt</td>
<td>.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>

coccygeal venipuncture into heparinized tubes (Vacutainer, Becton Dickenson, East Rutherford, NJ; 143 units heparin per 10 ml sample) and plasma harvested after centrifugation (3000 x g for 30 min) on each day of the experiment. In addition, blood was collected after the dietary treatments were initiated and allowed to clot at 4 °C overnight and centrifuged (as above) for the collection of serum. On the first day of the dietary change (day 6) as well as the day of ovariectomy (day 10), cows were fitted with jugular cannula and blood collected once every 10 minutes for 8 hours for analysis of concentrations of luteinizing hormone (LH) in plasma.

Ovaries were examined daily by ultrasonography from the time of Buserelin injection and CIDR insertion until ovariectomy. Ovaries were examined using a Equisonics LS 300A
linear array scanner equipped with a 7.5 Mhz transducer (Tokyo Kieki, Tokyo, Japan). Size and number of ovarian follicles greater than 3 mm were recorded, as well as the number and diameter of corpora lutea. Follicles were grouped into four follicular size classes for statistical analysis (class 1: 3 to 5 mm; class 2: 6 to 9 mm; class 3: 10 to 15 mm; class 4: > 15 mm). Size of the largest and second largest follicle also was recorded.

**Analysis of Blood Hormones and Metabolites**

**Plasma metabolites**

Plasma glucose was measured during the dietary period using the Sigma Chemical Co. (St. Louis, MO) kit No. 510 (glucose oxidase/peroxidase colorometric method). Twenty-five microliters was analyzed and concentrations of glucose estimated from a glucose standard solution. Plasma nonesterified fatty acids were measured in duplicate determinations of 25 ul of serum using the NEFA C kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma triglycerides were measured by the method described by Foster and Dunn (1973). Concentrations of triglycerides in the duplicate 50 ul samples were estimated using a triolein standard.

**Plasma hormones**

Concentrations of insulin and growth hormone (GH) in plasma were determined in nonextracted samples by competitive binding radioimmunoassay described by Collier et al. (1982).
All samples were measured in one assay and the intraassay coefficient of variation was 12.4 and 9.7% for insulin and GH assays, respectively. Plasma IGF-I was extracted in acid ethanol and assayed by radioimmunoassay as described by Lee et al. (in press). The intra- and interassay coefficients of variation were 4.2 and 7.5%, respectively. Plasma progesterone was measured in a single assay using procedures described previously (Knickerbocker et al., 1986b). Intraassay coefficient of variation was 6%. Plasma LH was measured using the technique described in Chapter 5. Intra- and interassay coefficients of variation were 9.6 and 8.3%, respectively. The concentration of LH at each time point was subjected to a peak identification algorithm (Pulsar program, Meriam and Wachter, 1982) for the determination of mean concentration, smooth mean concentration, number of episodic events (hormone peaks), peak amplitude, and peak length.

**Determination of Growth Factor Activity in Serum**

The growth factor activities in serum samples were determined using two cell lines (AKR-2B and 3T3 cells) which are stimulated mitogenically by different types of growth factors (AKR-2B cells are responsive to EGF while 3T3 cells are responsive to IGFs and insulin; Y. Ko and F.A. Simmen, unpublished observations). The same procedure initially described by Cera et al. (1987) was used (see also Simmen et al., 1988; Simmen et al., 1989). Cells were grown in either a high glucose Dulbecco's modified Eagle's medium (Sigma No.
D7777, Sigma Chemical Co., St. Louis, MO; AKR-2B cells) or a similar medium low in glucose (Sigma No. D5523; 3T3 cells) supplemented with 10% young calf serum (Gibco No. 230-6170) and 1% antibiotic/antimycotic solution (Sigma No. A9909). Stocks of cells were maintained in 100x20 mm petri dishes (Corning No. 25020-10, Corning Glass Works, Corning, NY) and passage occurred every 3 d using 1x trypsin (Sigma No. T5650) dissolved in calcium and magnesium-free Dulbecco's phosphate buffered saline (Sigma No. D5773). To assay growth factor activity of samples, cells were seeded into 24 well plates (Falcon No. 3047, Becton Dickinson, Lincoln Park, NJ) at a density of 35,000 cells per well (in 1 ml of medium containing 10% YCS). After cells reached confluency (approximately 3 d), new medium was added containing 2% YCS. Incubation continued for an additional 48 h at which time cells were treated with test solutions (i.e., serum samples) at a concentration of 5% (v/v). Samples were analyzed in triplicate. All related samples from the experiment were analyzed in a single assay. After 20 h of treatment, 1 uCi of [3H-methyl]-thymidine (Amersham Co., TRA.120, Arlington Heights, IL) was added to all wells. After 4 h, cells were washed using PBS and harvested using sequential 1 ml treatments with methanol (twice for 5 min), water (washed 4 times), and 5% trimethyl carboxylic acid (TCA; twice for 10 minutes). After the final TCA treatment, cells were washed with 300 ul of .3 N NaOH and the dissolved suspension pipetted into a scintillation vial.
and mixed with 4 ml scintillation cocktail (Scintiverse II, Fisher Chemical Co., Pittsburg, PA). Incorporated thymidine was estimated from B emissions (disintegrations per minute) recorded by liquid scintillation counting.

Ligand Blotting for Identification of IGF Binding Proteins

The qualitative array of IGF binding proteins (IGF-BP) was measured using the technique of ligand blotting first described by Hossenlopp et al. (1986). Serum or follicle fluid samples (3 ul) were diluted in 25 ul dissociation buffer (.173 M sodium dodecyl sulfate, .292 M sucrose, in .0625 M Tris-HCl, pH 6.8) and electrophoresed using a 12.5% acrylamide gel according to Roberts et al. (1984). The buffer system used was first described by Laemmli (1970). Electrophoresed samples were then transferred to nitrocellulose using an electroblotting technique (Western transfer) described previously (Roberts et al., 1984). The nitrocellulose filter was blocked using a 1% solution of nonfat dried milk for 1 h, washed and then incubated with approximately 800,000 dpm of $^{125}$I-IGF-I for 24 h. Following this incubation, the filters were washed repeatedly, dried, and exposed to radiographic film (Kodak film [X-OMAT], in an X-OMATIC cassette with enhancing screens) for approximately 7 to 9 d. Locations of bound IGF-I on the nitrocellulose (as elucidated by autoradiography) were assumed to represent IGF-BP. Other filters incubated with excess unlabelled IGF-I (0.1 ug) acted as negative controls. Radiographic films, with exposed
regions corresponding to IGF-BP, were subjected to densitometry analysis for quantification of the intensity of individual bands.

**Statistical Analysis**

Data were analyzed using the General Linear Models Procedure of SAS (1987). Hormone, metabolite, and cell assay data were analyzed as a split plot with repeated measures over time. The model included the effects of treatment, cow-within-treatment, day, the treatment-by-day interaction, and residual. The main effect of treatment was tested using cow-within-treatment as the error term while other effects were tested using the residual error term. The number of follicles within each size class after treatment with Buserelin was tested using a model which included the effects of treatment, cow-within-treatment, day, follicular size class, and interactions of these main effects. Data were analyzed for the entire experimental period (days 0 to 10) as well as only during the dietary treatment period (last 5 days of the experiment). Tests for homogeneity of regression (Wilcox et al., 1990) were employed where appropriate.

**Results**

**Follicular Populations and Plasma Progesterone**

Number of class 3 (10 to 15 mm) and class 4 (> 15 mm) follicles initially decreased after injection of Buserelin (day-by-class interaction, P<.05) to a minimum of .7 ± .3 and 0 ± .1 follicles per cow 2 d after injection (Figure 6-2).
Figure 6-2. Average number of class 2 (6 to 9 mm), class 3 (10 to 15 mm), and class 4 (>15 mm) follicles per cow after the initiation of follicle synchronization on day 0 (GnRH agonist injection [8 ug Buserelin]).

This decline in large follicles was followed by an increase in the number of class 2 follicles (6 to 9 mm) which became maximum on d 2 (4.7 ± .7 follicles per cow) and then declined to a minimum on d 7 (1.1 ± .7). Numbers of class 3 follicles per cow subsequently increased (until d 4 to 6) and then declined. Numbers of large follicles per cow (class 4) increased at the end of the synchronization period to a maximum of .6 ± .1 follicles per cow on d 10. Two cows ovulated large follicles which were present at the time of
Buserelin injection (Table 6-2). All of these induced corpora lutea were regressed when PGF was injected. Most large follicles present at the time of Buserelin injection were still detectable by ultrasound after 3 d.

The average number of follicles per cow increased during the dietary period in cows fed the low energy diet from 1.3 ± .2 on d 0 to 1.9 ± .2 on d 4. At the same time, average number of follicles per cow decreased in cows fed the high energy diet (treatment-by-day, P=.10) from 1.9 ± .2 on d 0 to 1.0 ± .2 on d 4. Treatment-by-class or treatment-by-day-by-class interactions were not detected (P>.15) in the analysis of follicular populations during the dietary period.

Concentrations of progesterone in plasma increased (P<.001) following injection of Buserelin and CIDR insertion to a maximum of 7.0 ng/ml after two days (Table 6-3). Following injection of PGF, mean plasma progesterone declined from 5.3 ng/ml (day 7) to 2.3 ng/ml (day 8). A further decline in progesterone occurred after removal of the CIDR on d 9 (1.2 ng/ml) to a concentration of .7 ng/ml on d 10.

**Energy Balance, Plasma Metabolites and Metabolic Hormones**

As expected, calculated energy balance was negative in cows fed the low energy diet and positive in cows fed the high energy diet (P<.001). Energy balance averaged +3.7 ± 1.4 Mcal/d for cows fed the high ration and -7.3 ± 1.4 Mcal/d for cows fed the low ration. Values for concentrations of nonesterified fatty acids, insulin, triglyceride, and glucose
Table 6-2. Changes in diameter and clarity of follicular fluid occurring in large follicles in lactating cows prior to (day 0) and after the injection of Buserelin (days 1 to 3). Number indicates diameter and letter indicates clarity of follicular fluid (F = clear, C = cloudy). ND = not detectable, CL = developed into a corpus luteum. LT = developed lutienized tissue.

<table>
<thead>
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<th>Cow Diet</th>
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<td>6 F</td>
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</tr>
<tr>
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<td>15 C</td>
<td>11 C</td>
<td>12 C</td>
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<td>10 F</td>
<td>10 C</td>
<td>8 C</td>
<td>6 C</td>
</tr>
<tr>
<td>1520 H</td>
<td>14 F</td>
<td>14 C</td>
<td>CL</td>
<td>CL</td>
</tr>
<tr>
<td>8920 H</td>
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<tr>
<td>8986 L</td>
<td>11 F</td>
<td>12 F</td>
<td>12 F</td>
<td>12 C</td>
</tr>
</tbody>
</table>

*Days after injection of 8 ug of Buserelin (GnRH agonist).*

*High energy diet (H) or low energy diet (L).*

in plasma are presented in Figure 6-3 (panels A, B, C, and D, respectively). During the dietary period, plasma NEFA tended to be higher (treatment, P=.10) for cows fed the low energy diet (548 ± 134 uEq/l) than for cows fed the high energy diet (325 ± 134 uEq/l) (Figure 6-3A; treatment-by-day, P<.02). Plasma insulin was similar between diets and averaged 1.7 ± .12 ng/ml for all cows (Figure 6-3B). Plasma insulin increased (day, P<.06) during the preovulatory (dietary) period and averaged 1.5 ± .2 ng/ml on d 2 and 2.3 ± .2 ng/ml on d 4 of the diets. Plasma triglycerides (Figure 6-3C)
Table 6-3. Plasma progesterone concentrations (ng/ml) in cows during the synchronization and dietary period.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Expt. day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diet day&lt;sup&gt;b&lt;/sup&gt;</th>
<th>High</th>
<th>Low</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDR IN&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 -5</td>
<td>5.9</td>
<td>4.3</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>6.4</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3 -3</td>
<td>7.4</td>
<td>5.1</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 -2</td>
<td>6.3</td>
<td>5.0</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 -1</td>
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<td>4.4</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 0</td>
<td>5.8</td>
<td>4.6</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>PGF&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7 1</td>
<td>6.0</td>
<td>4.6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 2</td>
<td>2.2</td>
<td>2.3</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>CIDR OUT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9 3</td>
<td>1.1</td>
<td>1.3</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 4</td>
<td>.4</td>
<td>1.0</td>
<td>.7</td>
<td></td>
</tr>
<tr>
<td>POOLED SEM</td>
<td></td>
<td>.9</td>
<td>.9</td>
<td>.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Day in relation to initiation of follicle synchronization.
<sup>b</sup>Day in relation to start of feeding of experimental diets.
<sup>c</sup>CIDR insertion and injection of 8 ug of Buserelin (GnRH agonist).
<sup>d</sup>Injection of 25 mg PGF (Lutalyse).
<sup>e</sup>Removal of CIDR device.

Tended to decline (treatment-by-day, P = .12) in those cows fed the low energy diets from a maximum of 21.2 ± 2.3 mg% (day 0 of dietary feeding) to a minimum of 9.2 ± 2.3 mg% (3 d after the initiation of dietary treatment). Plasma triglycerides remained unchanged in cows on the high energy diet (20.9 ± 1.0 mg%). Plasma glucose was not affected by dietary treatment and averaged 72.2 ± 2.0 and 72.1 ± 2.0 mg% for cows on high and low energy diets, respectively.

Concentrations of LH in plasma prior to the dietary treatments (day 0) are given in Figure 6-4 (panel A through E,
Figure 6-3. Average concentration of NEFA (μEq/l; Panel A), insulin (ng/ml; Panel B), triglyceride (mg%, Panel C), and glucose (mg%, Panel D) for cows (n=5 per diet) prior to and after the feeding of high and low energy diets.

low energy-fed cows) and Figure 6-5 (panel F through J, high energy-fed cows). Nine out of ten cows displayed pulsatile secretion of LH prior to the dietary treatments, however, one cow (8920; panel 4 J) had no LH pulses at any time, low basal LH, and no CL at the start of the experiment (determined by ultrasound). Therefore, cow 8920 was removed from subsequent LH and follicular analyses because of probable anestrus. Concentrations of LH in plasma after dietary treatments are
Figure 6-4. Concentration of LH (ng/ml) in cows (low energy diet, panels A to E) on the first day of dietary feeding (Day 0) during an 8 h LH blood sampling period (one sample every 10 minutes).

given in Figure 6-6 (panels A through E, low energy-fed cows) and Figure 6-7 (panels F through J, high energy-fed cows). Mean concentrations of LH (.70 vs 1.35 ng/ml; P<.07), smoothed mean concentrations of LH (.49 vs .86 ng/ml; P<.07) and number of LH peaks per h (.39 vs .75; P<.05) increased from d 0 (Table 6-4) to d 4 (Table 6-5). Cows fed the high-energy diet were similar to cows on the low energy diet in terms of mean concentrations of LH, smoothed mean concentrations of LH,
Figure 6-5. Concentration of LH (ng/ml) in cows (high energy diet, panels F to J) on the first day of dietary feeding (Day 0) during an 8 h LH blood sampling period (one sample every 10 minutes).

number of LH peaks, peak amplitude, and peak length. However, two low energy-fed cows (presented in Figure 6-6 D and E) did not have regular pulsatile gonadotropin secretion on d 4. Cow 8981 had low frequency pulses prior to feeding the low energy diet (Figure 6-4 D) but after 4 d of the diet had pulsatile secretion which ended 5 hours into the window bleed (Figure 6-6 D). In addition, low energy-fed cow 8986 had normal LH pulses (Figure 6-4 E) on d 0, but only a single LH pulse one
Figure 6-6. Concentration of LH (ng/ml) in cows (low energy diet, panels A to E) on the last day of dietary feeding (Day 4) during and 8 h LH blood sampling period (one sample every 10 minutes).

d prior to anticipated estrus (4 d later, Figure 6-6 E).

Plasma IGF-I decreased (P<.05) during the dietary period in cows fed the low energy diet (Table 6-6). Plasma IGF-I averaged 65.3 ng/ml for high energy-fed cows on d 0 and increased to 71.4 ng/ml on d 4. In contrast, IGF-I declined from 64.7 ng/ml (day 0) to 42.0 ng/ml (day 4) for cows fed the low energy diet. Two cows (8920 [high energy diet] and 8986 [low energy diet]) had IGF-I concentrations in plasma markedly
Figure 6-7. Concentration of LH (ng/ml) in cows (high energy diet, panels F to J) on the last day of dietary feeding (Day 4) during and 8 h LH blood sampling period (one sample every 10 minutes).

lower than other cows in this study.

Plasma GH was not different in cows on high or low energy diets (Table 6-7). Plasma GH averaged 3.92 ng/ml for cows on the low energy diet and 3.48 ng/ml for cows on the high energy diet. Two cows, 8920 [high energy diet] and 8986 [low energy diet] had concentration of GH in plasma markedly higher than for other cows on this study (mean = 11.42 and 8.46 ng/ml, respectively). There was a significant negative correlation
Table 6-4. Characteristics (determined by Pulsar Algorithm) of the concentrations of luteinizing hormone (LH) in plasma for an 8 h period on day 0 of dietary feeding for cows on the high (H) and low (L) energy diets (D). Mean ± SE = Least square mean ± standard error.

### Characteristic of LH Profiles

<table>
<thead>
<tr>
<th>D</th>
<th>Cow</th>
<th>Data Mean (ng/ml)</th>
<th>Smoothed Mean (ng/ml)</th>
<th>No. of Peaks (per h)</th>
<th>Amplitude (ng/ml)</th>
<th>Peak Length (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>956</td>
<td>.95</td>
<td>.82</td>
<td>.38</td>
<td>.89</td>
<td>36.7</td>
</tr>
<tr>
<td>L</td>
<td>1443</td>
<td>.63</td>
<td>.20</td>
<td>.38</td>
<td>3.27</td>
<td>60.0</td>
</tr>
<tr>
<td>L</td>
<td>1482</td>
<td>.36</td>
<td>.27</td>
<td>.25</td>
<td>.86</td>
<td>55.0</td>
</tr>
<tr>
<td>L</td>
<td>8981</td>
<td>.54</td>
<td>.49</td>
<td>.25</td>
<td>.45</td>
<td>50.0</td>
</tr>
<tr>
<td>L</td>
<td>8986</td>
<td>.77</td>
<td>.41</td>
<td>.88</td>
<td>1.26</td>
<td>38.0</td>
</tr>
</tbody>
</table>

Mean ± SE .7 ± .3 .4 ± .2 .4 ± .1 1.3 ± .4 48 ±11

| H | 1376| .30               | .21                   | .63                  | .35               | 38.0                 |
| H | 1446| 1.23              | .95                   | .25                  | 2.72              | 60.0                 |
| H | 1498| .61               | .50                   | .13                  | 1.54              | 60.0                 |
| H | 1520| .73               | .50                   | .63                  | 1.01              | 50.0                 |

Mean ± SE .7 ± .3 .5 ± .2 .4 ± .1 1.4 ± .4 52 ±13

between IGF-I and GH in plasma (P<.001; R²=.42; IGF-I = 76.3 - 4.39xGH). Daily changes in the insulin to GH ratio were not similar between groups (P<.05) with cows on the low energy diet having a declining ratio from 1.4 ± .4 to 1.1 ± .4 (days 0 to 4, respectively) and cows on the high energy diet having an increasing ratio (1.0 ± .4 to 1.6 ± .4; d 0 to 4, respectively).
Table 6-5. Characteristics (determined by Pulsar Algorithm) of the concentration of luteinizing hormone (LH) in plasma for an 8 h period after 4 days of dietary feeding for cows on the high (H) and low (L) energy diets (D).

<table>
<thead>
<tr>
<th>Characteristic of LH Profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>L</td>
</tr>
</tbody>
</table>
| Mean ± SE | 1.3 ± .3 | .8 ± .2 | .7 ± .1 | 2.0 ± .5 | 59 ± 11

| H | 1376 | .81 | .42 | .63 | 1.65 | 62.0 |
| H | 1466 | 1.24 | 1.04 | .75 | .87 | 36.7 |
| H | 1498 | 2.01 | 1.19 | .88 | 2.09 | 48.6 |
| H | 1520 | 1.48 | 1.05 | 1.00 | 1.62 | 27.5 |
| Mean ± SE | 1.4 ± .4 | .9 ± .2 | .8 ± .1 | 1.6 ± .6 | 44 ± 13

Growth of Largest and Second Largest Follicles

The dominant follicle (determined by ultrasonography) grew faster (P<.01, tested by homogeneity of regression) in cows fed the high energy diet. The dominant follicle in high energy-fed cows grew 6.8 ± .7 mm in 4 d and had an average growth rate of 1.8 mm/day (linear coefficient for continuous day variable) while the increase in size of the dominant follicle was less for low energy-fed cows (4.0 ± .6 mm) with an average growth rate of .8 mm/day (Figure 6-8A). Size of the
Table 6-6. Concentration of plasma IGF-I (ng/ml) in cows fed the high (H) and low (L) diets starting on dietary day 0.

<table>
<thead>
<tr>
<th>Cow</th>
<th>Diet</th>
<th>Dietary Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Meana</th>
</tr>
</thead>
<tbody>
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<td>956</td>
<td>L</td>
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<tr>
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<td>L</td>
<td>67.5</td>
<td>63.8</td>
<td>73.2</td>
<td>70.2</td>
<td>76.7</td>
<td>70.3</td>
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<tr>
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<td>L</td>
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<td>41.9</td>
<td>54.4</td>
<td>31.2</td>
<td>50.4</td>
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<td>70.6</td>
<td>64.0</td>
<td>77.7</td>
<td>50.4</td>
<td>70.4</td>
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<tr>
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<td>17.7</td>
<td>13.1</td>
<td>16.1</td>
<td>16.9</td>
<td>16.5</td>
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</tr>
<tr>
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<td>53.9</td>
<td>47.8</td>
<td>50.0</td>
<td>42.0</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>H</td>
<td>76.4</td>
<td>66.0</td>
<td>65.2</td>
<td>50.0</td>
<td>61.0</td>
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</tr>
<tr>
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<td>84.8</td>
<td>111.7</td>
<td>73.3</td>
<td>140.9</td>
<td>97.0</td>
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</tr>
<tr>
<td>1520</td>
<td>H</td>
<td>61.7</td>
<td>65.1</td>
<td>62.2</td>
<td>79.6</td>
<td>54.8</td>
<td>64.7</td>
<td></td>
</tr>
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<td>28.1</td>
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<td>21.8</td>
<td>16.6</td>
<td>20.0</td>
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</tr>
<tr>
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<td>69.3</td>
<td>69.2</td>
<td>66.4</td>
<td>71.4</td>
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</tr>
</tbody>
</table>

aAverage concentration of IGF-I for days 0 to 4.
bAverage concentration of IGF-I for cows within diets on each day.
cPooled SEM for diet-by-day interaction equalled 6.40; Diet-by-day trends differed (P<.05) when tested for homogeneity of regression.

second largest follicle on d 4 was $3.8 \pm 0.8$ mm less than its original size on d 0 of the trial for high energy-fed cows compared with $0.8 \pm 0.8$ mm less than its original size on d 0 for low energy-fed cows (Figure 6-8B). Therefore, the decline in the size of the nondominant (second largest follicle) was faster (P<.10; tested by homogeneity of regression) in those cows fed the high energy diet (-.9 mm/day vs -.3 mm/day; high vs low energy diets, respectively). Furthermore, the average number of follicles per cow increased during the dietary
Figure 6-8. Growth (change in size) of the preovulatory follicle (Panel A) and decline in size of the second largest follicle (Panel B) in cows fed the high (n=4, anestrus cow removed) and low (n=5) energy diets.

period in cows fed the low energy diet from 1.3 ± .2 on d 0 to 1.9 ± .2 on d 4. At the same time, average number of follicles per cow decreased in cows fed the high energy diet (treatment-by-day, P=.10) from 1.9 ± .2 on d 0 to 1.0 ± .2 on d 4.

Growth Factor Activity in Serum and Follicular Fluid

Growth factor activity, as measured by thymidine incorporation in AKR-2B and 3T3 cell lines, are presented in Table 6-8. For comparison, thymidine incorporation in untreated cells (0% serum) averaged 29,857 ± 2,467 and 1300 ± 95 dpm per 4 h for AKR-2B and 3T3 cells, respectively; while cells treated with 5% young calf serum averaged 556,839 ± 5892 and 198,751 ± 2437 dpm per 4 h for each cell line, respectively. There was no effect of treatment on ability of serum to increase thymidine incorporation by cells. There was, however, considerable cow variation (P<.001) with individual animals ranging from 88,938 ± 7,304 to 293,966 ±
Table 6-7. Concentration of plasma growth hormone (ng/ml) in cows fed the high (H) and low (L) diets starting on dietary day 0.

<table>
<thead>
<tr>
<th>Cow</th>
<th>Diet</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>956</td>
<td>L</td>
<td>2.1</td>
<td>1.5</td>
<td>2.5</td>
<td>3.6</td>
<td>7.3</td>
<td>3.4</td>
</tr>
<tr>
<td>1443</td>
<td>L</td>
<td>1.6</td>
<td>1.0</td>
<td>1.2</td>
<td>1.5</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>1482</td>
<td>L</td>
<td>2.7</td>
<td>3.4</td>
<td>1.5</td>
<td>1.9</td>
<td>3.6</td>
<td>2.6</td>
</tr>
<tr>
<td>8981</td>
<td>L</td>
<td>.6</td>
<td>.4</td>
<td>1.1</td>
<td>2.9</td>
<td>2.8</td>
<td>1.6</td>
</tr>
<tr>
<td>8986</td>
<td>L</td>
<td>10.6</td>
<td>7.2</td>
<td>11.2</td>
<td>7.7</td>
<td>5.6</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Mean<sup>bc</sup> 3.5  2.7  3.5  3.5  4.2

<table>
<thead>
<tr>
<th>Cow</th>
<th>Diet</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1376</td>
<td>H</td>
<td>2.1</td>
<td>1.8</td>
<td>3.5</td>
<td>2.2</td>
<td>8.6</td>
<td>3.6</td>
</tr>
<tr>
<td>1466</td>
<td>H</td>
<td>.7</td>
<td>1.7</td>
<td>1.9</td>
<td>1.7</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>1498</td>
<td>H</td>
<td>3.5</td>
<td>1.1</td>
<td>1.0</td>
<td>1.5</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>1520</td>
<td>H</td>
<td>1.1</td>
<td>2.5</td>
<td>.7</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>8920</td>
<td>H</td>
<td>26.2</td>
<td>7.6</td>
<td>7.7</td>
<td>7.5</td>
<td>8.1</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Mean<sup>bc</sup> 6.7  2.9  3.0  2.9  4.1

<sup>a</sup>Average concentration of GH for days 0 to 4.
<sup>b</sup>Average concentration of GH for cows within diets on each day.
<sup>c</sup>Pooled SEM for diet by day interaction equalled 1.38.

27,621 dpm for AKR-2B cells and 124,734 ± 16,716 to 254,960 ± 10,355 dpm for 3T3 cells. The main effect of day was significant (P<.07 for AKR-2B cells and P<.04 for 3T3 cells), with serum from all cows being less mitogenic 2 days after the diets started.

Growth factor activity in follicular fluid and serum as well as concentrations of IGF-I in follicular fluid on the day of ovariectomy are presented in Table 6-9. Data from seven cows are presented because the largest follicles in three cows
Table 6-8. Thymidine incorporation (dpm per 4 h) into cell lines treated with 5 % serum from cows fed high (H) or low (L) energy diets.

<table>
<thead>
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<th>Diet</th>
<th>Day</th>
<th>3T3</th>
<th>AKR-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0</td>
<td>173,227</td>
<td>150,436</td>
</tr>
<tr>
<td>L</td>
<td>1</td>
<td>214,299</td>
<td>163,044</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>146,811</td>
<td>99,360</td>
</tr>
<tr>
<td>L</td>
<td>3</td>
<td>164,764</td>
<td>155,287</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
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</tr>
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<td>H</td>
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<td>212,495</td>
<td>178,533</td>
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<td>216,801</td>
<td>140,670</td>
</tr>
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<td>2</td>
<td>144,087</td>
<td>111,944</td>
</tr>
<tr>
<td>H</td>
<td>3</td>
<td>214,320</td>
<td>186,272</td>
</tr>
<tr>
<td>H</td>
<td>4</td>
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</tr>
<tr>
<td></td>
<td>Pooled SEMb</td>
<td>21,046</td>
<td>26,432</td>
</tr>
</tbody>
</table>

Day relative to initiation of feeding of experimental diets.

Pooled standard error for diet-by-day interaction.

(1520, high energy diet; 956 and 1482, low energy diet) were broken at time of ovariectomy. Four of the seven largest follicles collected had estradiol to progesterone ratios in follicular fluid greater than 1.0 (3 of 4 high energy-fed cows and 1 of 3 low energy-fed cows). Thymidine incorporation in 3T3 and AKR-2B cells was lower after treatment with follicular fluid (P<.001) compared with treatment with serum from cows on the same day. The IGF-I concentration in follicular fluid ranged from 18.7 ng/ml to 131.6 ng/ml. The lowest concentrations of IGF-I in follicular fluid were associated
Table 6-9. Thymidine incorporation into 3T3 and AKR-2B cells treated with follicular fluid recovered from largest follicles at ovariectomy or serum from the same day from cows on high (H) and low (L) energy diets.

<table>
<thead>
<tr>
<th>Cow Size</th>
<th>E:P</th>
<th>IGF</th>
<th>AKR-2B Serum</th>
<th>AKR-2B FF</th>
<th>3T3 Serum</th>
<th>3T3 FF</th>
</tr>
</thead>
<tbody>
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<td>H1376</td>
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<td>121,253</td>
<td>11,862</td>
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<td>163,477</td>
<td>25,396</td>
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<tr>
<td>H1498</td>
<td>14</td>
<td>20.6</td>
<td>79,248</td>
<td>25,862</td>
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<td></td>
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<td>73,002</td>
<td>6,581</td>
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<tr>
<td>Mean</td>
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<td>99,411</td>
<td>32,793</td>
<td>89,106</td>
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<td>Grand Mean</td>
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<td>111,496</td>
<td>26,242</td>
<td>96,452</td>
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<td>0 % YCS</td>
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<td>5 % YCS</td>
<td></td>
<td></td>
<td>198,203</td>
<td></td>
<td>123,935</td>
<td></td>
</tr>
</tbody>
</table>

aDiameter (mm) of follicle.
bRatio of estradiol to progesterone in follicular fluid.
cConcentration of IGF-I (ng/ml) in follicular fluid.
dPooled SEM = 8894.

with lowest mitogenic activity (3T3 cells) in the follicular fluid samples tested. In addition, concentrations of IGF-I in follicular fluid were correlated with average IGF-I in plasma (P<.08; R²=.48; [IGF-I in follicular fluid] = 14.44 + .831 x [IGF-I in plasma]). There was no correlation (P>.10) between estrogen to progesterone ratio and IGF-I in serum or
follicular fluid, or diameter of the follicle and IGF-I in serum or follicular fluid.

Table 6-10. Densitometric analysis (results are in arbitrary absorbance units) for the series of insulin-like growth factor binding proteins (IGF BP) found in cows prior to (day 0) and after (day 4) being fed either the high or low energy diet.

<table>
<thead>
<tr>
<th>Day</th>
<th>Diet</th>
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<th>39</th>
<th>33</th>
<th>27</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Low</td>
<td>50.0(^a)</td>
<td>31.0</td>
<td>12.7</td>
<td>7.5</td>
<td>13.5</td>
</tr>
<tr>
<td>0</td>
<td>High</td>
<td>60.0</td>
<td>39.0</td>
<td>19.3</td>
<td>7.8</td>
<td>15.3</td>
</tr>
<tr>
<td>4</td>
<td>low</td>
<td>54.3</td>
<td>31.8</td>
<td>11.0</td>
<td>8.0</td>
<td>14.5</td>
</tr>
<tr>
<td>4</td>
<td>high</td>
<td>62.5</td>
<td>36.0</td>
<td>18.3</td>
<td>7.0</td>
<td>14.3</td>
</tr>
</tbody>
</table>

\(^a\)Pooled SE = 2.4.

IGF Binding Proteins

Plasma had a series of IGF binding proteins (IGF-BP) of molecular weights 44, 39, 33, 27, and 23 kDa. The qualitative array of IGF-BP observed in the plasma did not change (based on statistical analysis of densitometric data) from d 0 to 4 for cows on either the high or low energy diets (Table 6-10). The pattern of IGF-BP in follicular fluid collected from these cows was dissimilar from that of serum (P<.01; Table 6-11). Follicular fluid contained only small amounts of lower molecular weight species of IGF-BP (27 and 23 kDa) compared with serum.
Table 6-11. Densitometric analysis (results are in arbitrary absorbance units) for the series of insulin-like growth factor binding proteins (IGF BP) found in follicular fluid from two large (11 and 14 mm) follicles and serum from two control cows.

<table>
<thead>
<tr>
<th>Source</th>
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<th>33</th>
<th>27</th>
<th>23</th>
</tr>
</thead>
<tbody>
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<td>Control Serum</td>
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<td>27.5</td>
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<td>28.5</td>
</tr>
<tr>
<td>Follicular Fluid</td>
<td>49.5</td>
<td>26.0</td>
<td>22.5</td>
<td>1.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

^aPooled SE = 5.4

Discussion

Growth rates of the potential preovulatory follicle were slower in cows fed the low energy diet compared with cows fed the high energy diet. This finding suggests that acute growth of preovulatory follicles can be affected by short-term changes in energy balance. Others previously reported that short term energy restriction decreased ovulation rate in heifers treated with PMSG (Lamond, 1970). It is not clear what metabolic or hormonal factors cause this slower terminal growth or increase in size of large preovulatory follicles. Plasma IGF-I clearly declined in low energy-fed cows. In addition, the concentration of IGF-I in the follicular fluid and serum were highly correlated. Of interest was the fact that two cows (8920 [high energy diet] and 8986 [low energy diet]; Figure 6-6E and 6-7J) with abnormal (few or no pulses)
LH patterns had low IGF-I concentrations and high GH concentrations (see Table 6-6 and 6-7). Therefore, there may be an association between those factors controlling GH, IGF-I, and gonadotropin secretion as well as follicular growth and development. Furthermore, these two cows (8920 and 8986) had lowest 3T3 cell growth factor activity and lowest IGF-I concentration in follicular fluid (Table 6-9). These results yield a tentative relationship between nutrition, plasma LH, plasma IGF-I, plasma GH, and the mitogenic and hormonal composition of follicular fluid. One speculation may be that reduced nutrient intake alters LH secretion (as has been previously described, Imakawa et al., 1987a) as well as IGF-I in serum (also described by Gluckman et al., 1987; caused by reduced concentration of GH receptors in the liver) to markedly change the composition of follicular fluid (i.e., altered IGF-I concentration). This apparently can occur in estrogen active (see E:P ratio; Table 6-9; 8986) and inactive (8920) follicles. Similar experiments, with greater number of cows, need to be performed to substantiate the relationships suggested by the present study.

There was no significant difference in diameter of the dominant follicle at the start of the feeding trial. However, average diameter of dominant follicles in cows on the low-energy diet was approximately 2 mm larger at the start of the dietary feeding compared with dominant follicles in high energy-fed cows. Therefore, the average diameter of the
preovulatory follicle converged for high and low-energy fed cows and ultimately preovulatory follicles were similar in size after the 4 days of preovulatory follicular growth. An interesting question is whether or not these apparently larger follicles that occurred by chance in the low energy-fed cows reached maturity earlier and, therefore, stopped growing due to physiological mechanisms unrelated to the dietary treatments. Other studies using lactating cows treated with CIDR devices (see Chapters 2 and 7) have shown that large preovulatory follicles continue to grow to approximately 18 to 20 mm in the presence of progesterone. Therefore, since follicles in low-energy fed cows had slowed follicular growth at a considerably smaller size than 20 mm, the low energy treatment appeared to prevent the final growth and maturation of preovulatory follicles from 14 to 20 mm in diameter.

The second largest follicle decreased in size at a greater rate in cows fed the high energy ration. In addition, total number of follicles per cow increased during the preovulatory period in low-energy fed cows while decreasing in cows fed the high energy diet. The decrease in size of the second largest follicle during a follicular wave and changes in the total number of follicles on the ovary are methods used to evaluate follicular dominance. This is because dominant follicles are believed to prevent the growth and development of other follicles on the ovary. If these are accurate estimates of dominance, then cows on the high energy diet had
follicles that were better able to control the growth of smaller follicles on the ovary compared with low energy cows. This fact, combined with the faster growth rate of follicles in high energy-fed cows, provides additional evidence linking undernutrition with suboptimal follicular growth.

Changes in the dynamics of follicular populations within the ovaries of cows in this trial (Figure 6-2) were consistent with our knowledge of the effects a GnRH agonist on the ovary (Thatcher et al., 1989b; Wolfenson et al., 1990; Figure 6-2). Subsequent to Buserelin injection, there was a rapid decline in the number of large follicles (> 10 mm) which were either luteinized or ovulated (see Table 6-2). A decline in the number of these large follicles was followed by an increase in the number of class 2 follicles (6 to 9 mm) on the ovary. This increase probably was stimulated by the functional loss of large follicles (luteinized by Buserelin-induced LH release) which released these smaller follicle from the effects of follicular dominance (Ireland and Roche, 1987). Alternatively, small follicles may have been stimulated by GnRH injection independent of the effects of follicular dominance. Eventually, these smaller follicles moved into the larger class 3 follicles (10 to 15 mm) resulting in a decline in the number of class 2 follicles per cow. Finally, cows entered the preovulatory period and the number of class 4 follicles (> 15 mm) increased. Thus, due to the experimental
programming of follicular growth, either a class 3 or 4 follicle was present approaching the time of ovariectomy.

As expected, plasma progesterone remained high until the injection of PGF (Table 6-3). There was an initial increase in the concentrations of progesterone up to 2 days after CIDR insertion, associated primarily with the immediate release of progesterone from the CIDR. After the injection of PGF there was a decline in progesterone to approximately 2.3 ng/ml. This is a typical concentration of progesterone for cows that have had CIDR devices for 7 d (Lucy et al., 1990). Subsequent to CIDR removal progesterone declined to below 1 ng/ml.

Plasma hormones and metabolites changed appropriately for cows fed each diet. There was an immediate and sustained increase in the concentrations of NEFA in plasma in low energy-fed cows. Cows on the high energy diet also had increased NEFA on d 0, but this declined as feeding progressed. An increase in plasma NEFA represents greater lipid mobilization. Clearly, cows on the low energy diet were mobilizing lipids during this trial, suggesting that the diet did affect their general metabolism. It is unclear why cows on the high energy diet had increased NEFA on the first experimental day. This could have been related to the adjustment of high energy-fed cows to the feeding system (Calan gates) and the aggressive behavior of low energy-fed cows housed in the same barn. Dry matter intake remained constant in high energy-fed cows during the dietary period.
(15.7 ± 2.5 kg/d). Therefore, greater NEFA was probably related to reduced dry matter intake prior to the dietary period (not measured in the present study).

Plasma insulin and glucose was not affected by treatment. There was, however, an increase in insulin during the preestrual period. The possibility exists that increased concentrations of insulin in the blood around the time of estrus may modulate the growth and development of follicles. Direct effects of circulating insulin on the ovarian follicle have been reported in humans and may have some function in cattle prior to ovulation (Poretsky and Kalin, 1987). The role insulin played in follicular growth in this trial is unclear. Though concentrations of insulin were numerically higher for cows on the high energy diet (Figure 6-3B), this difference was not significant. As commonly observed in dairy cattle (Hart et al., 1978), concentrations of glucose in plasma were low and did not change appreciably during this trial. In contrast to the results for insulin and glucose, plasma triglycerides declined in cows fed the low energy diet. The seed lipid contained in the corn silage (low energy diet) was small compared with the seed lipid in the high energy diet (which contained nearly 50% grains). Reduced dietary lipid intake probably resulted in reduced concentrations of triglycerides in the blood of low energy-fed cows.

Our inability to detect differences in concentrations of GH among diets may have been related to the frequency of
sampling for GH. Growth hormone is secreted in a pulsatile manner from the anterior pituitary (Gluckman et al., 1987). Certainly, the best estimate of GH would be through the analysis of frequent samples and the evaluation of changes in GH baseline and pulse amplitude over time. Even with the infrequent sampling in this study, it appeared that 2 of 5 low energy-fed cows had increasing concentrations of GH across the dietary period.

Growth factor activity was measured, using cell lines, for two reasons. First, to determine if short-term dietary restriction could cause a decrease in mitogenic activity in serum (Salmon, 1975; Avasthy et al., 1986). This effect is well documented in laboratory animals which, when fasted for several days or placed on restricted diets, have a decrease in mitogenic activity of serum. Reduced mitogenic activity was associated with the development of binding proteins which inhibit the action of growth factors. Therefore, in the present study, cell lines were used because growth factor inhibitors can not be estimated by radioimmunoassay for IGF-I or insulin. The mitogenic activities in serum from cows on the high and low energy diets were similar in this trial. In addition, the series of low molecular weight IGF-BPs were also fairly constant. This fact may be related to failure to detect differences in mitogenic activity of serum. There exist five species of IGF-BP which corresponded closely to those in humans (Hossenlopp et al., 1987) and pigs (McCusker
et al., 1989). In neonatal pigs, 24 h of fasting causes an increase in the 29 kDa species and a decrease in other species (McCusker et al., 1989). In contrast, IGF-BP remained constant in cows on this trial fed the low energy diet. Perhaps more stringent dietary treatments (complete fasting) are required to observe changes in IGF-BP and mitogenic activity in serum in lactating dairy cows. If a relationship between dietary fasting, IGF-BP, and mitogenic activity of serum does exist in dairy cattle, then further experiments will be required to elucidate its existence.

Treatment of cells with follicular fluid from follicles collected at ovariectomy (> 11 mm) inhibited the growth of AKR-2B cells but resulted in stimulation of growth in 3T3 cells. This finding partially agrees with previous reports of the existence of mitogenic inhibitors in follicular fluid (Carson et al., 1986). Previously, these inhibitors were shown to act on 3T3 cells, but we did not find any inhibition of growth when 3T3 cells were treated with follicular fluid. The present study suggests that the inhibitor acts on a different cell line. The AKR-2B cells and the 3T3 cells are responsive to different classes of growth factors (EGF vs IGFs and insulin, respectively; Y. Ko. and F.A. Simmen, unpublished observations). Therefore, both cell lines were used in the present study so that classes of growth factors or growth factor inhibitors in follicular fluid could be identified. Since these cell lines respond to different growth factor
molecules the inhibitor present in follicle fluid may be specific for the AKR-2B cell line. One possibility is that TGF-β, which is an inhibitor of granulosa cell replication found in follicle fluid (Hammond et al., 1988), may actually be the inhibitor which we are measuring. If true, TGF-β is only affecting AKR-2B cells. Follicular fluid stimulated the growth of 3T3 cells but was not as stimulatory as serum. Therefore, serum either contains more factors that are stimulatory to 3T3 cell growth or there are inhibitors in follicular fluid that decreases the number of 3T3 cells undergoing a mitogenic response.

The pattern of IGF-BP in the follicular fluid was different from that in serum. It is clear that the follicular fluid is deficient in the lower molecular weight binding proteins. Perhaps, the absence of lower molecular weight species in follicular fluid has some physiological significance in cattle. Indeed, Ui et al. (1989) found that an inhibitor to FSH action, purified from porcine follicular fluid, had high sequence homology to a human IGF-BP (53 kDa; IGFBP 3). In addition to their antigonadotropic action, higher molecular weight binding proteins may be the source of the mitogenic inhibition in follicular fluid with lower molecular weight species serving alternate functions, either not associated with or detrimental to follicular growth. This possibility is not, however, supported by results from this study because equivalent amounts of high molecular weight IGF-
BP were present in cow serum (mitogenic) and follicular fluid (inhibitory to AKR-2B cell growth).

In conclusion, feeding a diet that was low in energy caused preovulatory follicles to grow at a slower rate and the second largest follicle to decrease in size at a slower rate when compared with cows maintained on a diet high in energy. Cows on the low energy diet had greater NEFA, a decrease in plasma triglycerides, and declining concentrations of IGF-I in plasma. In addition, concentrations of IGF-I in plasma and follicular fluid were correlated in cows studied in this trial. Changes in follicular growth were not associated with changes in concentrations of glucose, LH, mitogenic activity, or the qualitative array IGF-BP in serum. These results infer that IGF-I in plasma and follicular fluid may modulate the growth and development of follicles in cows in negative energy balance.
CHAPTER 7
EFFECT OF COMPOSITION OF DIET, ENERGY BALANCE, AND BOVINE SOMATOTROPIN ON OVARIAN FOLLICULAR DYNAMICS IN DAIRY CATTLE

Introduction

Administration of bovine somatotropin (bST) to increase milk production in dairy cows has been the subject of intensive research during the past decade (Peel and Bauman, 1987). Small quantities of bST, injected daily or biweekly, increases consistently the production of milk in dairy cattle. The mammary gland does not contain hormonal receptors for the bST molecule (Akers, 1985). Therefore, while the mechanism of action of bST is still under investigation, the general belief is that the insulin-like growth factor molecules (IGFs or somatomedins) released by several tissues (primarily liver) in response to bST, are mediating the galactopoietic effects of bST on the mammary gland (Gluckman et al., 1987). The study of bST and its effects on the nutrition and metabolism of the dairy cow is of obvious interest to lactational physiologists. However, other organs, including the uterus and ovaries of the cow, are potential sites of action for bST (or growth hormone, GH) or its associated IGFs (Hammond et al., 1988).

The actions of bST and/or the IGF-I molecule on ovarian function and the biology of the granulosa cell have been researched extensively (reviewed by Adashi et al., 1985a).
Three primary actions of IGF-I on granulosa cells have been elucidated through in vitro experimental approaches. First, treatment of granulosa cells with IGF-I causes increased cell replication (demonstrated in heifers, Savion et al., 1981). This finding is consistent with mitogenic activity of the IGF-I molecule. Secondly, IGF-I enhances steroid secretion by granulosa cells (Veldhuis et al., 1986a). This has been demonstrated for both progesterone and estrogen and suggests that IGF-I may stimulate early enzymatic processes in steroidogenesis (Veldhuis et al., 1986ab; Veldhuis et al., 1987). Finally, IGF-I, in the presence of follicle stimulating hormone (FSH), caused an increase in the concentration of luteinizing hormone (LH) receptors occupying rat granulosa cells in vitro (Adashi et al., 1985b). Therefore, the potential for enhanced action of gonadotropins on granulosa cells exposed to IGF-I through greater receptor content remains an important, unanswered scientific question.

In the lactating dairy cow, the stimulatory effects of bST on the function of the granulosa cell may interact with its metabolic and galactopoietic effects (Peel and Bauman, 1987). Injection of lactating dairy cows with bST enhances milk production at the initial expense of body tissue reserves (Bauman et al., 1985). This is because milk production increases almost immediately in cows treated with bST, while several weeks are required for dry matter intake (DMI) to increase in order to compensate for enhanced production (Peel
and Bauman, 1987). This temporary imbalance in milk energy output in relation to feed energy consumption results in a temporary decrease in energy balance and a compensatory mobilization of nutrients from adipose tissue (Bauman and Currie, 1981). Changes in animal energy balance appear to affect the function of the ovary in lactating dairy cows (reviewed by Butler and Smith, 1989). Specific examples of the effects of negative energy balance include delayed interval to first ovulation (see Chapter 2), fewer ovulatory-sized follicles (Chapter 4), slowed growth of the preovulatory follicle (Chapter 6), increased incidence of cystic follicles (Chapter 4; Kesler and Garverick, 1982), reduced progesterone production by the corpus luteum (CL; Villa-Godoy et al., 1988), reduced conception rate (Butler and Smith, 1989), and reduced responsiveness to estrous synchronization regimens (Stevenson et al., 1987). Therefore, it is possible that the stimulatory effects of bST on the granulosa cell, and the adverse effects of negative energy balance on the function of the ovary, may be positioned against each other in cows treated with bST.

One practical method to increase dietary energy intake and alter ovarian follicular dynamics is through the feeding of calcium salts of long chain fatty acids (CaLCFA). This high energy feed ingredient has the energy density of dietary fat and can be included in lactating cow diets without adverse effects on rumen fermentation (Chalupa et al., 1984; Jenkins
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and Palmquist, 1984; Chalupa et al., 1986; Grummer et al., 1988). One interesting side effect of fat feeding on reproduction (besides increased energy balance) is a general increase in the incidence of large follicles on the ovary (> 15 mm; Chapter 5) which may be translated into reduced fertility in some herds (Chapter 2). Adding CaLCFA to lactation diets will increase the energy density of the diet and therefore may offset some of the effects of negative energy balance on reproduction (Chalupa and Ferguson, 1988; Ferguson, 1988). Furthermore, feeding CaLCFA may be useful in bST-treated animals as a method to alleviate the initial reduction in energy balance associated with bST treatment and therefore offset potential negative effects of bST on the function of the ovary. The objectives of this research were 1) to determine if bST alters the growth and development of follicles during the first follicular wave, as well as during a preovulatory follicular wave in lactating as well as nonlactating dairy cows, 2) to determine if CaLCFA themselves, or additional energy provided by CaLCFA, mediate the previously observed effects of CaLCFA on the ovary, 3) to evaluate the effects of bST on the ovary in cows fed different dietary formulations with or without CaLCFA, and 4) to determine if energy balance affects ovarian follicular populations in cows treated with bST and fed different diets with or without CaLCFA.
Materials and Methods

Animals and Diets

Eighteen lactating Holstein cows (60 to 100 days into lactation) and six nonlactating, nonpregnant Holstein cows were used in this experiment. The experiment was conducted at the University of Florida Dairy Research Unit (Hague, FL) from March 15, 1990 to June 15, 1990. Cows were housed in a concrete-floored, open-air, free-stall barn with adjoining dirt lots. An ambient-temperature controlled, water sprinkler and fan system was in operation in the barn for the months of May and June for the purpose of cooling of the cows. Daily feed consumption of individual cows was measured using the Calan Gate System (American Calan, Inc., Northwood, NH). Cows were weighed weekly on two consecutive mornings immediately after the morning milking. Cows were milked twice daily and milk production was measured at each milking by electronic milk flow meters in the milking parlor. Milk composition (percentage milk fat and solids-not-fat percentage) was determined for four consecutive am and pm milkings by the Virginia DHIA milk testing laboratory (Blacksburg, VA). Feed samples (collected weekly) were dried (55 °C for 48 h) and composite samples were prepared on a monthly basis and analyzed at the Northeast DHIA forage testing laboratory (Ithaca, NY) for the determination of energy content (net energy of lactation; NEL). Energy balance (EB) was determined from dry matter energy intake, milk energy output, and
maintenance energy requirement as described in Chapter 4. Actual daily energy balance was subjected to a smoothing algorithm to remove extraneous values (outliers). Energy balance on a given day was calculated from the mean of the two previous days, the actual day, and the two subsequent days with the highest and lowest values removed.

Cows were fed ad libitum one of four diets beginning 30 d prior to the initiation of the first period and remained on the diets for the entire experiment. Nonlactating cows were fed perennial peanut hay (Arachis glabrata) while lactating cows were fed one of three diets (Table 7-1). Essentially these diets consisted of a control diet that did not contain CaLCFA (diet 1, NEL = 1.68 Mcal/kg), a diet which was equal in energy density to the control diet but contained CaLCFA at 2.2% of diet DM (diet 2, portion of ground corn, soybean meal, and whole cottonseed replaced with alfalfa hay, peanut hay, and CaLCFA), and a diet similar in ingredient makeup to diet 1, however, additional energy was provided using CaLCFA at 2.2% of diet DM (diet 3; NEL = 1.78 Mcal/kg). Estimate for the NEL of CaLCFA was 6.52 Mcal/kg (Andrew et al., 1990). Net energy of lactation of the diets was based on chemical analysis of corn silage, peanut hay, alfalfa hay, and whole cottonseed, and National Research Council (1989) values for concentrate ingredients. The chemical composition of experimental diets were balanced to be similar in% DM,% crude
protein, and% undegradable protein (Table 7-2). Ether extract was higher in diets containing CaLCFA.

Table 7-1. Ingredient composition of experimental diets fed to lactating cows.

<table>
<thead>
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<th>Ingredient</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
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<td>Corn silage</td>
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<td>33.4</td>
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<tr>
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<tr>
<td>Soybean meal</td>
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<td>2.7</td>
</tr>
<tr>
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<td>10.1</td>
<td>12.1</td>
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<tr>
<td>Blood meal</td>
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<td>2.7</td>
</tr>
<tr>
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<td>2.7</td>
</tr>
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<td>CaLCFA</td>
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</tr>
<tr>
<td>Sodium bicarbonate</td>
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<tr>
<td>Trace mineralized salt</td>
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<td>.5</td>
</tr>
<tr>
<td>Mineral premix</td>
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</tr>
<tr>
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<tr>
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</table>

Administration of bST and Reproductive Management

This experiment consisted of two experimental periods (19 days each in length). Cows were administered bst (daily injection, 25 mg recombinant-derived bst in phosphate-buffered saline; gift from the Monsanto Agricultural Company, St. Louis, MO) or diluent (saline) during one of the two experimental periods. Daily injections were given subcutaneously, behind the right or left shoulder (alternating days) in the afternoon (prior to ultrasonography) following
blood sampling. The experiment was a crossover design with respect to bST treatment, however, cows remained on the same diet for the entire experiment (i.e., across both periods, cows nested within diets).

Table 7-2. Chemical composition of experimental diets fed to lactating cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>51.68</td>
<td>51.72</td>
<td>51.71</td>
</tr>
<tr>
<td>Acid detergent fiber, % DM</td>
<td>22.60</td>
<td>26.40</td>
<td>22.40</td>
</tr>
<tr>
<td>Crude protein, % DM</td>
<td>19.40</td>
<td>20.90</td>
<td>19.40</td>
</tr>
<tr>
<td>Undegrad. prot., % CP</td>
<td>36.80</td>
<td>36.60</td>
<td>36.90</td>
</tr>
<tr>
<td>NE lactation, Mcal/kg</td>
<td>1.68</td>
<td>1.68</td>
<td>1.78</td>
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<tr>
<td>Ether extract, % DM(^a)</td>
<td>5.51</td>
<td>6.77</td>
<td>7.19</td>
</tr>
<tr>
<td>Calcium, % DM</td>
<td>.94</td>
<td>1.05</td>
<td>.95</td>
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<tr>
<td>Phosphorus, % DM</td>
<td>.54</td>
<td>.50</td>
<td>.54</td>
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<tr>
<td>Magnesium, % DM</td>
<td>.28</td>
<td>.24</td>
<td>.28</td>
</tr>
<tr>
<td>Sulfur, % DM</td>
<td>.25</td>
<td>.28</td>
<td>.25</td>
</tr>
<tr>
<td>Potassium, % DM</td>
<td>1.12</td>
<td>1.30</td>
<td>1.12</td>
</tr>
<tr>
<td>Sodium, % DM</td>
<td>.55</td>
<td>.57</td>
<td>.55</td>
</tr>
</tbody>
</table>

\(^a\)Calculated from NRC (1989).

Experimental periods were designed to specifically monitor growth and development of follicles during the first follicular wave during the estrous cycle, as well as during the preovulatory follicular wave. Approximately 3 weeks after the initiation of dietary treatments, a norgestomet capsule (Synchro-mate B implant, CEVA Laboratories Inc., Overland Park, KS) was implanted into the ear of all cows. Seven days later, cows were injected intramuscularly with 25 mg prostaglandin F\(_2\)a (PGF; Lutalyse, UpJohn Co., Kalamazoo, MI), and tail heads painted (Impervo Brand, Benjamin Moore,
Montvalo, NJ) as an aid for detection of estrus. Two days after PGF injection, norgestomet implants were removed, tailheads chalked (All-weather Paint Stik—Laco Industries Inc., Chicago, IL) and cows observed for estrus. The first period started (i.e., bST or saline injections initiated; day = 1) the day after synchronized estrus. On day 12 of the experimental periods, cows were injected with 25 mg of PGF to induce CL regression and a CIDR device (controlled internal drug release-Bovine; Carter-Holt Plastics Molding Co., New Zealand) containing 1.9 g progesterone was inserted into the vagina of all cows. Five days later (day = 17), CIDRs were removed and tail heads were painted as an aid to detection of estrus. The period ended when cows expressed estrus or were determined to have ovulated based on ultrasonographic examination. Approximately 2 weeks after the end of the first period, cows were injected with PGF to induce estrus and initiate the second period. Cows were managed during the second period exactly as the first and received a PGF injection on day 12 and CIDR insertion from days 12 to 17.

Ultrasonographic Examination, Blood Collection, and Analysis

Ovaries of cows were examined daily by ultrasonography using an Equisonic LS 1000 linear array ultrasound scanner equipped with a 7.5 Mhz probe (Tokyo Kieki Co., Ltd., Tokyo, Japan). Examinations involved the removal of fecal matter from the rectum, insertion of the probe into to the rectum, and positioning the probe adjacent to each ovary. Size and
number of ovarian follicles $\geq 3$ mm were recorded onto follicular maps. Large follicles ($\geq 6$ mm) were specifically localized on the ovary in an effort to follow the growth and development of individual large follicles. Size, number, and position of corpora lutea and large luteinized follicles were recorded also.

Ten milliliters of blood were collected from each cow on each day of the experiment. Blood was sampled from the jugular vein into evacuated heparinized tubes (Vaccutainer, Becton Dickenson, East Rutherford, NJ). Blood was placed on ice and immediately centrifuged at 3000 x g (4 °C) for 30 minutes. Plasma was harvested and stored at -20 °C until assay. Hormones and metabolites in plasma were analyzed in samples collected every other day (days 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19). Plasma glucose was measured using the glucose oxidase/peroxidase colorometric method (Sigma Chemical Co., St. Louis, MO; kit No. 510). Twenty-five microliters was analyzed and glucose concentration measured from a glucose standard solution. Total plasma cholesterol was measured in 10 ul samples based on a cholesterol standard curve (Sigma cholesterol calibrators No. C0534) using Sigma Chemical Co. kit No. 352. Plasma nonesterified fatty acids (NEFA) were measured in duplicate determinations of 25 ul of plasma (NEFA C kit; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Concentrations of insulin and growth hormone (GH) in plasma were determined in nonextracted samples by competitive binding
radioimmunoassay described by Collier et al. (1982). Intra- and interassay coefficients of variation, respectively, were 10.0 and 7.7% for the insulin assay and 11.2 and 14.7% for the GH assay. Plasma progesterone was measured using procedures described previously (Knickerbocker et al., 1986b). Intra- and interassay coefficients of variation were 6 and 15.0%, respectively.

Statistical Analysis

Data were analyzed primarily with one of two statistical models using the general linear models procedure of the Statistical Analysis System (SAS, 1987; unbalanced data sets) or the ANOVA procedure of SAS (balanced data sets; Tables 7-3 and 7-4). The first model (Table 7-3; Model 1) included the main effects of diet, cow-within-diet, treatment (bST or saline), period, day, interactions of these main effects, and residual. Energy balance, 4% fat-corrected milk (FCM) production, DMI, and body weight, as well as concentrations of plasma glucose, NEFA, cholesterol, GH, insulin, and progesterone were analyzed using this model. Single degree of freedom contrasts used for analyses of dependent variables were 1+2+3 vs. 4 (lactating vs. nonlactating), 1 vs. 2+3 (diet without CaLCFA vs. diets with CaLCFA), and 2 vs. 3 (lower energy density CaLCFA-diet vs. higher energy density CaLCFA-diet).

The second model (Table 7-4; Model 2) included the main effects of diet, group (which is treatment sequence across the
two periods, bST-saline or saline-bST), cow-within-diet-by-group, period, day, interactions of these main effects, and residual. The second model is used to characterize carry-over effects (period 1 to period 2) of treatment on follicular dynamics. The group-by-period interaction represents the main effect of treatment.

Table 7-3. Statistical model (model 1) for analysis of data (TRT = treatment, bST or saline).

<table>
<thead>
<tr>
<th>Source</th>
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<th>Error Term</th>
</tr>
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<tbody>
<tr>
<td>Diet</td>
<td>2</td>
<td>Cow(diet)</td>
</tr>
<tr>
<td>Cow(diet)</td>
<td>15</td>
<td>Residual</td>
</tr>
<tr>
<td>TRT</td>
<td>1</td>
<td>TRT<em>period</em>cow(diet)</td>
</tr>
<tr>
<td>Period</td>
<td>1</td>
<td>TRT<em>period</em>cow(diet)</td>
</tr>
<tr>
<td>Diet*TRT</td>
<td>2</td>
<td>TRT<em>period</em>cow(diet)</td>
</tr>
<tr>
<td>Diet*period</td>
<td>2</td>
<td>TRT<em>period</em>cow(diet)</td>
</tr>
<tr>
<td>TRT<em>period</em>cow(diet)</td>
<td>12</td>
<td>Residual</td>
</tr>
<tr>
<td>Day</td>
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<td>Day*cow(diet)</td>
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<tr>
<td>Diet*day</td>
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<td>Day*cow(diet)</td>
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<td>Day*cow(diet)</td>
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</tr>
<tr>
<td>Period*day</td>
<td>18</td>
<td>Residual</td>
</tr>
<tr>
<td>Diet<em>TRT</em>day</td>
<td>36</td>
<td>Residual</td>
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<tr>
<td>Diet<em>period</em>day</td>
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</tr>
<tr>
<td>Residual</td>
<td>216</td>
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</table>

Follicles observed on the ovary were separated into four follicular size classes. Class 1 was 3 to 5 mm, class 2 was 6 to 9 mm, class 3 was 10 to 15 mm, and class 4 was > 15 mm. Initially, the number of follicles on the ovary was analyzed using Models 1 and 2 which included the additional main effect of follicular size class. Follicular size class analyses were performed for the entire experimental period (days 1 to 19), prior to PGF injection (days 1 to 12), and after PGF injection.
Table 7-4 Statistical model (model 2) for analysis of data (Group = treatment sequence across periods, bST-saline or saline-bST).

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<tbody>
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</tr>
<tr>
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</tr>
<tr>
<td>Period</td>
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<td>Period<em>cow(diet</em>group)</td>
</tr>
<tr>
<td>Diet*period</td>
<td>1</td>
<td>Period<em>cow(diet</em>group)</td>
</tr>
<tr>
<td>Group*period</td>
<td>1</td>
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</tr>
<tr>
<td>Diet*day</td>
<td>36</td>
<td>Day<em>cow(diet</em>group)</td>
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<tr>
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<td>36</td>
<td>Day<em>cow(diet</em>group)</td>
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<tr>
<td>Diet<em>group</em>day</td>
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<td>Residual</td>
</tr>
<tr>
<td>Diet<em>period</em>day</td>
<td>36</td>
<td>Residual</td>
</tr>
<tr>
<td>Group<em>period</em>day</td>
<td>18</td>
<td>Residual</td>
</tr>
<tr>
<td>Diet<em>group</em>period*day</td>
<td>36</td>
<td>Residual</td>
</tr>
<tr>
<td>Residual</td>
<td>216</td>
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</tr>
</tbody>
</table>

(days 13 to 19). After this initial approach, analyses were run separately for follicles within each follicular size class. Size of the dominant follicle during the first follicular wave as well as during the preovulatory follicular wave were analyzed using the treatment model. The main effect of day subsequently was fit as a continuous variable for the analysis of the number of follicles within different size classes. Interactions of main effects with day were tested using the procedure of homogeneity of regression (Wilcox et al., 1990). The effect of energy balance on the number of
follicles within different size classes and growth of dominant follicles were tested using a third model which contained the effects of diet, cow-within-diet, treatment, day, interactions of the main effects, and residual. Energy balance was fit to this model as a continuous variable and the gain (change in residual sums of squares) from fitting individual EB curves for treatment, diets, and treatment x diets was tested (Wilcox et al., 1990).

Results
Production Responses to bST and Diets

Daily data for production responses to treatments for lactating and nonlactating cows are presented in Figure 7-1. Lactating cows treated with bST produced 10% more FCM compared with cows treated with saline (25.4 vs. 23.1 kg/day, SEM = .4, P<.01). As a result, bST-treated lactating cows were in lower EB during the later part of the period (treatment-by-day P<.01) as equivalent amounts of DM were consumed (20.2 and 20.7 kg/day, SEM=.4). Unexpectedly, lactating cows gained more BW during the period when injected with bST (treatment-by-day, P<.01).

Lactating cows, consuming different diets, had similar FCM production (24.3, 23.6, and 24.9 kg/day, SEM=.2), consumed similar amounts of DM (20.1, 19.7, and 20.7 kg/day, SEM=1.3), and had similar body weight (527, 537, and 527 kg, SEM=17; diets 1 to 3 respectively). In addition, increased production of FCM in response to bST was similar (P=.17) for control cows
Figure 7-1. Dry matter intake (panel A), fat corrected milk (B), energy balance (C), and body weight (D) for lactating (-----) and nonlactating (----) cows treated with bST (.) or saline (*) from day 1 to estrus (mean = day 19.5).

and cows fed CaLCFA (24.9 vs. 23.6 kg/day [6% increase] and 25.7 vs. 22.9 kg/day [12% increase], diet 1 and diets 2+3, respectively; Table 7-5). There were no diet-by-treatment-by-day interactions detected for FCM production, DMI, or BW. However, for lactating cows, there was a diet-by-day-by-treatment interaction for EB (P<.07; Figure 7-2) due to a greater reduction of EB in response to bST in cows consuming CaLCFA compared with control cows perhaps due to greater FCM production in cows treated with bST and fed CaLCFA.
Table 7-5. Mean amount and statistical contrasts for dry matter intake (DMI), milk production (MP), milk fat, milk solids non-fat, 4% fat corrected milk production (FCM), body weight (BW), and energy balance (EB) for lactating cows during the experimental period (TRT = treatment [bST [BST] or saline [SAL]]).

<table>
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<tr>
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<th>Diet 1</th>
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<td>SAL</td>
<td>BST</td>
<td>SAL</td>
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<td>20.9</td>
<td>20.6</td>
<td>.7</td>
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<tr>
<td>MP, kg/d</td>
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<td>3.32</td>
<td>3.33</td>
<td>3.12</td>
<td>.09</td>
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<tr>
<td>SNF, %</td>
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<td>8.40</td>
<td>8.34</td>
<td>8.28</td>
<td>8.26</td>
<td>8.30</td>
<td>.03</td>
</tr>
<tr>
<td>FCM, kg/d</td>
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<td>23.6</td>
<td>25.1</td>
<td>22.2</td>
<td>26.2</td>
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<td>BW, kg</td>
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<td>540</td>
<td>535</td>
<td>529</td>
<td>524</td>
<td>8</td>
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<tr>
<td>EB, Mcal/d</td>
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<td>7.8</td>
<td>6.3</td>
<td>8.5</td>
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<td>1v2+3</td>
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<td>DMI, kg/d</td>
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<td>MP, kg/d</td>
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<td>.94</td>
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<td>Milk Fat, %</td>
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<td>SNF, %</td>
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<td>BW, kg</td>
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<td>.48</td>
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<td>EB, Mcal/d</td>
<td>.15</td>
<td>.47</td>
<td>.22</td>
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Figure 7-2. Energy balance (Mcal/day) for cows fed diet 1 (panel A), diet 2 (B), diet 3 (C), or diet 4 (D; nonlactating) and treated with bST (••••) or saline (*) from days 1 to estrus (mean = day 19.5).

Plasma Hormones and Metabolites

Lactating cows treated with bST had higher concentrations of GH (9.5 vs. 3.2 ng/ml, SEM=.3; P<.01), glucose (68.5 vs. 66.1 mg%, SEM=.6; P<.05), and NEFA (282 vs. 182 uEq/l, SEM=11.8; P<.01) in plasma, and tended to have higher insulin concentration in plasma (.74 vs. .68 ng/ml, SEM=.03; P=.11) compared with saline-treated cows (Figure 7-3). However, plasma cholesterol was not affected by treatment (215 and 201 mg%, SEM=6). There was no effect of diet of lactating cows on plasma glucose (68.0, 66.1, and 68.0 mg%, SEM=1.3; diets 1 to
Figure 7-3. Plasma non-esterified fatty acids (A), glucose (B), growth hormone (C), and insulin (D) in lactating cows treated with bST (---.) or saline (*) or nonlactating cows treated with bST (-.-.-) or saline (*--*-).

3, respectively), NEFA (217, 255, and 225 uEq/l, SEM=18), insulin (.77, .65, and .71 ng/ml, SEM=.06), or cholesterol (185, 204, and 235 mg%, SEM=21). Cows fed CaLCFA (diets 2+3) tended to have higher plasma GH compared with cows fed the control diet (6.7 vs. 5.7 ng/ml, SEM=.4, P=.10). In addition, there were no treatment-by-diet or treatment-by-diet-day interactions detected for plasma GH, glucose, NEFA, insulin or cholesterol concentrations (Table 7-6).

In lactating cows, there was a significant effect of day on concentrations of glucose (P<.01) and insulin (P<.01) in
Table 7-6. Mean concentration and statistical contrasts for the plasma concentration of progesterone (P4, SEM=.47), growth hormone (GH, SEM=.57), insulin (Ins, SEM=.08), cholesterol (Chol, SEM=9), nonesterified fatty acids (NEFA, SEM=25), and glucose (Gluc, SEM=1.0) during the experimental period. TRT = treatment (bST [BST] or saline [SAL]).

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<td>SAL</td>
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<td>Chol, mg%</td>
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<td>184</td>
<td>216</td>
<td>192</td>
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<td>NEFA, uEq/l</td>
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<td>157</td>
<td>300</td>
<td>211</td>
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<tr>
<td>Gluc, mg%</td>
<td>69.8</td>
<td>66.1</td>
<td>66.7</td>
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P=

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<tbody>
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<tr>
<td>P4, ng/ml</td>
<td>.59</td>
<td>.01</td>
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<tr>
<td>GH, ng/ml</td>
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<td>Ins, ng/ml</td>
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<td>Chol, mg%</td>
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<td>Gluc, mg%</td>
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</table>
plasma. Glucose increased from a minimum of 65.7 mg% on day 1 to a maximum of 71.5 mg% on day 19. Concentrations of insulin increased from a minimum of .63 ng/ml on day 1 to a maximum of .79 ng/ml on day 15. As expected, concentrations of NEFA also changed with day of period (treatment-by-day interaction, P<.01) and reached a maximum of 386 uEq/l on day 7 in cows injected with bST (Figure 7-3B). Furthermore, NEFA and glucose increased immediately prior to estrus in all cows.

Compared with lactating cows (diet 1+2+3), nonlactating cows (diet 4) had higher concentrations of glucose (76.3 vs. 67.3 mg%, SEM=1.0; P<.01), lower cholesterol (91 vs. 207 mg%, SEM=16; P<.01), and tended to have lower concentrations of GH in plasma (5.4 vs 6.4 ng/ml, SEM=.4; P=.11). There was a diet (1+2+3 vs. 4)-by-period interaction for plasma NEFA concentration (P<.01). This was because nonlactating cows had elevated plasma NEFA in the first period (234 vs. 510 and 229 vs. 149 uEq/l, diets 1+2+3 vs. 4, periods 1 and 2, respectively). In addition, there was a diet (1+2+3 vs. 4)-by-treatment interaction (P<.01) for concentrations of insulin with the mean concentration of insulin being .68 vs. .74 ng/ml (SEM=.05) in saline vs. bST-treated lactating cows, while nonlactating cows treated with saline or bST had mean insulin concentration of .56 and 1.20 ng/ml (SEM=.05), respectively.

Plasma progesterone in lactating cows and nonlactating cows treated with bST or saline is presented in Figure 7-4 (samples assayed daily). There was no effect of bST treatment
on plasma progesterone prior to day 13 (endogenous progesterone; 4.80 vs. 4.57 ng/ml, SEM=.57, bST vs. saline) or after the injection of PGF on day 12 (progesterone derived from both endogenous sources as well as CIDR device; days 13 to 19; 2.59 vs. 2.61 ng/ml, SEM=.17, bST vs saline). There was a significant effect of lactational status on the concentration of progesterone prior to day 13 with lactating cows (diets 1+2+3) having lower progesterone than nonlactating cows (4.42 vs. 5.61 ng/ml, SEM=.28, P<.02). After day 12, concentrations progesterone in plasma were similar in
nonlactating and lactating cows (2.80 vs. 2.53 ng/ml, SEM=.13, respectively).

Effect of bST and Diet on Follicular Populations

The main effect of treatment sequence (i.e., group, model presented in Table 7-4) was not significant in the analyses of follicular size class data. Therefore, since carry-over effects of bST or saline treatment were not evident (i.e., group not significant), results are presented from analysis of data using the first model (Table 7-3). Mean numbers of follicles within different follicular size classes for bST- and saline-treated cows (days 1 to 19) are presented in Figure 7-5. Considering lactating cows prior to day 13, there was no significant effect of diet or treatment on the number of follicles within classes 1, 3, or 4. However, there was a significant diet-by-treatment-by-day interaction for the number of class 2 follicles prior to injection of PGF (Figure 7-6). Across diets and days (lactating cows only), bST increased the number of follicles in class 2 (2.2 vs. 1.6, SEM=.15, P<.01). There was no effect of bST on the number of class 2 follicles during the first 12 days in nonlactating cows (2.3 vs. 2.2, SEM=.2, bST vs. saline; Figure 7-6d). In addition, mean numbers of class 2 follicles prior to day 13 were similar in nonlactating cows (2.2, SEM=.2) and lactating cows treated with bST (2.2, SEM=.2; diet (1+2+3 vs. 4)-by-treatment, P<.05; Figure 7-5b) while saline-treated lactating
cows had fewer class 2 follicles (1.56, SEM=.2) compared with bST-treated lactating cows or nonlactating cows.

Figure 7-5. Mean number of class 1 (panel A), class 2 (B), class 3 (C), and class 4 (D) follicles in lactating cows treated with bST (---) or saline (*) or nonlactating cows treated with bST (-----) or saline (-----).

Among lactating cows, after the injection of PGF, there was no effect of diet or treatment on the number of follicles in class 1 (Figure 7-5A), while a diet-by-treatment-by-day interaction existed for the number of follicles in class 2 (Figure 7-6). There was no significant effect of diet or treatment on the average number of follicles in class 3 after day 12. However, tests of homogeneity of regression indicated that second order fitted curves for the bST and saline-treated
Figure 7-6. Mean number of class 2 follicles (6 to 9 mm) for cows fed diet 1 (A), diet 2 (B), diet 3 (C), or diet 4 (D; nonlactating) and treated with bST (•) or saline (*).

cows (pooled across diets [1+2+3] or fit for individual diets) were not parallel (P<.01 and P<.025, respectively). Furthermore, there was no effect of bST on the number of follicles in class 3 for nonlactating cows. The average number of class 3 follicles after day 12 was similar in nonlactating cows (1.45) and lactating cows treated with bST (1.55, SEM=.09; Figure 7-5c). However, lactating cows treated with saline had fewest class 3 follicles during this time (1.19, SEM=.09, diet (1+2+3 vs. 4)—by-treatment—by-day interaction for fitted curves, P<.01). Considering class 4 follicles (after PGF injection, lactating cows), there was a
Figure 7-7. Average number of class 4 (> 15 mm) follicles for cows fed diet 1 (o—o), diet 2 (x—x), diet 3 (+—+), or diet 4 (----; nonlactating).

tendency for cows treated with bST to have fewer class 4 follicles (.53 vs. .71, SEM=.08, P=.12). In addition, there was a significant diet-by-day (P<.08; Figure 7-7) and a treatment-by-day (P<.08) interaction for class 4 follicles after day 12. Nonlactating cows had fewer class 4 follicles compared with lactating cows (.62 vs .35, SEM=.11, P<.03).

Effect of EB on Number of Follicles within Size Classes

Linear regression (lactating cows) of EB on the average number of follicles within different size classes are presented in Table 7-7 (data is graphed in Figures 7-8 [days 1 to 12] and 7-9 [days 13 to 19]). Interactions of treatment (pooled across diets 1, 2, and 3) with EB were not significant
Table 7-7. Linear regression of EB on numbers of follicles within different size classes (lactating cows; Number = $B_0 + B_1 \times EB$).

<table>
<thead>
<tr>
<th>Class</th>
<th>Days</th>
<th>Treatment</th>
<th>$B_0$</th>
<th>$B_1$</th>
<th>$P&lt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1-12</td>
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<td>.051</td>
<td>.181</td>
</tr>
<tr>
<td>2</td>
<td>1-12</td>
<td>SAL</td>
<td>2.10</td>
<td>-.062</td>
<td>.099</td>
</tr>
<tr>
<td>2</td>
<td>13-19</td>
<td>BST</td>
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<td>.064</td>
<td>.207</td>
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<tr>
<td>2</td>
<td>13-19</td>
<td>SAL</td>
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<td>-.040</td>
<td>.336</td>
</tr>
<tr>
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<td>BST</td>
<td>.64</td>
<td>.0095</td>
<td>.546</td>
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<tr>
<td>3</td>
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<td>SAL</td>
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<td>-.042</td>
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<tr>
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<td>BST</td>
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<td>.046</td>
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<td>SAL</td>
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<td>BST</td>
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</tr>
<tr>
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<td>SAL</td>
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<td>.015</td>
<td>.066</td>
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<td>13-19</td>
<td>BST</td>
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<td>13-19</td>
<td>SAL</td>
<td>.81</td>
<td>-.010</td>
<td>.461</td>
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</tbody>
</table>

Regression coefficients for the effect of energy balance on the number of follicles per cow (dependent variable).

Follicular size class, class 2 = 6 to 9 mm; class 3 = 10 to 15 mm; and class 4 = > 15 mm.

Day of period.

BST = bovine somatotropin; SAL = saline.

For class 1 follicles prior to or after day 12 of the period. There existed a treatment-by-EB interaction for class 2 follicles with the average number of class 2 follicles increasing with increasing EB in BST-treated cows while decreasing in saline-treated cows ($P<.01$). This interaction was consistent (sign of $b_1$ similar) before as well as after...
Figure 7-8. Linear regression of EB (days 1 to 12) on numbers of follicles in different size classes (A, class 1; B, class 2; C, class 3; D, class 4) in lactating cows treated with bST (BST) or saline (SAL).

day 12 (P<.05). However, slopes of these lines were not different from zero. Effect of EB on numbers of follicles in classes 3 and 4 differed with respect to treatment according to day of period. Prior to day 13, there was a significant treatment-by-EB interaction for class 3 follicles (P<.03). Increasing EB was associated with fewer class 3 follicles in cows treated with saline, while no effect of EB on number of follicles per cow was evident in bST-treated cows. In addition, class 4 follicles increased with increasing EB for cows treated with saline (but not bST) during the same period (treatment-by-EB, P<.01). After day 12 there was no
Figure 7-9. Linear regression of EB (days 13 to 19) on numbers of follicles in different size classes (A, class 1; B, class 2; C, class 3; D, class 4) for lactating cows treated with bST (BST) or saline (SAL).

interaction of treatment with EB for class 3 or class 4 follicles. R-square of statistical models increased by 0, 3, 3, and 2% (classes 1, 2, 3, and 4) and 0, 4, 1, and 0% after fitting the treatment-by-EB interaction for each class before and after day 12, respectively.

There was no interaction of diet (lactating cows, pooled across treatments) and EB for class 1 follicles before or after day 12. There was a significant diet-by-EB interaction (P<.01) for class 2 follicles prior to day 13 with diet 1 cows not responsive to EB (Y = 1.62 + .028 x EB; \( P = .49 \); Y = number of class 2 follicles per cow), diet 2 cows having greater
numbers of class 2 follicles with increasing EB ($Y = .71 + .134 \times EB; P=.074$) and diet 3 cows having fewer class 2 follicles with increasing EB ($Y = 3.73 - .164 \times EB; P = .058$). After day 12, the diet-by-EB interaction was not significant. Effects of EB on the number of class 3 and 4 follicles within diets before and after day 12 are presented Table 7-8 (data is graphed in Figure 7-10). In general, effects of EB on the average number of follicles per cow were similar before (diet-by-EB, $P<.03$) and after (diet-by-EB, $P<.10$) injection of PGF (day 12). For diet 1, numbers of class 3 follicles decreased with increasing EB, while the number of class 4 follicles increased with increasing EB. Among cows fed diet 2, responsiveness of follicular populations to EB were opposite that of diet 1 (i.e., more class 3 follicles with increasing EB, and fewer class 4 follicles with increasing EB). Responsiveness of class 3 and class 4 follicles to changes in EB for cows fed diet 3 were intermediate to diets 1 and 2. R-square of statistical models increased by 3 and 6% (class 3), and 2 and 4% (class 4) after fitting the diet-by-EB interaction before and after day 12, respectively.

Significant diet-by-treatment-by-EB interactions existed for class 1 follicles before ($P<.10$; $Y_{diet1,bST} = 3.98 + .14 \times EB [P=.26]$, $Y_{diet2,bST} = 5.05 + .14 \times EB [P=.43]$, and $Y_{diet3,bST} = 5.41 + .05 \times EB [P=.76]$ vs. $Y_{diet1,SAL} = 6.18 - .10 \times EB [P=.42]$, $Y_{diet2,SAL} = 2.53 + .37 \times EB [P=.06]$, and $Y_{diet3,SAL} = .69 + .28 \times EB [P=.03]$, diets 1, 2, and 3, respectively, bST vs. saline, $Y =$
Table 7-8. Linear regression of EB on numbers of follicles within different size classes for lactating cows fed different diets (Number = $B_0 + B_1 \times EB$).

<table>
<thead>
<tr>
<th>Class $^b$</th>
<th>Days $^c$</th>
<th>Diet</th>
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<th>$B_1$</th>
<th>P $^&lt;$</th>
</tr>
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<tbody>
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<td>.29</td>
<td>.053</td>
<td>.084</td>
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<td>1-12</td>
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<td>.56</td>
<td>-.006</td>
<td>.799</td>
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<td>.72</td>
<td>.114</td>
<td>.009</td>
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<td>.69</td>
<td>.078</td>
<td>.080</td>
</tr>
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<td>.16</td>
<td>.002</td>
<td>.816</td>
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<td>-.024</td>
<td>.130</td>
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<td>.02</td>
<td>.022</td>
<td>.081</td>
</tr>
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<td>.50</td>
<td>.013</td>
<td>.501</td>
</tr>
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<td>13-19</td>
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<td>1.07</td>
<td>-.055</td>
<td>.022</td>
</tr>
<tr>
<td>4</td>
<td>13-19</td>
<td>3</td>
<td>.70</td>
<td>-.015</td>
<td>.546</td>
</tr>
</tbody>
</table>

$^a$Regression coefficients for the effect of energy balance on the number of follicles per cow (dependent variable).

$^b$Follicular size class, class 2 = 6 to 9 mm; class 3 = 10 to 15 mm; and class 4 = > 15 mm.

$^c$Day of period.

number or class 1 follicles per cow), as well as after day 12 (P < .05; $Y_{\text{diet1, bst}} = 5.42 - .041 \times EB$ [P = .80], $Y_{\text{diet2, bst}} = 6.89 - .233 \times EB$ [P = .20], and $Y_{\text{diet3, bst}} = 4.97 + .075 \times EB$ [P = .70] vs. $Y_{\text{diet1, sal}} = 8.60 - .364 \times EB$ [P = .005], $Y_{\text{diet2, sal}} = 4.25 + .100 \times EB$ [P = .53], and $Y_{\text{diet3, sal}} = 2.66 + .122 \times EB$ [P = .42], diets 1, 2, and 3, respectively, bst vs. saline, $Y$ = number of class 1
follicles per cow). In addition, there was a significant diet-by-treatment-by-EB interaction for class 3 follicles after day 12 (P<.01; \( Y_{diet1,bST} = 1.71 - .014 \times EB \ [P=.78] \), \( Y_{diet2,bST} = .276 + .234 \times EB \ [P=.001] \), and \( Y_{diet3,bST} = 1.41 + .016 \times EB \ [P=.776] \) vs. \( Y_{diet1,SAL} = 1.36 - .041 \times EB \ [P=.28] \), \( Y_{diet2,SAL} = 1.15 + .040 \times EB \ [P=.39] \), and \( Y_{diet3,SAL} = -.06 + .100 \times EB \ [P=.028] \), diets 1, 2, and 3, respectively, bST vs. saline, \( Y \) = number of class 3 follicles per cow). All other diet-by-treatment-by-EB interactions for classes 2, 3, or 4, before or after day 12 were not significant.
Figure 7-11. Mean diameter (mm) and fourth-order fitted curves for the first dominant follicle in cows fed diet 1 (o), diet 2 (x), diet 3 (+), or diet 4 (.; nonlactating).

Growth of Dominant Follicles

Average diameter of the first dominant follicle for each day of the period as well as the fourth-order fitted curves for these data is given in Figure 7-11 (diets 1, 2, 3, and 4). Among lactating cows, there was no effect of diet (11.9, 11.6, and 11.0 mm, SEM=.9, diets 1 to 3, respectively), or treatment (11.7 and 11.3 mm, SEM=.5, bST and saline) on the mean size of the first dominant follicle (across all days). However, growth rate differed (P<.01) among lactating cows fed different diets, as well as among lactating and nonlactating cows (Figure 7-12). Growth of the first wave dominant follicle was similar in bST- and saline-treated cows.
Figure 7-12. Growth rate of first-wave dominant follicles in lactating cows (diets 1 to 3) fed different diets and nonlactating cows (diet 4).

However, a diet-by-day interaction (P<.01) existed for lactating cows with diet 2 cows having larger follicles after day 8 of the period. Furthermore, first dominant follicles of nonlactating cows grew to a smaller size compared with first dominant follicles in lactating cows (diet 1+2+3 vs. 4, diet-by-day interaction, P<.001) while no diet-by-treatment interaction existed for the size of the first dominant follicle in lactating vs. nonlactating cows. Maximum size of first-wave dominant follicles was smaller for cows treated with bST compared with saline (16.3 vs.17.1 mm, SEM=.4). Furthermore, the maximum size of the first wave follicles was smaller (P<.001) in nonlactating cows compared with lactating
cows (diets 1+2+3; Table 7-9). Cows fed CaLCFA (diets 2+3) had dominant follicles of equivalent size to control cows (diet 1), while cows fed diet 2 had larger dominant follicles compared with cows fed diet 3 (P<.08; Table 7-9).

Table 7-9. Maximum size of the dominant follicle in the first follicular wave or on the day of ovulation (preovulatory follicle) in cows fed different diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>First Wave Follicle&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Preovulatory Follicle&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Control (Diet 1)</td>
<td>17.8</td>
<td>.7</td>
</tr>
<tr>
<td>CaLCFA (Diet 2)</td>
<td>18.8</td>
<td>.8</td>
</tr>
<tr>
<td>CaLCFA (Diet 3)</td>
<td>16.7</td>
<td>.8</td>
</tr>
<tr>
<td>Non-lactating</td>
<td>14.4</td>
<td>.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>1+2+3 vs. 4, P<.001; 2 vs. 3, P<.08.

<sup>b</sup>1 vs. 2+3, P<.08.

Day of initiation of the second follicular wave was similar for cows treated with bST or saline (11.1 vs 11.7, SEM=.4, Table 7-10). Four out of 18 (22%) lactating cows treated with saline and 1 out of 18 (6%) lactating cows treated with bST did not have a second follicular wave (i.e., first dominant follicle eventually ovulated). Across diets, there were no significant differences in the average day of initiation of the second follicular wave (12.0, 11.3, 11.4, and 10.9, SEM=.7, diets 1 to 4, respectively; Table 7-11).
However, variance associated with mean was similar for cows in diets 1 (variance = 1.6) and 4 (variance = 1.5), but greater (P<.05) for cows fed diets 2 (variance = 6.6) and 3 (variance = 5.6). Finally, among cows having a second follicular wave (lactating and nonlactating), a higher proportion ($X^2 = 4.42; P<.05$) of double ovulations occurred in cows initiating the second follicular wave after day 12 (PGF) compared with those initiating the second follicular wave prior to day 13 (number with two ovulations/total number of cows (%) = 2/25 (8%) vs. 6/18 (33%); before day 12 vs. after day 12, respectively).

Table 7-10. Day of initiation of second follicular wave [number of cows (%)] for lactating cows (1+2+3) and nonlactating cows (4) treated with BST or saline.

<table>
<thead>
<tr>
<th>Daya</th>
<th>BST 1+2+3</th>
<th>BST 4</th>
<th>BST Total (%)</th>
<th>Saline 1+2+3</th>
<th>Saline 4</th>
<th>Saline Total (%)</th>
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</thead>
<tbody>
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<td>0 (0)</td>
<td>1 (6)</td>
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<tr>
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<td>2 (11)</td>
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<td>3 (13)</td>
<td>1 (6)</td>
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<td>1 (4)</td>
</tr>
<tr>
<td>10</td>
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<td>2 (8)</td>
<td>1 (6)</td>
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<td>2 (11)</td>
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<tr>
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<td>1 (4)</td>
<td>4 (22)</td>
<td>0 (0)</td>
<td>4 (17)</td>
</tr>
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</table>

aDay of period.
Table 7-11. Day of initiation of second follicular wave [number of cows (%)] for lactating cows (diets 1, 2, and 3) and nonlactating cows (diet 4).

<table>
<thead>
<tr>
<th>Diet</th>
<th>1</th>
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<td>1 (8)</td>
<td>1 (8)</td>
<td>0 (0)</td>
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<td>1 (8)</td>
<td>2 (17)</td>
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</table>

Size of the preovulatory follicle was similar for cows treated with bST or saline (13.2 and 13.3 mm, SEM=.7, bST vs. saline, pooled across days) and also for cows fed different diets (12.2, 13.8, 12.8, and 14.0 mm, SEM=.9, diets 1 to 4 respectively; Figure 7-13, day means and second-order fitted curves). Growth rate was also similar across diets and treatments (Figure 7-14). Size of the preovulatory follicle at estrus was not different in bST and saline-treated cows (17.9 and 18.1 mm, SEM=.1, bST and saline, respectively) but cows fed CaLCFA (diets 2+3) had larger (P<.08) preovulatory follicles compared with cows fed the control diet (diet 1; Table 7-9). Sizes of the preovulatory follicle were similar for lactating (diets 1+2+3) and nonlactating cows (diet 4).
Figure 7-13. Mean diameter and second-order fitted curves for preovulatory follicles in cows fed diet 1 (o), diet 2 (x), diet 3 (+) or diet 4 (.; nonlactating).

Effect of EB on the Growth of Dominant Follicles

There was a diet-by-treatment-by-EB interaction (P<.001) for the growth of the first-wave follicle as well as the preovulatory follicle (Table 7-12; data is graphed in Figure 7-15 [first wave dominant follicle] and Figure 7-16 [second wave dominant follicle]). Energy balance did not affect the size of the first-wave dominant follicle for cows fed diets 1 or 4 treated with either bST or saline. However, cows fed CaLCFA (diets 2 and 3) were similar in that EB had no effect on the size of the first-wave dominant follicle in cows treated with bST, while for cows treated with saline, average size of the first-wave dominant follicle was greater for cows
Figure 7-14. Growth rate (mm/day) of preovulatory follicles in lactating cows (diets 1 to 3) fed different diets and nonlactating cows (diet 4).

in higher EB. As with the first dominant follicle, EB did not affect the growth of the second dominant follicle (preovulatory) in cows fed diet 1 or 4 (diet-by-treatment-by-EB; P<.05) although the response of dominant follicle size was influenced by EB and was similar for cows fed CaLCFA (diets 2 and 3). However, results were opposite to that of the first wave follicle. The bST-treated cows were responsive to EB (larger follicles for cows in higher EB), while there was little change in follicle size in response to EB for cows treated with saline. Model R-square increased by 2 and 1% after fitting the diet-by-treatment-by-EB interaction for the first and second wave dominant follicle, respectively.
Table 7-12. Linear regression of EB on the size of the first or second dominant follicle for cows on diets 1 to 4 treated with BST or saline (size = \( B_0 + B_1 \times EB \)).

<table>
<thead>
<tr>
<th>Dominant Follicle</th>
<th>Diet</th>
<th>Treatment</th>
<th>Regression$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( B_0 )</td>
</tr>
<tr>
<td>First</td>
<td>1</td>
<td>BST</td>
<td>12.79</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>SAL</td>
<td>11.65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BST</td>
<td>16.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SAL</td>
<td>12.38</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BST</td>
<td>12.63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>SAL</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>BST</td>
<td>11.73</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>SAL</td>
<td>10.77</td>
</tr>
</tbody>
</table>

$^a$Estimated regression line for the effect of energy balance on the size of the first or second dominant follicle (dependent variable).

$^b$BST = bovine somatotropin, SAL = saline.

Interval to Estrus and Ovulation Rate

There was no difference in interval to estrus after removal of the CIDR for cows receiving different treatments. Average intervals to estrus for cows treated with bST or saline were 2.6 and 2.3 days (SEM=.3), respectively (Table 7-13). However, cows fed different diets were not similar in terms of intervals to estrus which averaged 2.9, 2.0, 2.8, and
Figure 7-15. Linear regression of EB on the size of the first wave dominant follicle for bST- and saline-treated cows fed diets 1 (A), 2 (B), 3 (C), and 4 (D, nonlactating).

2.1 days (SEM=.3) for diets 1 to 4, respectively. Nonlactating cows were in estrus sooner after CIDR removal than lactating cows (P<.09) and cows fed diet 2 were in estrus sooner than cows fed diet 3 (P<.07). The proportion of cows have single, double, or no ovulation after each period is given in Table 7-14. Cows fed different diets and receiving different treatments were similar with respect to ovulation rate after each period. For all cows, anovulation (no ovulation by period day = 30) occurred in 5 of 24 bST-treated cows (21%) and 3 of 24 saline-treated cows (13%).
Figure 7-16. Linear regression of EB on the size of the preovulatory follicle for BST- or saline-treated cows fed diets 1 (A), 2 (B), 3 (C), or 4 (D, nonlactating).

Table 7-13. Interval to estrus after CIDR removal [number of cows (%)] for lactating cows (1+2+3) and nonlactating cows (4) treated with BST or saline.

<table>
<thead>
<tr>
<th>Hours</th>
<th>BST 1+2+3</th>
<th>BST 4</th>
<th>Total(%)</th>
<th>BST 1+2+3</th>
<th>BST 4</th>
<th>Total(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>1(17)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>48</td>
<td>6(33)</td>
<td>4(66)</td>
<td>10(42)</td>
<td>10(56)</td>
<td>5(83)</td>
<td>15(62)</td>
</tr>
<tr>
<td>72</td>
<td>6(33)</td>
<td>2(33)</td>
<td>8(33)</td>
<td>2(11)</td>
<td>0 (0)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>96</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>120</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>No Estrus</td>
<td>5(28)</td>
<td>0 (0)</td>
<td>5(21)</td>
<td>3(17)</td>
<td>0 (0)</td>
<td>3(13)</td>
</tr>
</tbody>
</table>
Table 7-14. Number of occurrences of single corpora lutea (single), multiple corpora lutea (multiple) or no ovulation (no ovul) following four ovulations (diets 1 to 4; one prior to each of two periods, one after each of two periods) or subsequent to treatment (BST or saline [SAL]).

<table>
<thead>
<tr>
<th>Type</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Treatment BST</th>
<th>Treatment SAL</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>18</td>
<td>14</td>
<td>17</td>
<td>65 (68%)</td>
</tr>
<tr>
<td>Multiple</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>21 (22%)</td>
</tr>
<tr>
<td>No Ovul</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>10 (10%)</td>
</tr>
</tbody>
</table>

Discussion

Production responses of lactating cows to BST were as expected. A 10% increase in FCM production was a typical response to BST treatment of dairy cows (Peel and Bauman, 1985). There was no significant increase in DM intake in association with BST treatment. This suggests that the treatment periods were too short for cows to compensate for enhanced FCM production with greater feed intake. This disproportionate increase in FCM production in the absence of enhanced DMI resulted in a large decrease in energy balance in cows treated with BST. However, BST-treated cows were not in negative energy balance during this trial.

As expected, treatment of lactating cows with BST resulted in higher concentrations of GH, glucose, NEFA, and insulin in plasma compared with lactating cows treated with
saline. Diets had little effect on these blood hormones and metabolites, although there was a tendency for cows fed CaLCFA to have higher concentrations of GH in plasma compared with cows fed the control diet. In contrast to a previous report (Carroll et al. [in press]), there was not a detectable change in concentrations of cholesterol in the blood of cows fed CaLCFA. Therefore, it appears that formulation of CaLCFA diets may specifically influence responses in terms of blood cholesterol. Of interest was the fact that plasma glucose and insulin increased across the experimental period of the estrous cycle in lactating and nonlactating cows. This is a novel finding and suggests that the general metabolism of the cow may change across days of the estrous cycle. One possible explanation for this phenomenon is that reduced feed intake as well as milk production around estrus results in a decrease in EB and an associated depression in blood glucose and insulin which recovers gradually across the estrous cycle. This is supported by gross examination of EB profiles from cows on this trial (individual data not shown) in which EB is quite cyclical and clearly decreases around estrus in most cows. Consistent with reduced EB around estrus, NEFA in plasma increased on day 19 of the period suggesting that cows were mobilizing lipids immediately prior to estrus.

Based on the hormonal and metabolic data presented in this study, it is clear that nonlactating cows are a poor model for reproductive questions pertaining to lactating cows.
Differences in hormones and metabolites between nonlactating and lactating cows were dramatic. Nonlactating cows had higher plasma glucose and progesterone, and lower GH and cholesterol compared with lactating cows. Differences in glucose may relate to metabolic differences between the two animal models. Nonlactating cows do not have glucose requirements for milk synthesis and therefore may have reduced glucose turnover resulting in higher concentrations of glucose in plasma. Also of interest was the fact that plasma insulin in nonlactating cows increased by 114% in response to bST, while only 9% for lactating cows. Reduced responsiveness of insulin to bST in lactating cows may be a reflection of reduced insulin synthesis, storage, and release in these cows. This seems likely because lactating cows normally have chronically low concentrations of insulin in the blood during the early postpartum period (see Chapter 4).

Progesterone was higher in nonlactating cows compared with lactating cows. This may be caused by a reduced rate of metabolism of progesterone or a greater production of progesterone from the CL. Concentrations of progesterone in plasma after day 12 (CIDR progesterone only) were similar for lactating and nonlactating cows, suggesting that clearance rate of progesterone was the same. Therefore, greater concentrations of progesterone in nonlactating cows from days 1 to 12 were probably related to enhanced production by the CL. Concentrations of cholesterol in plasma, although
markedly reduced in nonlactating cows on this trial, appeared to be adequate to support production of progesterone by the CL of nonlactating cows. This finding argues against the concept that cholesterol is limiting to progesterone production by the CL in cattle (Williams, 1989).

Unfortunately a significant period-by-diet interaction existed for plasma NEFA when values for lactating cows were compared with those for nonlactating cows. This reflects management decisions for the nonlactating cows prior to their entry into the trial. The nonlactating cows were fed a lactating cow diet before the initiation of peanut hay feeding. As would be expected, the nonlactating cows gained considerable body condition while on the lactating cow diet (approximately 3 weeks). Nonlactating cows lost body condition through the first period when fed peanut hay. Therefore, nonlactating cows had high NEFA in plasma at this time. Nonlactating cows had apparently adjusted to the peanut hay during the second period, and had plasma concentrations of NEFA considerably lower than those for lactating cows.

Cows treated with bST had more class 2 (6 to 9 mm) follicles during the first follicular wave (i.e., prior to day 13 of the period). The stimulation of follicles by bST, prior to day 13, was restricted to the class 2 size. This finding strongly supports the view that growth factors are critical to the function of follicles on the ovary (Adashi et al., 1985a). Class 2 follicles represent those follicles which are
undergoing recruitment and selection early during the follicular wave phase (Ireland and Roche, 1987). Therefore, follicles undergoing recruitment appear to be very sensitive to changes in growth factors within the blood. However, class 2 follicles did not move into larger size classes during this period (see class 3 follicles, days 1 to 12). Therefore, there appears to be a block to continued development of these follicles. It is plausible that although these follicles can be recruited, only a single follicle is selected eventually. Therefore, no stimulation of larger size classes of follicles is observed with bST treatment. Class 2 follicles in nonlactating cows were not responsive to bST. Indeed, average number of class 2 follicles in nonlactating cows (across treatments) and the average number of class 2 follicles in bST-treated lactating cows were very similar. This may suggest that growth factors (GF; perhaps IGF-I) limit the number of class 2 follicles being recruited in lactating cows and that these GF are not limiting in nonlactating individuals. Given the previously described relationship between animal energetics and growth factors in the blood (see Chapter 6), it seems possible that lactating cows may have reduced GF activity in blood compared with nonlactating cow and this may limit recruitment of class 2 follicles.

After day 12 the relationship between bST treatment and the number of follicles within the different size classes changed. While the number of class 2 follicles was still
elevated at the initiation of the second follicular wave (although to a smaller extent), there was a marked movement of follicles out of class 2 into class 3 during this time period (immediately after PGF, see days 13 to 18). Therefore, under certain physiological conditions (in this case luteolysis followed by supplemental progesterone), it appears that bST-stimulated class 2 follicles can move into larger size classes. One speculation might be that enhanced LH pulse secretion (known to occur in response to PGF and progestogen treatment: Roberson et al., 1989) may have stimulated the movement of smaller follicles into larger size classes. Furthermore, an increase in the number of LH receptors on the granulosa cell, shown in vitro after IGF-I treatment (Adashi et al., 1985b) may have made follicles in bST-treated cows more responsive to LH. The population of class 3 follicles in nonlactating cows was not responsive to bST. However, nonlactating cows (pooled across treatments) and bST-treated lactating cows were very similar in the average number of class 3 follicles across the experimental period. As stated above, this may reflect the deficiencies of growth factors in the blood of lactating cows which limit the number of follicles moving into class 3.

Although enhanced movement of follicles into class 3 in bST-treated cows was detected, an increase in ovulation rate for bST-treated cows was not found in this trial. This experiment was not designed to detect differences in ovulation
rate among treatments or diets. However, one known side effect of bST is an increase in ovulation rate in some herds. Therefore, the detection of changes in the number of large follicles on the ovary in response to bST is consistent with the concept of enhanced ovulation rate in bST-treated cows. This was not detected in this trial either because of too few cows or a physiological block to the ovulation of extra follicles which developed on the ovary due to our management of the estrous cycle with PGF and CIDR treatment.

Energy balance affected how follicles responded to the bST treatment. For class 2 follicles, there was a significant treatment-by-EB interaction with bST-treated-cows in the highest EB having the greatest number of class 2 follicles. In contrast, effects of EB on the number of class 2 follicles was opposite for saline-treated cows. These relationships were consistent before and after day 12. Enhanced action of bST in cows in higher EB may suggest that mediators of the action of bST on the follicle are responsive to changes in EB of the cow. One of the prime candidates for this effect may be IGF-I, since the release of IGF-I in response to GH is known to be responsive to changes in EB (Gluckman et al., 1987). Therefore, cows in higher EB may be releasing more IGF-I, resulting in enhanced growth of class 2 follicles. For larger size classes of follicles (classes 3 and 4), the treatment-by-EB interaction was more subtle. Before day 13, there was essentially no effect of EB on the number of class
3 and 4 follicles for bST-treated cows, while class 3 follicles declined with increasing EB and class 4 follicles increased with increasing EB in saline-treated cows. Therefore, greater EB in saline cows was associated with movement of follicles from class 3 to class 4 follicles. Since the regression coefficient of the number x EB curves for bST-treated cows was near zero for classes 3 and 4, bST treatment prevented the movement of follicles in association with changes in EB. The presence of additional growth factors in the blood of bST-treated cows may have compensated for the effects of EB on the movement of large follicles across classes at this time. After day 12, greater EB was associated with more class 3 follicles in bST-treated cows. This may have been related to the movement of bST-stimulated class 2 follicles into larger size classes under the influence of enhanced gonadotropin secretion which was not present prior to day 13.

As an alternative to the systemic effects of bST on the concentration of IGF-I in blood, bST may be directly affecting the local production of IGF-I by granulosa cells. Growth hormone will cause the release of IGF-I by granulosa cells (Hammond et al., 1988) and therefore paracrine actions of bST should be considered. It is possible that the action of bST on the follicle occurs by a twofold mechanism. First, bST results in the release of IGF-I from the liver and this IGF-I may pass from the blood into the follicular fluid to influence
the local concentrations of IGF-I in the follicle. In addition, bST may be causing the local production of IGF-I by granulosa cells. Therefore, bST would be acting in a paracrine manner. Perhaps changes in EB regulate the paracrine as well as the systemic secretion of IGF-I. This possibility should be tested using the analysis of mRNA for IGF-I from granulosa cells of cows in positive and negative energy balance.

Previous findings that cows fed CaLCFA had more large follicles on the ovary (Chapter 5) were verified in this trial. Cows on diet 2 had a greater number of class 4 follicles compared with cows on diets 1 or 3. In addition, there was a diet-by-day interaction detected where maximum size of the first follicular wave dominant follicle tended to be larger in cows fed diet 2 and the preovulatory follicles also tended to be larger in these cows. These effects were due to increased growth rate of dominant follicles in cows fed diet 2 which led to larger follicles. Large follicles may have a negative effect on fertility because they may antagonize maternal recognition of pregnancy through enhanced estrogen secretion (Thatcher et al., 1989ab). This concept is supported by the fact that cows fed CaLCFA were less fertile (Chapter 2). The fact that diet 2 cows and not diet 3 cows had a greater population of large follicles on the ovary indicates that feeding CaLCFA does not necessarily lead to the development of large follicles on the ovary. Indeed, in this
trial, cows on diet 3 had the smallest frequency of class 4 follicles. Therefore, specific formulation of the diet may be critical to results observed on follicular populations. It is possible that the higher forage to concentrate ratio in diet 2, in combination with the CaLCFA led to the development of large follicles on the ovary. Fitting the diet-by-EB interaction to these data (days 1 to 12 and 13 to 19) revealed that there were marked differences between diet 1 (control) and diet 2 (equal energy, containing CaLCFA) in terms of the effects of EB on the number of large follicles. While follicles moved out of class 3 into class 4 with increasing EB in the control diet, the opposite relationship occurred in diet 2. Therefore, for those cows fed diet 2, large class 4 follicles were less prevalent for cows in higher EB. This supports that low EB is a causative factor in the development of large follicles in cows fed CaLCFA (diet 2). Diet 3 cows were similar to diet 2 after day 12 in responsiveness to EB, suggesting some commonality among the two CaLCFA diets. It appears, therefore, that effects of diet on the population of follicles on the ovary cannot be dissociated from EB. This is consistent with findings presented in Chapter 4 indicating that dietary differences in the number of follicles within classes were explained by differences in EB. In the present study, these data suggest that the greater population of large follicles in cows fed CaLCFA might not be detected in later lactation cows in highest EB. Therefore, a window of
susceptibility to the effects of CaLCFA may exist during early lactation. Since this window coincides with the breeding period in most herds, more important understanding of the effects of CaLCFA on the ovary seems warranted. Indeed, the Illinois herd (data presented in Chapter 2) may have been extremely sensitive to the effects of CaLCFA because they were fed to cows in negative energy balance. Therefore, the findings that CaLCFA altered the reproductive characteristics of cows in this herd may relate to the effects of EB and CaLCFA on the population of follicles on the ovary.

Nonlactating cows in this trial had first-wave dominant follicles considerably smaller than for lactating cows. This result demonstrates the acute difference between lactating and nonlactating cows for the study of follicular dynamics. One unanswered question, however, is how the diet of the nonlactating cows influenced the growth of the first wave dominant follicle. Given the fact that the nonlactating cows were fed a diet devoid of seed lipids, it may be that the results related more to the nutrient composition of the diets than to actual differences among lactating and nonlactating cows. Irrespective of questions pertaining to the nonlactating cow diet used in this trial and its direct effects on growth of the first dominant follicle, it is clear that diets should be strictly controlled in future studies to insure accurate duplication of experimental results.
The second follicular wave was initiated at a similar time in cows regardless of treatment or diet. Five cows (four bST and one saline) did not have a second follicular wave and therefore failed to generate two dominant follicular waves. However, the treatment sequence (PGF [day 12] + CIDR [day 12 to 17]) successfully synchronized two follicular waves in 90% of the cows treated. Therefore, this experimental method to study follicular growth and development in cows is applicable to further studies. This appears essential because the spontaneous expression of two or three follicular waves would have reduced our experimental sensitivity. Across diets, average day of initiation of the second follicular wave was similar. However, the variance associated with the mean day that the second wave started was greater in cows on diets 2 and 3. Therefore, growth and development of follicles for cows fed CaLCFA was consistently less programmed. One interesting finding of this trial was the interaction of day of wave with day of period. In fact, a higher proportion of double ovulations occurred in cows having their first follicular wave after day 12 of the period. This finding may relate to the method used to program the ovary and changes in gonadotropin secretion after day 12. Possibly, higher LH pulse frequency recruited more follicles into large size classes. These findings could be applicable to the study of superovulation in cattle. One reasonable method to superovulate cows may be to program maximal LH secretion
(using PGF and CIDR) around the time of FSH injections to take advantage of endogenous gonadotropins during superovulation.

Dietary EB and treatment directly affected the size of the first and second dominant follicle in cows fed CaLCFA in this trial. Essentially, before day 12 (diets 2 and 3, only), saline-treated cows in higher EB had larger first wave dominant follicles. These effects were not small with the mean adjusted size of the first wave dominant follicle increasing by 1 mm for every 2 Mcal change in EB (see Diet 3, saline). This association was not present in bST-treated cows. The effect of EB on the preovulatory follicle (i.e., second wave) was consistent with respect to the diets. Cows fed diet 1 and 4 were not responsive to changes in EB across treatments while cows fed CaLCFA (diets 2 and 3) were similar in their responses to bST or saline. However, the opposite relationship was found with respect to treatment. In other words, bST-treated cows had larger second wave dominant follicles with increasing EB while saline-treated cows were unresponsive to the effects of EB. As with the first dominant follicle, the effects of EB were not small and the mean adjusted size of the second dominant follicle was nearly 1 mm larger for every 2 Mcal of EB increase. The consistent effects of EB within treatments in CaLCFA-fed cows indicates that CaLCFA in all dietary formulations had an effect on the growth and development of dominant follicles with respect to EB. Perhaps higher fat content in these diets influences the
growth and development of dominant follicles and these effects are modulated by the EB of the cow. Less clear is why cows fed CaLCFA responded so differently to bST or saline for the first wave compared with the second wave of follicles. Results for the first wave follicles are consistent with data from Chapter 6 (i.e., smaller dominant follicles in cows in lower EB). The preovulatory follicle in that study (Chapter 6) grew in the presence of a CL, and it was most likely similar to the first wave follicle presented here. Therefore, these previous findings were validated partially by results from this trial. The preovulatory follicle of cows fed CaLCFA responded to changes in EB only in the presence of bST. The difference in the effects of EB for the first wave follicles compared with the second wave follicle probably relate to the timing of changes in EB experienced by these cows. Clearly the first wave follicle of bST-treated cows grew at a time when mean EB was higher compared with when the second wave follicle grew (i.e., late during the period). Possibly, the reduced EB in bST-treated cows allowed for the detection of EB-by-treatment interactions for the preovulatory follicle. Perhaps the additional gonadotropin support after day 12 standardized the growth and development of all preovulatory follicles in saline-treated cows regardless of EB. In contrast, for bST-treated cows, in lower EB, secretion of gonadotropins may have been compromised in cows in extremely low or negative EB (Imakawa et al., 1987b). Therefore, we detected a significant
effect of EB for cows at this time with cows in lower EB having smaller preovulatory follicles.

In conclusion, injection of cows with bST increased the number of class 2 follicles within the first follicular wave. However, these follicles did not move into larger size classes until after the injection of PGF and insertion of the CIDR. Energy balance interacted with bST to determine the magnitude of effects on average number of class 2 follicles per cow. This may relate to releasable IGF-I in response to GH injection. Feeding CaLCFA resulted in an increase in the size and number of largest follicles on the ovary, and dietary formulation with CaLCFA determined this effect. Cows fed CaLCFA and in the lowest EB had the highest frequency of class 4 follicles. Size of dominant follicles was not affected directly by bST treatment. However, EB interacted with treatment to determine the size of dominant follicles in cows fed CaLCFA. Dominant follicles in control fed cows were unresponsive to the effects of EB. These results indicate that 1) bST has effects on the growth and development of follicles in the cow, 2) EB can modulate many of the effects of bST and 3) diet composition (with or without CaLCFA) appears to be a significant determinant of the growth and development of follicles in the cow. Therefore, continued research utilizing in vitro approaches and targeting the recruited population of follicles as well as the dominant ovarian follicle seems to be warranted.
CHAPTER 8
GENERAL CONCLUSIONS

The study of ovarian function in the postpartum dairy cow involves integration of the disciplines of nutrition, energy metabolism, lactation, and reproductive physiology. Dairy cows have undergone intensive genetic selection for improved milk production in the past 30 years (Smith, 1986). This has resulted in tremendous gains in average production per cow and represents one of the best examples of improved animal performance through genetic selection in animal agriculture. These gains have been combined with better nutritional management allowing for the full phenotypic expression of milk production traits. Even with current levels of production, higher yields per cow can be expected with new advances in biotechnology and growth factor biology which could result potentially in an immediate 10% improvement in animal productivity.

The trend towards higher producing cows represents an important challenge to reproductive physiologists. In response to the energetic demands of early lactation, dairy cows must mobilize energy from body tissue reserves to compensate for their inability to consume sufficient energy in feed (Bauman and Currie, 1980). Therefore, instead of simply limiting production to the amount of feed energy
which they ingest, dairy cattle will produce milk up to their genetic potential at the expense of body tissue. Cows mobilize adipose tissue, sometimes in excess of 50 kg (Chapter 2), during early lactation and are in negative energy balance (EB) because they are consuming less energy than they are utilizing. During or immediately after the period of negative energy balance, dairy farmers are attempting to breed cows for the subsequent lactation. Loss of body weight is antagonistic to reproductive performance in most species (Richards et al., 1989ab) and dairy cattle are not unique in this respect (Chapter 2). Therefore, the relationship between negative EB and reproduction represents an important problem for study in dairy cattle. The purpose of the experiments described in this dissertation was to study how energy metabolism, nutrition, and ovarian function are tied together in the postpartum cow, and to explore potential mediators of their interactions.

One good indicator of postpartum ovarian function is the interval from calving to first ovulation. Postpartum anestrus (lack of follicular growth leading to ovulation) was found to occur in those cows consuming the least dry matter and producing the least amount of milk (Chapter 2). These findings are contrary to the general belief that high producing cows are susceptible to anestrus during the postpartum period (Butler et al., 1981). Actually, those cows not consuming sufficient energy were most likely to be
anestrus. Therefore, feeding strategies to improve interval to first ovulation should be aimed at providing the best quality forages and concentrates to maximize energy intake in the early postpartum period. Primiparous cows studied in Chapter 2 lost more body weight and were in greater negative EB than multiparous cows. Furthermore, primiparous cows had an interval to first ovulation that was considerably longer than that for multiparous cows. One hypothesis for these results is that the severity of negative EB experienced by the primiparous cows resulted in a delayed interval to first ovulation. Therefore, primiparous cows, as a group, were highly susceptible to postpartum energy deficiency. In the future it may be necessary to specifically target the feeding of primiparous cows to insure that first ovulation occurs prior to initiation of the breeding period (40 days postpartum).

The effect of EB on the populations of ovarian follicles was a major question explored in this dissertation. It appears from the work presented in Chapters 4, 6, and 7, that ovarian follicular populations of cattle are responsive to changes in EB. Cows in negative EB had a greater number of small ovarian follicles, and fewer large ovarian follicles during the early postpartum period, (Chapter 4). In contrast, those cows in positive EB had fewer small follicles and more large follicles indicating that small follicles were moving into larger size classes. This may lead to earlier postpartum
ovulation in cows in positive EB because these larger follicles represent a pool of potential ovulatory follicles. Later during the postpartum period, preovulatory follicles grew slower in cows placed into negative EB (Chapter 6). An interesting question that remains to be answered is whether slower growing follicles, which may occur as a consequence of negative EB, are able to trigger endocrine events associated with estrus and ovulation and release oocytes of normal fertility. This is an obvious question that should be addressed in the future.

The exact mechanism by which EB determines the growth and development of follicles currently is being elucidated. Based on the work presented in this dissertation and work by numerous other researchers (see reviews by Butler and Smith, 1989; Short et al., 1990) three different axes, through which EB affects the ovary, may be hypothesized. First, there appear to be direct effects of EB on the secretion of gonadotropins from the pituitary. In the early postpartum cow (Chapter 5), significant correlations were detected between EB, luteinizing hormone (LH) secretion, and the size of the largest follicle on day 10 postpartum. In some manner, the hypothalamus and/or pituitary gland are responding to changes in EB. It has been hypothesized by others that insulin (Baskin et al., 1987), through diffusion into the cerebral spinal fluid, is signaling higher brain centers and integrating animal energetics with brain function. Concentrations of
insulin in the blood of the postpartum cows are low, but were responsive to changes in EB (Chapter 4) which makes the hormone a good candidate for these actions. However, a direct effect of insulin in the hypothalamus on the secretion of LH by farm animals has not yet been demonstrated. Therefore, the action of insulin on gonadotropin secretion remains an open scientific question.

The second hypothetical axis by which EB affects the development of ovarian follicles is through changes in the concentration of growth factors in the blood. There is good clinical evidence that the concentration of insulin in the blood dramatically affects the growth of ovarian follicles (Poretsky and Kalin, 1987). In addition, across several species (including mice [Eisen et al., 1973; Eisen et al., 1978], sheep [Zavy et al., 1989], and cattle [Echternkamp et al., 1990]), multiple or enhanced ovulation rates were associated with elevated IGF-I in the blood, which suggests that this potent ovarian growth factor (Adashi et al., 1985a) can affect the number of follicles recruited and ovulated. The results presented in this dissertation support the concept that growth factors in the blood will influence the growth of ovarian follicles. First, when cows were placed into negative EB (Chapter 6) the preovulatory follicles grew at slower rates. In addition, concentrations of IGF-I in plasma of these cows declined. A decrease in IGF-I in association with negative EB was not unexpected (Gluckman et al., 1987).
However, we found a high correlation between the concentration of IGF-I in plasma and follicular fluid from large follicles in these cows. Therefore, changes in concentrations of growth factors in the blood were associated with changes in growth factors in follicular fluid which may have acted to alter the growth rate of preovulatory follicles on the ovary. An alternative explanation for these results is the paracrine regulation of growth factor secretion by granulosa cells. Perhaps, changes in energy balance may affect both the systemic and paracrine (within the follicle) secretion of IGF-I. This possibility needs to be explored in the future may represent an exciting new area of postpartum research.

Additional evidence supporting direct affects of growth factors on the function of the ovary were found when recombinant bovine somatotropin (bST or growth hormone; Chapter 7) was administered to lactating cows. Bovine somatotropin caused an increase in the number of 6 to 9 mm follicles on the ovary. This represents a stimulation of the pool of ovarian follicles undergoing active recruitment early during a follicular wave. Therefore, an increase in concentration of bST in the blood changed patterns of ovarian follicular development. One interesting finding from this study was that the follicular dynamics of lactating cattle treated with bST were similar to that for nonlactating cattle. This suggests that the growth and development of follicles in lactating dairy cows may be limited by growth factors in the
blood. These additional growth factors may either be present in the blood of nonlactating cows or may not be necessary for the maximal growth and development of follicles in the nonlactating cows.

The third axis by which EB may influence the growth and development of follicles is through effects on the concentration of glucose, nonesterified fatty acids, or additional unknown hormones or metabolites in the blood. This may have direct or indirect (i.e., via gonadotropins) effects on the ovary. The experiments presented in this dissertation were not designed to examine this axis directly. However, in the dairy cattle studied, concentrations of glucose in the blood were low and not responsive to changes in EB (see Chapters 4 and 6). The exact role of identified or unidentified hormones or metabolites in the function of the pituitary, hypothalamus, ovary, and/or uterus represents an exciting area of future research.

Several practical feeding strategies may improve ovarian function in the postpartum cow. First, feeding cows more energy (i.e., providing fat in the diet) and reducing negative EB may prove to be beneficial to postpartum reproduction (Ferguson, 1988). The effect of including calcium salts of long chain fatty acids (CaLCFA) in diets of postpartum cows was examined in Chapters 5 and 7. Fat feeding may achieve the additional goal of enhancing the postpartum concentration of prostaglandin \( F_{2\alpha} \) (PGF) in peripheral by improving the content
of fatty acid precursors of PGF in the ration (see Chapter 3). When a soybean oil emulsion was infused into heifers, the concentrations of PGF metabolite (PGFM) increased in the blood and ovarian follicular growth was greater (Chapter 3). However, when CaLCFA were fed to postpartum dairy cows, the population of ovarian follicles changed, but no effect on PGFM was detected. The effect of CaLCFA on follicular populations was especially obvious between 40 and 60 days postpartum when the incidence of large follicles (>15 mm) increased in CaLCFA-fed cows. This may be a concern to the producer because large estrogen active follicles on the ovary may antagonize maternal recognition of pregnancy (Thatcher et al., 1989a). This could act to depress fertility in certain herds. In fact, overall fertility was reduced in one herd fed CaLCFA (Chapter 2).

After analysis of the data from the experiment presented in Chapter 4, it was not clear whether the CaLCFA-diet itself or the additional dietary energy provided in the diet had affected the population of ovarian follicles. Therefore, the final study (Chapter 7) was designed to specifically test whether CaLCFA themselves or additional energy provided in certain CaLCFA diets were critical to the effects of CaLCFA on the population of ovarian follicles. Interestingly, energy, CaLCFA, and diet composition seemed to be causative factors in the development of large follicles on the ovaries of cows fed CaLCFA. Indeed, cows developed additional large follicles in response to one but not both of the CaLCFA rations which were
formulated. This suggested that the formulation of the diet was critical to the previous effects which were observed. However, several other aspects of reproductive physiology, including the effects of EB, were similar in cows fed different CaLCFA rations. Therefore, it appears that there are some general effects of CaLCFA with respect to EB, while other effects of CaLCFA (i.e., increased incidence of large follicles on the ovary) are only manifested in certain diets.

Based on the results presented in this dissertation, there appear to be measurable effects of EB on the growth and development of follicles in postpartum dairy cows. These effects may be mediated by several mechanisms including changes in circulating growth factors (i.e., the IGF's and insulin). Feeding strategies for postpartum cows, and more specifically CaLCFA, affected the population of ovarian follicles. Future scientific research should explore the effects of growth factors and diet on the granulosa and thecal cells which comprise the ovarian follicle. In this manner, the basic molecular mechanisms responsible for the physiological and endocrinological results described in this dissertation may be elucidated. These studies will provide basic knowledge for the scientific community as well as practical knowledge for animal agriculture, especially the dairy industry. Through these lines of research, the ultimate goal of improved postpartum reproductive efficiency may be realized through nutritional management.
REFERENCES


growth factor I as an enhancer of androgen biosynthesis by cultured rate ovarian cells. Endocrinology 122:1603.


McNeilly, A. S. 1985. Effect of changes in FSH induced by bovine follicular fluid and FSH infusion in the preovulatory phase on subsequent ovulation rate and corpus luteum function in the ewe. J. Reprod. Fertil. 74:661.


factor preparations on luteinizing hormone receptor induction in granulosa cell cultures. Biol. Reprod. 30:603.


cyclic cow: dependence upon the period of the cycle. Endocrinology 107:498.


Schallenberger, E., D. Schams, and K. Zottmeir. 1978. Response of lutropin (LH) and follitropin (FSH) to administration of gonadoliberin (GnRH) in pregnant and postpartum cattle including experiments with prolactin suppression. Theriogenology 10:35.


Tanabe, T. Y., J. F. Hokanson, and L. C. Griel. 1968. Minimal exogenous progesterone requirements for maintenance of


Umphrey, J. 1988. Effects of whole cottonseed, Megalac, or the combination on lactational performance, digestibility of nutrients and relative levels of selected hormones in response to a glucose challenge of dairy cows during summer months. M. S. Thesis. Auburn University, Auburn, AL.


BIOGRAPHICAL SKETCH

Matthew Christian Lucy, son of Russell and Evelyn Lucy, was born on June 4, 1960, in Syracuse, New York. He attended elementary and secondary schools at Baldwinsville, New York, graduating from C. W. Baker High School in 1978.

He attended Cornell University from September, 1978, to December, 1981, and received his Bachelor of Science degree in animal science from the College of Agriculture and Life Sciences. While a student at Cornell, he met Jacqueline S. Jamieson whom he married in August, 1984.

He enrolled in graduate school at Kansas State University in the Department of Animal Science and Industry in the fall of 1983. He served as a graduate research assistant while fulfilling the requirements for the Master of Science degree which he completed in August, 1985. His master's thesis was entitled "Control of Intervals to First Service and Attempts to Improve Fertility in Dairy Cattle Using Prostaglandin F2α and Gonadotropin-Releasing Hormone."

In September, 1987, he began his doctoral program under the direction of Dr. William W. Thatcher in the Department of Dairy Science at the University of Florida, Gainesville. He completed his dissertation and was awarded the degree of Doctor of Philosophy in December, 1990.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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