CONTROL OF OVARIAN FOLLICULAR GROWTH AND STEROIDOGENESIS IN NUTRITIONALLY ANESTROUS BEEF COWS

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NOMENCLATURE

ANOVA	analysis of variance
BW	body weight
CL	corpus luteum
FFL	follicular fluid
FSH	follicle stimulating hormone
g	gram
GLM	general linear model
GnRH	gonadotropin-releasing hormone
h	hour
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
kg	kilogram
LH	luteinizing hormone
μg	microgram
min	minute
mm	millimeter
mL	milliliter
ng	nanogram
RIA	radioimmunoassay

CHAPTER I

INTRODUCTION

The largest contributor to reproductive inefficiency in beef cows is animals that are noncyclic during the breeding season. When cattle are subjected to conditions that alter their reproductive cycles and subsequent pregnancy, proficiency and profitability are lost. Folliculogenesis, or ovarian follicular growth, is essential to ensure that normal estrous cycles in cattle will occur. The events leading to ovulation of the mature follicle are dependent upon the secretion of gonadotropin-releasing hormone (GnRH) to stimulate release of the gonadotropins from the anterior pituitary. The response of the ovarian thecal and granulosa cells to the stimulation of LH and FSH and their interaction with growth factors such as insulin-like growth factor-I, and the subsequent production of ovarian steroids are also dependent on GnRH (Hansel and Convey, 1983; Guilbault et al., 1993; for reviews see, Fortune, 1994; Spicer and Echternkamp, 1995).

Nutritional status and dietary composition play an essential role in the physiological control of cattle, especially in the regulation of the reproductive processes (Wiltbank et al., 1962; Selk et al., 1988; Richards et al., 1986). Small adjustments in the diet do not appear to cause any critical problems, however, reproductive performance in cattle may be reduced if animals are subjected to nutritional levels that are severely restricted. How these changes in nutrition seem to alter the factors that influence reproduction is unclear. However, changes in the concentration of numerous hormones and metabolites in systemic blood during underfeeding have been documented

(Richards et al., 1991, McGuire et al., 1992; Richards et al., 1995).

Because reproductive efficiency is the main factor that limits overall production efficiency, it is crucial that the ovarian processes that control cyclicity and follicular growth be understood in an effort to assess and/or improve reproductive management. The exact mechanism(s), however, by which the reproductive process is affected by nutrition has not been completely characterized. Therefore, the objectives of this study were to determine the effects of pulsatile and continuous infusion of exogenous GnRH on the initiation of estrous cycles, uterine and ovarian weights, numbers and size of ovarian follicles, and concentrations of estradiol, androstenedione, progesterone, and insulin-like growth factor-I in the follicular fluid of nutritionally anestrous cows.

CHAPTER II

REVIEW OF LITERATURE

OVARIAN FOLLICULAR FUNCTION IN CATTLE

Ovarian follicular growth

One of the main functions of the mammalian ovary is to maintain continuous folliculogenesis. Folliculogenesis is defined as the development of small follicles which grow into larger, graafian follicles that ultimately result in ovulation. This sequence of events is essential for the reproductive process to take place. Follicular development and ovulation are regulated by a combination of interactions between hormones, growth factors, cell to cell communication systems and gene products (Roche and Boland, 1991).

Throughout the estrous cycle of cattle, ovarian follicles grow, regress and are continuously replaced by other large follicles (Spicer and Echternkamp, 1986). Follicles can be divided into three classes of sizes based upon the growth changes observed in the follicular epithelium. These classes include: 1) primordial or primary follicles, 2) growing follicles, and 3) graafian follicles. Primordial, or small follicles, are formed in the ovary during embryonic development (for review see, Hansel and Convey, 1983), average about 133,000 at birth (Erickson, 1966), and do not change in number after birth until about 14 years of age at which time primordial follicle numbers begin to decline in cattle (Erickson, 1966). Therefore, primordial follicles may exist in a

resting state for many years in the ovarian cortex without showing any signs of growth. The growing follicles originate from the pool of primordial follicles but do not have a developed antrum or theca layer. As the follicle continues to grow, two layers from surrounding connective tissue of the stroma form around the follicle. These layers are referred to as the theca externa and theca interna (for review see, Hisaw, 1947). Once the follicle develops an antrum it is considered a tertiary follicle which continues to grow and form a graafian follicle that is capable of ovulating (Rajakoski, 1960).

During the past three decades, controversy has existed with regard to the pattern of antral follicular growth that occurs during repetitive estrous cycles in cattle (for review see, Spicer and Echternkamp, 1986). The two most popular theories that developed during this time were: 1) continuous follicular growth and regression throughout the estrous cycle with one wave (i.e., one increase in the numbers of large graafian follicles) occurring during the last half of the cycle (Choudary et al., 1968; Dufour et al., 1972), and 2) follicular growth and regression occurring in two sequential waves, one during the first week of the estrous cycle and the second starting about the twelfth day of the cycle after the first wave has become atretic (Rajakoski, 1960).

More recently, transrectal ultrasound evaluation of ovarian structures in cattle has provided an accurate, sequential record of ovarian activity and follicle growth, therefore revealing whether the turnover of ovarian follicles is continuous or whether waves of follicular growth occur at specific times during the estrous cycle (Sirois and Fortune, 1988; Driancourt et al., 1991; Beal et al., 1992). Specifically, use of transrectal ultrasonography to monitor follicular dynamics in cattle has allowed identification of one-, three- and four-wave follicular growth patterns (Sirois and Fortune, 1988; Adams et al., 1992; Adams et al., 1994; for review see, Fortune, 1994) in addition to the two-wave pattern initially proposed by Rajakoski (1960) and subsequently by Ireland and Roche (1982). Follicular growth may occur via a growth pattern that includes one to four follicular waves which will vary from study to study

(Table 1). On average, 53% of the animals studied had two waves versus 45% for three waves, with the remaining 2% of cows having either one or four waves (Table 1). Interestingly, dietary intake influenced the proportion of cattle with two versus three waves and thus may account for the variation that occurs between studies (Murphy et al., 1991). In animals exhibiting three-waves of follicular growth, follicles begin growing around days 2, 9, and 16 of the estrous cycle, whereas, in animals exhibiting a four-wave follicular growth pattern, follicular growth begins around 2, 8, 14, and 17. This is in contrast to the two-wave pattern where follicular growth begins on approximately days 3 and 11 (Sirois and Fortune, 1988; for review see, Fortune, 1994).

During each follicular wave, one follicle is selected and becomes larger, thereby, "dominating" the other subordinate follicles. The dominant follicle from each follicular wave continues to grow and differentiate presumably, in an attempt to prepare for ovulation. Although not well characterized biochemically, the selected follicle is functionally different from its sister follicles due to the fact that it secretes increased amounts of estradiol (Ireland and Roche, 1983; Guilbault et al., 1993). Once the dominant follicle has been selected, it apparently prevents further growth and differentiation of the other subordinate follicles and has been hypothesized to prevent further follicular recruitment due to its increased production of inhibins and estradiol (Sirois and Fortune, 1988; Martin et al., 1991; Guilbault et al., 1993; Adams et al., 1994; for review see, Fortune, 1994).

Ovarian follicular steroidogenesis

The ovary is capable of producing many hormones, several of which are classified as steroid hormones. Steroid hormones are responsible for many physiological actions in target tissues including cell division, tissue differentiation, synthesis of proteins, and contraction of smooth muscle (Hafez, 1993). Ovarian follicular fluid is a

rich source of the steroid hormones that are produced by the ovary. Follicular fluid concentrations of steroids vary widely between follicles and the intrafollicular environment of steroids is proposed to be an important regulator of follicular development (Henderson et al., 1982). The steroid hormones that have been identified in bovine follicular fluid include: pregnenolone, progesterone, 20β-hydroxy-pregn-4-en-3-one, 17α-hydroxyprogesterone, androstenedione, testosterone, estrone, and estradiol-17β (Short, 1962). Of the steroids listed above, estradiol-17β, androstenedione and progesterone are synthesized in the greatest concentrations (Fortune and Hansel, 1985).

The primary estrogen found in bovine ovarian follicular fluid is estradiol-17β. Estradiol, along with luteinizing hormone (LH) and follicle stimulating hormone (FSH), have roles in the growth and development of follicles and are also important for the differentiation of granulosa cells (Merz et al., 1981; for review see, Richards and Hedin, 1988). The increased capacity of follicles to secrete estradiol seems to be due to an increase in the ability of theca cells to respond to LH by secreting androgens and to the ability of granulosa cells to aromatize the androgens to estradiol (McNatty et al., 1984a; for review see, Fortune, 1994;). Just prior to estrus, follicular estrogens begin to increase indicating that the large "estrogen-active" follicles are responsible for most of the estradiol that is being secreted at this point in the estrous cycle (England et al., 1973; Dieleman et al., 1983b; Ireland et al., 1984; McNatty et al., 1984a). In the cow, the synthesis of ovarian estrogens is thought to be regulated by circulating concentrations of FSH as FSH stimulates aromatase activity in cultured bovine granulosa cells (Spicer et al., 1994a), and increases the percentages of estrogen-active follicles if administered during the first 3 days of the estrous cycle (Goulding et al, 1991). In addition, large follicles tend to have greater concentrations of estradiol than smaller follicles (Short, 1962; Ireland et al., 1979; Staigmiller et al., 1982; Henderson et al., 1982; Spicer et al., 1988; Spicer and Enright, 1991) and estrogen levels are

increased in the large, preovulatory follicles that are present at estrus (Martin et al., 1991; Rutter and Manns, 1991; Einspanier et al., 1993) while the large, atretic follicles contain only low levels of estradiol and lack gonadotropin binding sites (Merz et al., 1981; Guilbault et al., 1993). The increased estradiol production that occurs in large follicles *in vivo*, also occurs *in vitro* (Staigmiller et al., 1982; Kruip and Dieleman 1989). Concentrations of steroids, specifically androstenedione and estrone, in follicular fluid were several-fold higher before the LH surge than during the luteal phase (Dieleman et al., 1983a), indicating that preovulatory follicles are more steroidogenically active than the larger luteal phase follicles. Thus, atresia and lack of LH receptors in follicular granulosa cells appears to be related to decreased estradiol production by the follicle (Merz et al., 1981; Guilbault, et al. 1993), but the hormonal factors controlling these events remain to be determined. Follicular estrogens begin to rapidly decrease at the time of the LH surge after estrus due to luteinization of granulosa and theca cells (Fortune, 1986; for review see, Fortune, 1994).

In addition to estrogens, androgens are involved in the regulation of follicular growth and steroidogenesis. Because androgens are the precursors of estrogens, changes in the concentrations of ovarian androgens could reflect periods of follicular development during the bovine estrous cycle. The primary androgen produced by the bovine ovary is androstenedione. In a study conducted by Henderson and coworkers (1982) androstenedione concentrations decreased significantly as follicle size increased whereas estradiol concentrations were significantly increased. Their results indicate that as follicle size increases, the steroid environment of follicular fluid changes from being predominately androgenic to predominantly estrogenic. Additionally, it appears as if follicular growth waves may correspond to the rise and fall of ovarian androgen secretion, as measured in ovarian (Wise et al., 1982) and jugular (Kanchev and Dobson, 1976) vein plasma, during different days of the bovine estrous cycle. In the cow, the synthesis of ovarian androgens is thought to be regulated by the circulating

concentrations of LH. This became evident in a study by McNatty et al. (1984b) where LH treatment caused a 3- to 5-fold increase in thecal androstenedione production.

The theca and granulosa cells located in the ovary are responsible for the dynamic process of ovarian steroidogenesis. The interaction that exists between the two cell types is explained by the "two-cell" theory. According to this theory, two different cell types are dependent upon one another in order to produce their products. In the case of the ovary, this relationship is evident in the production of estradiol. Here, androgens, such as androstenedione and testosterone, are produced and released from one cell type, the theca, and diffuse to the membrana granulosa where they are aromatized to estradiol by a second cell type, the granulosa (for reviews see, Richards, 1980; Richards and Hedin, 1988). Theca cells do not have the capability to aromatize the androgens that they produce, whereas granulosa cells have the ability to aromatize androgens to estradiol but they lack the ability to produce androgens. In addition, granulosa cells enhance the ability of the theca cells to produce androstenedione by supplying them with progestin precursor (Fortune 1986). The synthesis of androstenedione takes place via the pathway of 17α-hydroxylase cytochrome P450 and through the aromatase cytochrome P450 pathway following the sequence of cholesterol → pregnenolone → 17α-hydroxypregnenolone → dehydroepiandrosterone → androstenedione → testosterone or estrone → estradiol. This sequence of conversions is referred to as the "Δ^5-pathway" and is the favored pathway for androgen conversion in the bovine as evidenced using cell culture experiments in which pregnenolone was found to be the preferred substrate for androstenedione synthesis by theca cells (Short, 1962; Lacroix et al., 1974; Dieleman et al., 1983a; Fortune, 1986; Cupps, 1991) Androgen conversion from 17α-hydroxypregnenolone occurs through cytochrome P450 activity in the thecal cells (Rodgers et al., 1986; Hinshelwood, 1993); this enzyme is absent in granulosa cells. The enzyme 17,20 lyase is responsible for the carbon cleavage that is necessary to form androstenedione and is present in thecal cells only, whereas the

granulosa cell possess the aromatase enzyme that is needed for conversion of androstenedione to estradiol (Cupps, 1991). Cell culture experiments have also confirmed that concentrations of estradiol similar to those found in preovulatory follicles prior to the LH surge, inhibit the secretion of progesterone by bovine theca and granulosa cells as compared to control cultures (Fortune and Hansel, 1979; Langhout et al., 1991). In turn, the androgens produced by the thecal cells enhance the ability of the granulosa cells to make pregnenolone (Fortune, 1986) although testosterone has no effect on progesterone production by bovine granulosa cells *in vitro* (Henderson and Franchimont, 1983; Spicer et al., 1993).

Because granulosa cells are the principal site of follicular androgen aromatization, with estradiol being the major product, an increase in granulosa cell numbers and/or aromatase activity could account for the decline in follicular fluid concentrations of androstenedione and the increase of estradiol concentration that is associated with increased follicle size (Henderson et al., 1982). Likewise, McNatty et al. (1984a) proposed that follicular fluid obtained from follicles measuring 2 - 4.5 mm during proestrus contained high concentrations of testosterone and androstenedione and low concentrations of estradiol; they also discovered that small follicles possess low aromatase activity in granulosa cells but do, however, have an LH-responsive theca. These results would indicate that early developing antral follicles have the ability to synthesize androgen before sufficient aromatase activity is present in the granulosa cells. Therefore, the increasing concentration of estradiol, via conversion of androgen in follicles larger than 4.5 mm, is likely a result of the activation of the aromatase activity in granulosa cells. Luteinizing hormone is responsible for the cytochrome P450 17,20 lyase activity in theca cells whereas FSH is responsible for aromatase activity in the granulosa cells (Cupps, 1991).

Progesterone is the primary progestin found in ovarian follicular fluid. Both the theca interna and granulosa cells are capable of secreting progesterone, however, in

experiments conducted by Fortune and Hansel (1979), progesterone production was more abundant in granulosa cell preparations. During the preovulatory sequence of follicular development, the endocrine system quickly changes to become more suited for fertilization and the subsequent pregnancy. A preovulatory decrease in follicular fluid estrogen concentrations occurs during this time while there begins to be an increase in progesterone concentrations in bovine follicular fluid at about 14 to 20 hours after the LH surge (Wise et al., 1986). Concentrations of follicular fluid progesterone vary between size of follicles, day of the estrous cycle, and coexistence with corpora lutea (Spicer and Geisert, 1992; Takagi et al., 1993). Concentrations of progesterone are greatest between days 5 to 10 of the cycle and decrease dramatically between days 18 to 20 in small follicles. In medium follicles, concentrations increase 4-fold from days 1 to 10 and then remain elevated for the rest of the estrous cycle, and concentrations of progesterone are greater in large follicles from days 1 to 10 than from days 11 to 20 (Takagi et al., 1993). Overall, there are more progestins present in the follicular fluid of small follicles than in medium-sized follicles and large follicles (Ireland et al., 1979) and in large follicles on the ovary ipsilateral to a functional CL (Spicer and Geisert, 1992). Follicle-stimulating hormone plays an important role in progesterone synthesis by granulosa cells (Spicer et al., 1993) whereas LH stimulates thecal cell progesterone synthesis (McNatty et al., 1984b). Therefore, increased gonadotropin secretion ensures maximal steroidogenesis by the developing follicle by increasing androgen precursors (progesterone, via P450 side-chain cleavage) coincidently with stimulation of aromatase activity and 17,20-lyase activity.

Ovarian insulin-like growth factor system

Insulin-like growth factors (IGFs) are a family of small homologous, single chain polypeptides that are structurally and functionally similar to insulin. Two different

forms of IGFs have been isolated in the bovine, designated as IGF-I and IGF-II (McGuire et al., 1992; for review see, Spicer and Echternkamp, 1995). The IGFs were originally termed somatomedins (mediators of the effects of somatotropin) because they appeared to be involved in the somatogenic actions of growth hormone (for review see, Jones and Clemmons, 1995). This family of peptides was believed to include compounds designated as somatomedins A, B, and C, basic somatomedin, non-suppressible insulin-like activity (NSILA) somatomedin and multiplication-stimulating activity (MSA) somatomedin. Purification and structural determination has revealed that somatomedins A and C, basic somatomedin, and NSILA are all the same peptide and are now referred to collectively as IGF-I. MSA was later identified as IGF-II (for review see, Baxter, 1988). In cattle, the IGFs are thought to be important mediators of lactation and reproduction, in addition to growth (for reviews see, Davis, 1988; McGuire et al., 1992). The liver is believed to be the primary source of circulating IGF-I although many tissues, including the ovary, are capable of producing IGF-I (Spicer et al., 1993; for review see, Spicer and Echternkamp, 1995). Thus, the IGFs are able to exert their action through endocrine, autocrine, and/or paracrine mechanisms. The IGF autocrine/paracrine system is composed of IGF-I and IGF-II, specific receptors for the IGFs and a family of proteins known as insulin-like factor binding proteins (IGFBPs) which regulate the availability of the IGFs to their target cells (for review see, Giudice, 1992; Jones and Clemmons, 1995).

Follicular fluid is a particularly rich source of IGF in ovarian tissue. Concentrations of IGF-I are greater in follicular fluid from gonadotropin-stimulated and large follicles than in small or medium-sized follicles indicating a positive correlation between follicular diameter and concentrations of IGF-I (Hammond et al., 1991; Spicer and Enright, 1991; Einspanier et al., 1993). Ovarian granulosa cells from a number of species, including cattle, secrete and respond to IGF-I (Spicer et al., 1993; for review see, Spicer and Echternkamp, 1995), thereby, allowing IGF-I to influence an array of

processes within the ovary. Although FSH and LH were once thought to be the primary regulators of cyclic ovarian follicular development, it now seems as if locally-produced substances, such as IGF-I and inhibin, may also play a role in this regulatory process (Webb et al., 1994). Insulin-like growth factors have direct effects on cultured ovarian cells including: stimulation of granulosa cell mitogenesis, granulosa and luteal cell progesterone production, and thecal cell androgen production (Hammond et al., 1991; for reviews see, Giudice, 1992; Spicer and Echternkamp, 1995). These studies provide evidence that IGF-I may exert local effects on bovine follicular function, however, a systemic effect of IGF-I on follicular function is also possible.

As mentioned, insulin-like growth factor-I has been found to affect steroid production in the ovary. It is able to stimulate estradiol production by ovarian granulosa cells to some degree but does not appear to be as potent a stimulator of estradiol production as is insulin (Spicer et al., 1993; Spicer et al., 1994a). Also, it has been determined that IGF-I is inhibitory to insulin-stimulated estradiol production by granulosa cells of both small and large bovine follicles (Spicer et al., 1994a). In contrast, IGF-I consistently stimulates progesterone production by granulosa cells (Schams et al., 1988; Spicer et al., 1993) and its effect is much more potent than the stimulatory effects of insulin. Furthermore, FSH treatment enhances the stimulatory effect of IGF-I on progesterone production (Spicer et al., 1993). Additionally, physiological concentrations of IGF-I had no effect on basal androstenedione production in cattle, however, it did act to synergistically enhance LH-induced androstenedione production by thecal cells from large ($\geq$ 8 mm) bovine follicles (Stewart et al., 1995). Collectively, these studies provide evidence that IGF-I, acting with gonadotropins, is able to promote thecal cell androgen production. Follicle size also appears to be an important factor in the regulation of interactions between IGF-I and the gonadotropins since FSH and LH are able to enhance the mitogenic effect of

IGF-I in granulosa cells from small (< 5 mm) but not large (> 10 mm) bovine follicles (Gong et al., 1993).

Nutrient intake and nutritional status are as important as growth hormone (GH) in controlling the ovarian and systemic concentrations of IGFs. Animals receiving a restricted diet have decreased concentrations of IGF-I as compared to those on a diet to maintain body weight despite increased GH concentrations (Ronge and Blum, 1989; Elsasser et al, 1989; Breier and Gluckman, 1991; Richards et al., 1991; McGuire et al., 1992; Thissen et al., 1994; Richards et al., 1995). Changes in nutrition, such as those seen in animal production systems, appear only to have minimal effects on circulating IGF while strict feed restriction, such as fasting, may cause concentrations of IGF-I in plasma to decline nearly 80%, possibly due to a decrease in the number of GH receptors in the liver (Breier and Gluckman, 1991; McGuire et al., 1992). Insulin-like growth factor-I concentrations are, however, able to rebound to normal circulating levels after realimentation (Merimee et al., 1982; Richards et al., 1995). Dairy cows in negative energy balance had lower serum IGF-I concentrations than did cows in positive energy balance even though their dry matter intake was similar (Spicer et al., 1990). Additionally, circulating concentrations of IGF-I were reduced in growing cattle when they were severely underfed (Breier, et al., 1986) . In contrast, other studies have reported that reduced intake does not reduce concentrations of IGF-I. Spicer et al, (1991) indicated that IGF-I concentrations in plasma and follicular fluid were not reduced in heifers that were fed a moderately restricted diet to lose body weight for 10 weeks (ADG = -.31 kg/d) compared to heifers fed to gain body weight (ADG = .96 kg/d). In a subsequent study, however, Spicer et al., (1992) found that short-term fasting for 48 but not 24 hours significantly decreased plasma IGF-I concentrations without affecting intraovarian IGF-I concentrations in heifers. Therefore, nutritional regulation of IGF-I, in both serum and follicular fluid, is probably dependent upon the severity and the length of nutrient restriction.

The IGFBPs are high affinity, soluble carrier proteins that circulate in the blood and extracellular fluids. Insulin-like growth factor binding proteins, like IGFs, are primarily produced by the liver, however, most other tissues can also produce IGFBPs. Functions of IGFBPs include: 1) acting to transport proteins in plasma and to control the efflux of IGFs from the vascular compartment, 2) extending the half-lives of IGFs and regulating their metabolic clearance, 3) providing a means of tissue- and cell type-specific localization, and 4) directly regulating the interaction of the IGFs with their receptors and therefore indirectly controlling biological actions (Bourner et al., 1992; for review see, Jones and Clemmons, 1995). The IGFBPs are thought to exert both stimulatory and inhibitory effects on the actions of IGF-I. The actions of the IGFBPs seem to be dependent on the type and concentration of IGFBP, the duration of exposure, and whether IGFBPs are associated with cellular membranes (Froesch et al., 1985; Hossner et al., 1988; Rosenfeld et al., 1990; Clemmons and Underwood, 1991; Thissen et al., 1994; for reviews see, Giudice, 1992; Cohick and Clemmons, 1993; Rechler, 1993). One important role of IGFBPs in the autocrine or paracrine actions of IGFs is to keep IGFs secreted by a cell retained locally so that their actions are exerted on the same or adjacent cells (Breier and Gluckman, 1991).

To date, six different IGFBPs have been identified. These binding proteins exist in the hypothalamus, anterior pituitary, serum, and follicular fluid in several species (Clemmons and Underwood, 1991; Funston et al., 1993). Insulin-like growth factor binding proteins-1, -4, and -6 have been identified in both the human and the rat but have not been isolated in cattle. Insulin-like growth factor binding protein-2, IGFBP-3, and IGFBP-5, however, have been found in cattle. Overall, bovine IGFBP-2 is about 89% homologous with the IGFBP-2 of humans and rats, while IGFBP-3 possesses about a 77% homology with the IGFBP-3 found in human, rats, and pigs (Bourner et al., 1992; for review see, Rechler 1993). Insulin-like growth factor binding protein-3 is the major carrier of IGF-I and is most likely responsible for the stability of IGF-I in

blood (Clemmons and Underwood, 1991; Vicini et al., 1991). The IGFBPs are present in both dominant and subordinate follicles of cattle, however, follicular fluid from small follicles appears to have about twice the total IGFBP activity as compared with follicular fluid from large follicles (Echternkamp et al., 1994; Stanko et al., 1994).

Nutrient intake also appears to be a regulator of systemic concentrations of IGFBPs since serum IGFBP-2 and IGFBP-3 change with feed restriction (Breier and Gluckman, 1991; Clemmons and Underwood, 1991; Thissen et al., 1994). Concentrations of IGFBP-2 are at a maximum in dairy cattle during early lactation when cows are in a negative energy balance, whereas concentrations of IGFBP-2 are at a minimum during the dry period when IGF-I is elevated (Clemmons and Underwood, 1991; Vicini et al., 1991; Thissen et al., 1994). Because many factors may be involved in regulating serum and/or follicular fluid levels of IGFBPs, including nutritional status, peptide hormones, proteases, chronologic age, and stage of the estrous cycle (for review see, Rechler 1993), changes in the local production of IGFBPs could have the potential to regulate the availability of IGF to ovarian cells (Hammond et al., 1991; for review see, Giudice, 1992).

Most of the actions of both IGF-I and IGF-II are mediated by IGF receptors. Receptors for the IGFs are present in various types of ovarian cells including granulosa and thecal cells (McArdle et al., 1991; Spicer et al., 1994b). Two types of IGF receptors have been identified, type-1 and type-2 receptors. The effects of IGF-I are mediated by the high affinity type-1 receptors, which are abundant in ovarian granulosa and thecal cells (Spicer et al., 1994b; for review see, Spicer and Echternkamp, 1995). The type-2 receptor, on the other hand, binds only IGF-II and does not cross react with IGF-I or insulin (Breier and Gluckman, 1991; Cohick and Clemmons, 1993; Thissen et al., 1994; for reviews see, Giudice, 1992; Jones and Clemmons, 1995). These studies also indicate that type-1 IGF-I receptors in granulosa cells are hormonally regulated and

increase as small antral follicles develop into graafian follicles (for review see, Spicer and Echternkamp, 1995).

Ovarian follicle gonadotropin receptors

For any hormone to have an effect on its target tissue it must be bound to a receptor. Because the gonadotropins, LH and FSH, are essential to follicular growth, the understanding of the availability and specificity of their receptors is also important. The responsiveness of follicles depends, in part, on the changes in concentration of hormone receptors (or binding sites) in the cellular membranes of the follicles. Therefore, changes in follicular function may be associated with changes in the numbers of gonadotropin receptors within the ovarian follicle (for review see, Spicer and Echternkamp, 1986). Receptors for LH and FSH in the ovary are located in the thecal and/or granulosa cells. FSH binds almost exclusively to granulosa cells of medium and large sized follicles while LH binds to both the thecal and granulosa cells of large follicles (Channing and Kammerman, 1974; Ireland and Roche, 1983). In addition to their presence in the cellular membranes of follicles, FSH and LH receptors are also present in bovine corpora lutea (Rao et al., 1979; Spicer et al., 1981; Manns et al., 1984).

The stage of maturity of follicles determines the number of LH receptors that are present on the granulosa cells. In cattle, concentrations of LH receptors in thecal and granulosa cells from large follicles increase greatly between the onset and peak of the LH surge and decrease just prior to ovulation (Ireland and Roche, 1982; Staigmiller and England, 1982; Staigmiller et al., 1982; Walters et al., 1982b; Ireland and Roche, 1983). Concentrations of FSH receptors in granulosa cells of large follicles, on the other hand, do not change during the estrous cycle (Ireland and Roche, 1983). This finding

suggests that maturation of ovarian follicles is not associated with changes in the concentrations of FSH receptors in granulosa cells as is the case with LH receptors.

Spicer et al. (1986b) also observed that cows injected with GnRH exhibited an increase in LH but not FSH binding sites in large follicles. Additionally, concentrations of FSH receptors were more abundant in estrogen-active than estrogen-inactive follicles, whereas concentrations of LH receptors were greater in estrogen-inactive than estrogen-active follicles. From these results, it was proposed that the combination of increased estradiol production and FSH secretion may have caused the increase that was observed in numbers of FSH receptors in the estrogen-active follicles. The increase in LH receptors in estrogen-inactive follicles in GnRH treated cows indicates that increased numbers of LH receptors are necessary for the increased estradiol production and FSH binding during the development of dominant follicles since the capacity of follicles to secrete estradiol appears to be due to the ability of thecal cells to respond to LH by secreting androgens (McNatty et al., 1984a; for review see, Fortune, 1994). Increased LH binding enhances LH action and is, therefore, able to ensure maximal estradiol production in the developing dominant follicle by increasing concentrations of androgen precursors.

EFFECT OF EXOGENOUS GnRH ON OVARIAN FOLLICULAR FUNCTION

Gonadotropins are required for folliculogenesis. In addition, there are intragonadal factors that act locally and/or systemically to modulate gonadotropin action at the ovarian level (Hammond et al., 1991; Guilbault et al., 1993). Gonadotropin-releasing hormone (GnRH) is necessary for the induction of the preovulatory surge of gonadotropins in most species. It appears that development of pulsatile LH release in

the postpartum cow occurs in response to pulsatile endogenous GnRH secretion and is a requirement for the initiation of ovarian activity. Also, the frequency of pulses may be important in determining the timing of the first ovulation post partum (Peters et al., 1981). In cattle, FSH and LH surges are induced by increasing quantities of GnRH acting upon a sensitized pituitary gland (Hansel and Convey, 1983). Administration of 500 μg GnRH to suckled beef cows approximately 20-30 days post partum and a second injection approximately 10 days later results in follicular growth and apparently normal ovulation and CL development (Webb et al., 1977). Pulsatile injections of 500 ng GnRH at two hour intervals for 96 hours increased gonadotropin secretion and caused a more rapid return to an ovulatory follicle (Walters, et al., 1982). In addition, ovulation was induced within 8 days in 73% of postpartum beef cows as compared to 18% of suckled control cows, however, most treated animals failed to exhibit estrus at the first ovulation (Walters, et al., 1982). Similar results have been reported in suckled postpartum beef cows when utilizing a method of single injection of either 250 or 500 μg GnRH as opposed to pulsatile injection (Webb et al., 1977; Troxel and Kesler, 1984). In contrast, neither follicular growth nor ovulation in postpartum anovulatory beef cows was induced by pulsatile administration of 500 ng GnRH within 4 days of treatment, inspite of the fact that both LH and FSH pulses were increased (Spicer et al., 1986a).

A single injection of 150 μg of exogenous GnRH administered to postpartum anestrous beef cows resulted in increased release of LH compared with control cows and cows subjected to short-term calf removal within 15 minutes after treatment, although, maximum concentrations of LH occurred at 120 minutes post-injection (Smith et al., 1983). Furthermore, 82% of cows injected with 300 μg GnRH 24 hours after calf removal had formed corpora lutea by 24 days postpartum, as opposed to 69% of control cows (Smith et al., 1983). Other studies have documented that GnRH given at two hour intervals almost doubles the frequencies of LH and FSH pulses in postpartum

cows as compared to controls (Walters et al., 1982b; Spicer et al., 1986a). Besides increasing gonadotropin pulses, increased concentration of progesterone precedes increased concentrations of estradiol in follicular fluid of cows treated with GnRH (Spicer et al., 1986b). In addition, multiple, low-dose injections of GnRH increased estradiol concentrations in large follicles after 96 hours in 78% of cows as opposed to 14% of cows injected with saline. This increase in estradiol did not occur, however, after 48 hours of GnRH administration. The increase in estradiol was also associated with increased numbers of LH and FSH binding sites in the theca and granulosa of cows injected with GnRH (Spicer et al., 1986b). Increased pulsatile LH and FSH release, therefore illicits an increase in LH and FSH receptors in the largest follicle which, in turn, generates a response causing increased secretion of estrogen (Roberts and Funston, 1993; for review see, Funston et al., 1995). Therefore, it is apparent that estradiol is needed for the sensitization of the pituitary so that GnRH may stimulate the preovulatory surge of LH and FSH. Without estradiol, the GnRH-induced release of LH and FSH is reduced in cattle (Kesner et al., 1981; Kesner and Convey, 1982).

Gonadotropin-releasing hormone may have a direct effect on the ovary by stimulating steroidogenesis. *In vitro* treatment with 10 or 1,000 ng/mL of GnRH significantly increased progesterone and estradiol secretion by bovine granulosa cells (Sirotkin et al., 1994). Therefore, it was proposed that GnRH could have a stimulatory effect on the enzymes that catalyze the early stage (progesterone production) as well as the late stage (aromatase activity) of steroidogenesis. Although results from a previous experiment indicated that neither bovine follicular nor luteal ovarian tissue possessed GnRH receptors (Brown and Reeves, 1983), GnRH-like proteins have been found to be present in bovine ovarian tissues, but not follicular fluid (Ireland et al., 1988). Additionally, granulosa cells contain greater concentrations of the GnRH-like peptides than do other tissues (Ireland et al., 1988). These results, collectively with the fact that

GnRH treatment induced bovine ovarian steroidogenesis (Sirotkin et al., 1994), indicate that GnRH may have direct ovarian effects in cattle.

Studies to determine the effect of exogenous GnRH administration on concentrations of gonadotropins have also been conducted in nutritionally anestrous cattle. As cows become anestrus, due to depletion of nutrients, they exhibit reduced concentrations of serum LH and fewer pulses of LH (Imakawa, et al., 1986; Richards, et al., 1989). Pulsatile infusion of GnRH stimulates pulsatile LH secretion and resumption of luteal activity in cows that are nutritionally anestrus (Bishop and Wettemann 1993; Vizcarra, 1994). The infusion of GnRH increased the amplitude of LH but not the pulse frequency over control cows infused with saline (Bishop and Wettemann 1993). Additionally, fewer cows infused with saline had luteal activity during or after infusion compared with cows that were given a pulsatile infusion of 2.0 μg GnRH every fourth hour or hourly. There was also a tendency for more cows that received the pulsatile injection every hour to have luteal activity compared with cows receiving a pulsatile injection every fourth hour. Furthermore, more of the cows injected every hour than every fourth hour maintained luteal activity during and after the infusion period (Bishop and Wettemann, 1993). In a similar study, Vizcarra (1994) discovered that treatment of nutritionally anestrous cows with GnRH given as a pulse every hour or infused continuously resulted in increased mean concentrations of LH but not FSH during the 13-day infusion period as compared to controls. However, LH secretion was intermediate in cows infused every fourth hour and did not differ from controls, cows pulsed once every hour or continuously infused cows. Additionally, cows fed restricted diets release more LH in response to a single 100 μg (i. m.; Rasby et al., 1991) or a 200 μg (i. v.; Whisnant et al., 1985) injection of exogenous GnRH as compared to cows in moderate to fat body condition. Results from these studies indicate that decreased body energy reserves influence reproduction by inhibiting the

release of GnRH from the hypothalamus and that pulsatile secretion of GnRH is necessary to stimulate pulsatile LH secretion and follicular growth in cattle.

CONCLUSIONS

The development of ovarian follicles is essential for the dynamic process of reproduction to take place. Follicular development and ovulation are regulated by a combination of interactions between hormones, growth factors, and cell to cell communication systems. Gonadotropin-releasing hormone (GnRH) is necessary for the release of the gonadotropic hormones, LH and FSH. The gonadotropins stimulate the thecal and granulosa cells, located within the ovary, to produce the steroids that regulate ovarian growth. The insulin-like growth factor system is also an important regulator of ovarian follicular growth. Insufficient energy and protein intake have been found to affect the concentrations of IGF-I and numerous other hormones and metabolites in serum, however, the mechanisms by which nutrition influences the reproductive process have not yet been well defined.

Table 1. Summary of follicular wave characteristics in cattle as determined by ultrasound.

Authors	Year	Breed	Type	No. Cattle	% with 1-Wave	% with 2-Waves	% with 3-Waves	% with 4-Waves
Sirois & Fortune	1988	Holstein	Heifers	10	0	20	70	10
Savio et al.	1988	Friesian x Hereford	Heifers	13	4	15	81	0
Knopf et al.	1989	Holstein	Heifers	10	0	90	10	0
Taylor & Rajamahendran	1991	Holstein and Ayrshire	Cows	10	0	81	19	0
Murphy et al.	1991	Friesian x Hereford-FG[a]	Heifers	5	0	80	20	0
		Friesian x Hereford-FL[b]	Heifers	7	0	29	71	0

[a]FG = fed to gain body weight

[b]FL = fed to lose body weight

CHAPTER III

INFLUENCE OF EXOGENOUS GONADOTROPIN RELEASING HORMONE (GnRH) ON OVARIAN FUNCTION IN NUTRITIONALLY ANESTROUS COWS

Abstract

To determine the effect of exogenous GnRH on ovarian function, 32 nutritionally anestrous beef cows were divided into four treatment groups: Control group received saline injections at 1 pulse/4 h for 13 d, GnRH-4 received 2 μg of GnRH at 1 pulse/4 h (2 μg infused in 1.8 mL of saline over 5 min) for 13 d, GnRH-1 received 2 μg of GnRH at 1 pulse/h, infused as in GnRH-4, for 13 d, and GnRH-C received a continuous infusion of 2 μg of GnRH (a total of 2 μg in 34 mL, hourly) for 13 d. On the last day of treatment, cows were slaughtered, and uteri and ovaries were removed. Uterine and ovarian weights were recorded and ovarian follicles were measured. Follicular fluid (FFL) samples (n=188) were collected for analysis of concentrations of estradiol, progesterone, androstenedione, and IGF-I. Percentages of ovulatory cows were 0%, 12.5%, 75%, and 25% for Control, GnRH-4, GnRH-1, and GnRH-C cows, respectively, and were different ($P < .01$). Due to the large percentage of ovulatory GnRH-1 cows, this treatment group was omitted from the analysis, as were cyclic cows from the GnRH-4 and GnRH-C treatment groups. Average uterine and ovarian weights were 306 ± 26 g and 13.9 ± 1.4 g, respectively, and did not differ between groups. However, the difference between right and left

ovarian weights were different ($P < .01$). Number of small (1 to 4.9 mm) and large ($\geq$ 5 mm) follicles on the ovarian surface averaged 37.4 ± 9.4 and $3.0 \pm .8$ respectively, per cow and were not affected by treatment. There was a trend ($P < .10$) for a treatment effect on concentrations of estradiol in FFL. Average FFL estradiol concentrations (ng/mL) were 1.68, 6.39, and 2.22 ± 1.31 for Control, GnRH-4, and GnRH-C groups, respectively. Concentrations of progesterone (64.5 ± 13.4 ng/mL), androstenedione (20.9 ± 3.6 ng/mL) and IGF-I (13.4 ± 1.7 ng/mL) in FFL were not influenced ($P > .10$) by treatment. Overall, there was an effect ($P < .01$) of follicle size on the concentrations of androstenedione with small follicles having greater concentrations than large follicles, but this difference was only evident in GnRH-4 cows. Follicle size did not affect concentrations of estradiol or progesterone. A trend ($P < .10$) for a treatment x size interaction was apparent for IGF-I, with greater IGF-I concentrations in large follicles than in small follicles of GnRH-4 but not in Control or GnRH-C groups. We conclude that pulsatile infusion of GnRH into nutritionally anestrous cows results in increased FFL estradiol concentrations in anovulatory cows without affecting concentrations of progesterone, androstenedione, or IGF-I in FFL.

Introduction

Severe nutrient restriction and loss of body energy reserves causes cessation of estrous cycles in cattle (Richards et al., 1989; Bishop and Wettemann, 1993). Restricted dietary intake also reduces the maximum diameter and persistence of dominant follicles (Murphy et al., 1991). Cows that become anestrus, due to depletion of nutrients, exhibit reduced concentrations of serum LH and fewer pulses of LH as they approach anestrus (Imakawa et al., 1986; Richards et al., 1989). The pulsatile infusion of gonadotropin-releasing hormone (GnRH), however, is able to

stimulate pulsatile LH secretion and resumption of luteal activity in cows that are nutritionally anestrus (Bishop and Wettemann, 1993; Vizcarra, 1994) and in suckled beef cows (Walters, et al., 1982; Edwards, et al., 1983). Additionally, cows fed restricted diets release more LH in response to exogenous GnRH than do cows in moderate to fat body condition consuming normal rations (Whisnant, et al., 1985; Rasby, et al., 1991).

Body energy reserves and weight loss also influence the postpartum anestrous interval in beef cattle (Wiltbank, et al., 1962; Selk et al., 1988; for review see, Dunn and Kaltenbach., 1980). As is the case with nutritionally anestrous cows, increased pulsatile release of LH is required for initiation of estrous cycles in postpartum anestrous beef cows (Rawlings et al., 1980; Riley et al., 1981; for review see, Wettemann, 1980). These increased pulses of LH appear to act directly on the ovary to stimulate growth of estrogen-active follicles prior to ovulation (Walters et al., 1982a; Spicer et al., 1986).

The effect of pulsatile and continuous GnRH infusion on pulse amplitude and pulse frequency of LH and FSH, and luteal activity of nutritionally anestrous cows has recently been determined (Bishop and Wettemann, 1993; Vizcarra, 1994). However, the effect of pulsatile vs continuous GnRH infusion on ovarian activity and follicular fluid hormone concentrations in nutritionally anestrous cows has not been evaluated. Therefore, the objectives of this experiment were to determine the effects of pulsatile and continuous infusion of exogenous GnRH on the initiation of estrous cycles, uterine and ovarian weights, ovarian follicle numbers and size, and concentrations of estradiol, androstenedione, progesterone, and insulin-like growth factor-I in the follicular fluid of nutritionally anestrous cows.

Materials and Methods

Animals and Treatments. Thirty-two nonlactating, Hereford x Angus cows exhibiting normal estrous cycles were used. Cows were maintained in a drylot that was subjected to enviromental conditions, and fed a restricted diet until they became anestrus. The diet was comprised of 2.72 kg of prairie hay and 35 g of a mineral mix. When ambient temperature was below 0°C, an additional 1.4 kg of hay was provided to each cow per day. The diet was designed so that cows would lose approximately 1% of their initial BW per wk. Measurements of BW and body condition score (BCS; 1 = emaciated and 9 = obese; Wagner et al., 1988) were taken every 14 d. Blood samples were collected once weekly via jugular venipuncture in 10 mL vacutainer tubes containing EDTA to prevent clotting. Blood samples were placed on ice and centrifuged (3000 x g for 20 min) within 4 h after collection. Plasma was decanted and stored at -20°C until progesterone concentrations were quantified. Cows were determined to be anestrus when concentrations of progesterone in blood (Coat-A-Count progesterone kit, Diagnostic Products Corporation, Los Angeles, CA) were less than 1 ng/mL for three consecutive weeks.

Shortly after cows were determined to be anestrus (1 to 3 wk), they were transported to an indoor barn where treatments would be administered. The cows were acclimated to the indoor barn for two days prior to initiation of the 13-d treatment. Treatments were administered on October 1, 1993 to the first group of cows and the last group of cows commenced treatments on August 30, 1994. Over the course of the experiment, cows were divided into two different seasonal blocks based on the date when they were administered treatments. Four cows from each treatment group were represented in each of the seasonal blocks. Seasonal block 1 consisted of cows (n=16) that were subjected to treatment during

autumn/winter/spring months (October through May) while seasonal block 2 consisted of those animals (n=16) that were placed on treatment during summer months (June through August).

During treatment, cows were confined indoors in individual stalls where environmental factors were controlled (21 $\pm$ 4°C, 50 $\pm$ 10% relative humidity and 14 h of light). Each cow was fed a diet of 5.5 kg of prairie hay and 35 g of a mineral mix daily at 0900 h to maintain body weight and nutritional anestrus. A polyvinyl jugular cannula (i.d. 1.68 mm, o.d. 2.39 mm, Bolab, Lake Havasu City, AZ) was inserted into each external jugular vein for hormone infusion and collection of blood. Cannulas were applied 2 d before initiation of treatment.

Cows were randomly assigned to one of four treatment groups: saline (1.8 mL/h) infused (i.v.) at 1 pulse/4 h , GnRH (2 μg in 1.8 mL of saline; Sigma Chemical, St. Louis, MO) infused at 1 pulse/4 h (GnRH-4), GnRH (2 μg) infused at 1 pulse/h (GnRH-1), and continuous infusion of GnRH (GnRH-C; 2 μg). Cows were exposed to treatment for 13 d, beginning at 0800 h on d 0 and treatment was continued until 0800 h on d 13 . All pulsatile infusions (control, GnRH-4, GnRH-1) were administered via Harvard Infusion pumps (Model 931, Harvard Infusion/Withdrawal Pump, South Natick, MA) controlled by an automatic timer (Model CD-4, ChronTrol, Lindburg Ent., San Diego, CA). The pump-timer unit was calibrated to deliver the assigned pulse of saline or GnRH over a 5 min interval. The continuous infusion of GnRH was disbursed using a peristaltic pump (Manostat Pump, Fisher) that delivered 34 mL/h. Heparin (1 USP/mL) and penicillin (50 units/mL) were added to sterile saline to prevent clotting and bacterial contamination of cannulas during infusion.

Plasma samples were collected from each animal on d 12 of treatment and stored at -20°C until IGF-I concentrations were quantified by radioimmunoassay

(RIA). Serum samples were taken at 10 min intervals for 8 h on day -1, 0, 2, 4 and 12. Concentrations of LH and FSH were reported by Vizcarra (1994).

On d 13 cows were removed from treatment and taken to a local abattoir. The uteri and ovaries were removed, placed on ice and transported to the laboratory. The right and left ovaries were separated from the uteri, trimmed of any excess fat and/or tissue, and weighed. The uteri (including cervix but not vagina) were also trimmed and their weights were recorded. Follicles on each ovarian surface were counted and measured using calipers (calibrated to 0.05 mm) and recorded as either small (1 to 4.9 mm) or large ($\geq$ 5 mm). A sketch was drawn of each ovary and the location of each follicle, corpus luteum, and corpus albican was recorded. Follicular fluid (FFL) was aspirated from each follicle using a 1 mL single-use syringe (B-D Tuberculin Syringe and PrecisionGlide Needle), and placed in 12 x 75 mm polystyrene culture tubes. The follicular fluid from follicles on the same ovary measuring $\leq$ 4.9 mm was pooled within the same tube while fluid from each follicle measuring $\geq$ 5 mm was stored individually. Tubes were capped and placed on ice until all follicles had been aspirated (< 2 h). Samples were frozen at -20°C until assayed for hormone concentrations.

Radioimmunoassays. Radioimmunoassays of IGF-I were performed on both plasma and FFL samples while estrogen, progesterone, and androstenedione concentrations were quantified with RIAs using only FFL samples. Concentrations of IGF-I in plasma and FFL were determined by RIA after acid-ethanol extraction as described previously (Echternkamp et. al., 1990). Intra- and interassay coefficients of variation were 12.8% and 16.2%, respectively. Concentrations of estradiol and progesterone in FFL were quantified using previously described RIAs (Spicer and Enright, 1991). Intra- and interassay coefficients of variation were 10.3% and 24.7% for estradiol and 19.2% and 18.7% for progesterone, respectively. Concentrations of androstenedione in FFL were determined using solid-phase RIA kits (ICN

Biomedicals, Costa Mesa, CA). Serial dilutions (.4, .8, 1.25, 2.5, 5.0, 7.5, and 10.0 μl) of bovine follicular fluid displaced ^{125}I-labeled androstenedione from the antiserum to produce a binding curve parallel to the standard curve. Intra- and interassay coefficients of variation were 11.4% and 15.9%, respectively.

Statistical Analyses. Hormone data within each treatment were grouped into two classes based on follicle size of 1 to 4.9 mm (small) or $\geq$ 5 mm (large). In addition, data were categorized within two different seasonal blocks with Block 1 = October to May and Block 2 = June to August. Analyses of variance (ANOVA; SAS, 1988) with treatment, follicle size, and seasonal block as main effects and their interactions were used to determine the FFL concentrations of estradiol, androstenedione, progesterone, and IGF-I using the GLM Procedure (SAS, 1988). When analyzing hormones in FFL (estradiol, androstenedione, and progesterone), if heterogeneous variance was present, data were normalized by transformation to ln (x + 1). Although transformed values were used for statistical analyses the values reported herein are taken from absolute data. Means for all data are reported as least-squares means. Specific differences between means were determined using PDIFF (SAS, 1988) if significant main effects were observed.

Results

Number of Cyclic Cows. A cow was considered to be cyclic when a CL was present on the ovary upon visual examination after slaughter. The percentages of ovulatory cows were 0%, 12.5%, 75%, and 25% for cows in Control, GnRH-4, GnRH-1, and GnRH-C groups, respectively. GnRH-1 was different ($P < .01$) from all other groups but Control, GnRH-4, and GnRH-C cows did not differ from one another (Figure 1). Due to its large percentage of ovulatory cows, the GnRH-1 treatment group was omitted from the analysis as were cyclic cows from the GnRH-4

and GnRH-C groups. The cows from these groups were dropped from the analyses because of concerns that they may have misrepresented values of follicle numbers and hormone concentrations.

Uterine and Ovarian Weights. Average uterine weights were 335 ± 25, 304 ± 25, and 278 ± 27 g for Control, GnRH-4, and GnRH-C groups, respectively and were not different ($P > .10$). Ovarian weights also did not differ ($P > .10$) among treatment groups with values for total ovarian weights averaging 14.3 ± 1.3, 13.5 ± 1.4, and 14.0 ± 1.5 g for Control, GnRH-4, and GnRH-C, respectively (Table 2). For comparison, uterine and ovarian weights in cyclic GnRH-1 cows averaged 394 ± 35 and 18.5 ± 1.3 g, respectively. A treatment effect ($P < .01$) was evident when differences between right and left ovarian weights were analyzed. The right ovaries of cows in the Control and GnRH-C groups were heavier than the left ovaries of cows in these same treatment groups. Average weights of the right and left ovaries were 7.9 ± .7 and 6.4 ± .5, and 7.8 ± 1.1 and 6.1 ± .6 g for the Control and GnRH-C groups, respectively. The left ovaries of GnRH-4 cows, however, were heavier than the right ovaries. Right and left ovarian weights averaged 6.3 ± .5 and 7.2 ± 1.0 g, respectively.

Numbers of Follicles. Treatment had no effect ($P > .10$) on the number of small or large follicles present on the ovarian surface. Numbers of small follicles per cow were 36.5 ± 8.7, 38.8 ± 9.4, and 36.8 ± 10.1 for Control, GnRH-4, and GnRH-C cows, respectively, while numbers of large follicles per cow for Control, GnRH-4, and GnRH-C cows were 2.5 ± .7, 3.4 ± .8, 3.2 ± .8, respectively (Table 2). For comparison, number of small and large follicles on the ovarian surface of cyclic GnRH-1 cows averaged 33.3 ± 11.0 and 4.0 ± 1.8, respectively.

Concentrations of Estradiol in Follicular Fluid. There was a trend for level of GnRH treatment to affect FFL estradiol concentrations. Treatment of GnRH-4 had greater concentrations (6.39 ± 1.26 ng/mL) and differed ($P < .07$) from Control

and GnRH-C groups which contained estradiol concentrations of 1.68 ± 1.31 and 2.22 ± 1.53 ng/mL, respectively (Figure 2). There was no difference (P > .10), however, in the concentrations between Control and GnRH-C. Follicular fluid estradiol concentrations also did not differ (P > .10) between small (2.75 ± 1.08 ng/mL) and large (4.11 ± 1.08 ng/mL) follicles. There was no season x follicle size interaction apparent for estradiol concentrations, however, season did affect (P < .01) the amount of estradiol present in FFL. Pooled across follicle size, the cows on treatment during the autumn/winter/spring months (October to May) had greater estradiol concentrations (5.98 ± 1.24 ng/mL) than those cows that were treated during the summer months (June to August; 1.81 ± .95 ng/mL; results summarized in Table 3). There was a trend (P < .06) for follicular fluid concentrations of estradiol to reflect a treatment x season interaction. Those follicles from GnRH-4 cows in the autumn/winter/spring season contained greater FFL estradiol (12.69 ± 1.80 ng/mL) than did FFL from all other treatment groups and seasons. This trend was also evident in cyclic GnRH-1 cows (Table 3).

Concentrations of Progesterone and Androstenedione in Follicular Fluid. There was no effect (P > .10) of GnRH treatment on the amount of progesterone or androstenedione present in FFL. Progesterone concentrations were 64.7 ± 12.7, 71.0 ± 11.9 and 57.9 ± 15.5 ng/mL (Figure 2) and androstenedione concentrations were 23.1 ± 3.6, 22.0 ± 3.3, and 17.7 ± 3.9 for Control, GnRH-4, and GnRH-C groups, respectively (Figure 3). Progesterone values were 50.6 ± 10.8 and 78.5 ± 11.1 ng/mL for small and large follicles, respectively and were not different (P > .10). In contrast, follicle size significantly affected (P < .01) the amount of FFL androstenedione concentrations. Averaged across treatment groups, small follicles contained 23.8 ± 2.8 ng/mL while large follicles contained 18.1 ± 3.2 ng/mL, (Figure 3). Cows that were on treatment during the autumn/winter/spring months tended (P < .07) to have greater FFL concentrations of androstenedione than did cows that were subjected to

treatment during the summer months (22.4 $\pm$ 3.4 vs 19.6 $\pm$ 2.5 ng/mL). However, this trend was not evident in all treatment groups or follicle sizes (Table 3). Although both follicle size and season exerted some effect on androstenedione concentrations, a season x follicle size interaction was not observed ($P > .10$). Likewise, there was no treatment x follicles size x season interaction ($P > .10$).

Concentrations of Insulin-Like Growth Factor-I in Follicular Fluid and Plasma. Although concentrations of IGF-I in FFL were not affected ($P > .10$) by either treatment or follicle size (Figure 4), a trend ($P < .06$) was apparent for a treatment x follicle size interaction in FFL concentrations of IGF-I. Concentrations of IGF-I in small follicles (9.9 $\pm$ 2.0 ng/mL) were less ($P < .05$) than those found in the large follicles (15.7 $\pm$ 2.1 ng/mL) of GnRH-4. In contrast, FFL concentrations of IGF-I in small and large follicles did not differ ($P > .10$) within or between Control and GnRH-C cows. Concentrations of IGF-I in plasma were 10.9 $\pm$ 1.9, 10.5 $\pm$ 2.1, and 9.4 $\pm$ 2.2 for cows in the Control, GnRH-4, and GnRH-C groups, respectively, and were not affected ($P > .10$) by treatment.

Discussion

Results of the present study revealed that 1) hourly pulses of GnRH but not continuous infusion of GnRH or once-per-4 hour pulses of GnRH induced ovulation, 2) GnRH treatments had no effect on uterine and total ovarian weight or on the number of small and large follicles, 3) GnRH treatments did have an effect on the difference between right and left ovarian weights and the concentration of estradiol in ovarian FFL, 4) follicle size had an effect on androstenedione concentrations in ovarian FFL, 5) concentrations of IGF-I in FFL were influenced by a treatment x size interaction, and 6) season in which treatment was initiated had an effect on the FFL

concentrations of estradiol and androstenedione but did not affect concentrations of progesterone or IGF-I.

Administration of pulsatile GnRH injections, given at one pulse per hour, was able to induce ovulation in the majority (i.e., 75%) of nutritionally anestrous cows, as determined by the presence of a corpus luteum (CL) at slaughter. Resumption of estrous cycles occurred at a much lower rate (i.e., $\leq$ 25%) in cows that were given pulsatile injections of GnRH every four hours or in those cows that were continuously infused with GnRH. The finding that hourly pulses of GnRH induced ovulation is in agreement with results from Riley et al. (1981) who found that pulse patterns of LH release in postpartum acyclic beef cows occurred synchronously in response to GnRH injections of 5 μg/2 h for 48 hours and was able to stimulate pulsatile patterns of LH release comparable to those seen during the normal follicular phase. Infusion of GnRH at this dosage also resulted in a subsequent ovulation in 80% of the treated postpartum acyclic beef cows (Riley et al., 1981). Suckling beef cows injected with GnRH, in doses of .25, .5, 1.0, 2.5, 3.0, or 5.0 μg/1 or 2 hours for 2 to 4 days did not significantly increase the number of ovulatory cows over controls within the 2 to 4 day treatment period. However, 55% of the cows injected with either 1 μg/hour or 3 μg/hour of GnRH for 96 hours exhibited an increase in the frequency of LH pulses (Edwards et al., 1983). Eighty-eight percent of postpartum milked cows exhibited a distinct pulsatile pattern of LH within 20 days after calving whereas suckled postpartum cows had fewer and less distinct LH pulses as well as reduced plasma LH concentrations (Peters et al., 1981). Furthermore, 71% of the cows with more rapid LH pulse frequencies (4 to 10 per 8-h period) resumed ovarian activity within 25 days postpartum, while cows with less frequent pulse frequencies (0 to 2 per 8-h period) failed to ovulate within this same time period (Peters et al., 1981). Based on these results it was proposed that the development of a pulsatile pattern in the postpartum anestrous cow is a prerequisite for early return to ovarian activity and that the

frequency of pulses may be important in determining the timing of the first ovulation post partum (Peters et al., 1981). The results of the present study confirm this hypothesis since neither GnRH pulses given every fourth hour nor continuous infusion of GnRH had a significant effect on the number of ovulatory cows. Collectively, these results imply that ovarian activity is able to be initiated by administration of GnRH pulses. However, the frequency of the pulses is a major determinant of cyclicity.

Upon visual examination, we found that treatment of nutritionally anestrous cattle with exogenous GnRH did not affect the number of small or large follicles present on the ovary. These results are in agreement with other data collected in postpartum anestrous cattle in which follicle numbers were not affected by GnRH-induced changes in gonadotropin secretion within 4 days after initiation of treatment (Spicer et al., 1986a). Administration of GnRH did not affect total ovarian weight, however, it did have an effect on the difference between right and left ovary weight. In the present study, the weight of the right ovary was heavier in 55.6% of the control and continuously infused animals when these two treatment groups were pooled together. To our knowledge, this is the first report of ovarian weight differences in nutritionally anestrous cows. Similarly, Rajakoski (1960) observed that the right ovary comprised 57.1% of the total ovarian weight in cyclic heifers, and postulated that the left ovary is less functional than the right ovary in cattle due to its placement in the body cavity. Because the left ovary is positioned near the rumen it could have restricted blood flow and therefore exhibit decreased follicular/luteal activity. In support of this hypothesis, previous studies have shown that the percentage of large normal follicle formation in the right ovary is 66.7% (Rajakoski, 1960) and that the most recently formed CL is present on the right ovary in 63.9% and 58.5% of the cases in heifers and in postpartum cows, respectively (Rajakoski, 1960; Graves et al., 1968). Therefore, the greater weight of the right ovary appears to depend upon the

weight of the larger follicles and/or the heavier CL found on the right ovary. Results from the present study, however, revealed that there were no differences between the right and left ovary in regard to the size of the largest follicle present on the ovary at slaughter (data not shown) or in the numbers of small and large follicles. Thus, differences in the right and left ovary also may reflect general ovarian function not just follicular activity.

The fact that treatment had a tendency to affect estradiol concentrations was not suprising since estradiol secretion has previously been shown to be induced with low-dose injections of GnRH in postpartum anovulatory cows (Walters et al., 1982b; Spicer et al., 1986a). It would be expected that follicle size would influence the amount of estradiol in follicular fluid due to an increase in the number of granulosa cells present in the larger follicles, therefore allowing for an increase in the aromatization of androstenedione for subsequent conversion to estradiol. In fact, it has been documented that follicular fluid from large follicles ($\geq$ 8 mm) contains more estradiol than small follicles ($<$ 8 mm) in cattle during the follicular phase of the estrous cycle as well as in postpartum anovulatory cattle treated with GnRH (Short, 1962; Ireland et al., 1979; Henderson et al., 1982; Staigmiller et al., 1982; Spicer et al., 1986a; Spicer et al., 1988; Spicer and Enright, 1991). Interestingly, however, follicle size did not have an effect on FFL estradiol concentrations in this study. Why estradiol concentrations in large follicles were reduced compared with values previously reported and not greater than concentrations in small follicles is not known. Whether the reduced FFL androstenedione concentrations in large versus small follicles is related causually to the reduced FFL estradiol concentrations in large follicles remains to be elucidated. In this same study, Vizcarra (1994) found that there was no increase in FSH concentrations when animals were either pulsed or continuously infused with 2.0 μg exogenous GnRH. Therefore, he assumed that FSH was not a rate-limiting factor for induction of ovulation in nutritionally anestrous

cows. However, pulsatile GnRH treatment in postpartum anestrous cows induced pulsatile FSH secretion in addition to LH secretion, and after 4 d of treatment increased FFL estradiol concentrations (Spicer et al., 1986a; Spicer et al., 1986b). This increase in FSH secretion was thought to play an important role in the observed ovarian response. In particular, the increase in FFL estradiol in postpartum cows was associated with increased numbers of FSH and LH receptors in granulosa cells (Spicer et al., 1986b), which depend on increased FSH and LH secretion (for review see, Richards, 1980). Additionally, aromatase activity increased with increasing follicle size in granulosa cells from healthy but not atretic follicles on a per cell basis (McNatty et al., 1984a). Also, androgens can be readily synthesized by LH-stimulated thecal tissues under *in vitro* conditions (McNatty et al., 1984b). Spicer et al. (1987) found that estradiol concentrations are reduced in medium-sized atretic follicles as compared to normal follicles. As large follicles degenerate, the theca interna is no longer capable of secreting androgens in response to LH. All the follicles of anovulatory cattle in this study were biochemically atretic using a progesterone to estradiol ratio of > 1 as described by Ireland and Roche (1982), and thus it is likely that most of the large follicles were atretic and possessed granulosa cells with decreased aromatase activity. Another possible explanation could be that the granulosa cells in this animal model are immature due to nutrient depletion and are, therefore, unable to fully respond to the stimulation of FSH and/or LH.

There was no treatment effect on the concentrations of progesterone and androstenedione in follicular fluid. Nutritional status does not appear to limit the synthesis of these two steroids since the values reported in this study are comparable to previously reported concentrations in normally cycling cattle (Fortune and Hansel, 1985; Spicer and Enright, 1991; Spicer and Geisert, 1992). In postpartum anovulatory cattle, however, pulsatile administration of GnRH affects progesterone concentrations in medium and large follicles within 96 and 48 hours after treatment,

respectively (Spicer et al., 1986b). Furthermore, treatment with GnRH does not affect androstenedione concentrations in medium and large follicles of postpartum anovulatory cows (Spicer et al., 1986b). The finding that follicular size had an effect on androstenedione concentrations is in agreement with results of McNatty et al. (1984a) in which small follicles contained significantly greater concentrations of androstenedione than large follicles. Collectively, these studies provide evidence that early developing antral follicles are able to synthesize androgens and progestins before aromatase activity is developed in the granulosa cells. The activation of aromatase activity in granulosa cells at the expense of androgens also explains why estrogen concentrations normally increase and androstenedione concentrations decrease as follicles increase in size (McNatty et al., 1984a). Why FFL androstenedione concentrations decreased without a concomitant increase in estradiol concentrations in the present study remains to be determined.

Concentrations of IGF-I in FFL and plasma of cattle used in this study were drastically reduced compared with concentrations in normally cycling cows and in cows subjected to short-term fasting (Spicer and Enright, 1991; Spicer and Geisert, 1992; Spicer et al., 1992). Previously, we have shown that nutritionally anestrous cattle have significantly reduced serum IGF-I concentrations than normal cyclic cattle fed to maintain body weight (Richards et al., 1991; Richards et al., 1995). It is also known that serum IGF-I concentrations are reduced in cattle during long-term (> 4 weeks) feed restriction (Brier et al., 1986; Houseknecht et al., 1988; Elsasser et al., 1989). Because IGF-I enhances FSH-induced progesterone production by bovine granulosa cells (Spicer et al., 1993) and enhances LH-induced androstenedione production by bovine thecal cells *in vitro* (Stewart et al., 1995), perhaps reduced estradiol concentrations in FFL are due to reduced blood and FFL IGF-I concentrations. Indeed, after 24 and 48 hours of fasting in heifers, serum IGF-I concentrations decreased coincident with decreased FFL estradiol concentrations

(Spicer et al., 1992). Furthermore, it was postulated by Richards et al. (1991) and Enright et al. (1994) that the production of estradiol by the ovaries of cyclic cattle may enhance the secretion of IGF-I by the liver and that this communication is uncoupled with undernutrition (Richards et al., 1995). Averaged across treatment groups, IGF-I concentrations were not different between small and large follicles. Likewise, there was not a significant ($P > .10$) correlation between concentrations of IGF-I and diameter of follicles ≥ 5 mm. This finding conflicts with other data that indicate that IGF-I increases as follicular diameter increases (Spicer et al., 1988; Echternkamp et al., 1990; Spicer et al., 1991). Although neither treatment nor size affected FFL concentrations of IGF-I, there was a trend for an interaction of these two variables such that GnRH-4 cows had greater IGF-I in large versus small follicles. Perhaps increased LH secretion induced by GnRH-4 enhanced production of IGF-I by large follicles as concentrations of IGF-I in FFL of large follicles were greater than serum IGF-I concentrations.

Gonadotropin-releasing hormone may have had an effect by directly affecting the ovary by stimulating steroidogenesis. In a recent study, it was determined that treatment with 10 or 1,000 ng/mL of GnRH significantly increased progesterone and estradiol secretion by bovine granulosa cells (Sirotkin et al., 1994). Therefore, it was proposed that GnRH could have a stimulatory effect on the enzymes that catalyze the early stage (progesterone production) as well as the late stage (aromatase activity) of steroidogenesis. Although results from a previous experiment indicated that neither bovine follicular nor luteal ovarian tissue possessed GnRH receptors (Brown and Reeves, 1983), GnRH-like proteins have been found to be present in bovine ovarian tissues, but not follicular fluid (Ireland et al., 1988). Additionally, it was found that granulosa cells contain greater concentrations of the GnRH-like peptides than do other tissues (Ireland et al., 1988). These results, collectively with the fact that GnRH treatment was able to induce bovine ovarian cell steroidogenesis (Sirotkin et

al., 1994), indicate that GnRH may have direct ovarian effects in cattle. However, further studies in these areas are needed before a definitive conclusion can be drawn.

The effect due to season on the levels of estradiol and androstenedione was such that concentrations of both hormones were greater when treatment was initiated during autumn/winter/spring months (October to May) than in summer months (June to August). Wolfenson et al., (1995) also noticed a decrease in plasma concentrations of estradiol during the second half of the first follicular wave in lactating dairy cows that were subjected to heat stress. These results conflict with data presented by McNatty et al., (1984c) who found that FFL estradiol concentrations as well as *in vitro* granulosa cell estradiol production and thecal cell androstenedione production were not affected by season, although plasma LH peak frequency was affected. The capacity of bovine luteal cells to produce progesterone was impaired by both long-term seasonal heat stress and short-term incubation temperature effects (Wolfenson et al., 1993). In contrast, progesterone concentrations were not altered by season in this study. Although effects due to season are not well defined, it appears that seasonal differences in bovine ovarian activity do exist (McNatty et al., 1984c; Wolfenson et al., 1993; Gilad et al., 1993; Badinga et al., 1993; Wolfenson et al., 1995) and that these differences are probably a result of seasonal differences in gonadotropin secretion and/or steroid production. Alternatively, seasonal changes in melatonin, known to occur in most mammals including cattle (Tamarkin et al., 1985; Schillo et al., 1992), may be involved. Melatonin stimulated androstenedione synthesis by human ovarian stroma *in vitro* (MacPhee et al., 1975). Also, melatonin stimulated progesterone production by human and bovine granulosa cells *in vitro* (Webley and Luck, 1986). Other hormones in addition to melatonin, such as thyroxine, that change with season in cattle may also be involved (Richards et al., 1995). However, further research is needed to clarify such possibilities.

Previously, experiments have been conducted to evaluate the effects of pulsatile GnRH treatment on ovarian activity of postpartum anestrous cows (Riley et al., 1981; Walters et al., 1982; Edwards et al., 1983; Spicer et al., 1986a; Spicer et al., 1986b). However, these GnRH treatments were administered in the presence of a suckling stimulus. Nutritionally anestrous cows provide a desirable model in which to compare ovarian response to exogenous GnRH, infused either as pulses or through continuous infusion, because of the absence of the suckling stimulus and presence of a calf. The tendency for intravenous pulsatile infusion of GnRH, at 2.0 μg per 4 hours, to increase the concentrations of estradiol in FFL, and at 2.0 μg hourly, to induce ovulation in nutritionally anestrous cows indicates that pulsatile secretion of GnRH and subsequent LH secretion is necessary for the stimulation of follicular steroidogenesis and ovulation in cattle.

Implications

Beef cows that are subjected to nutritionally deficient feeding regimens exhibit thin body condition and subsequently become anestrus due to a lack of dietary energy. Reduced ovarian activity in nutritionally anestrous cows is most likely due to a reduced secretion of luteinizing hormone, since exogenous GnRH treatment was able to induce LH secretion and ovulation. The mechanisms through which nutrient deficiency limits reproductive processes are not well defined, however, it appears that adequate amounts of nutrients are essential for optimal reproductive performance. Pulsatile infusion of GnRH tends to increase FFL estradiol concentrations and causes resumption of luteal activity in nutritionally anestrous cows, indicating that pulse frequency of GnRH is an important factor in the regulation of ovarian function in beef cattle.

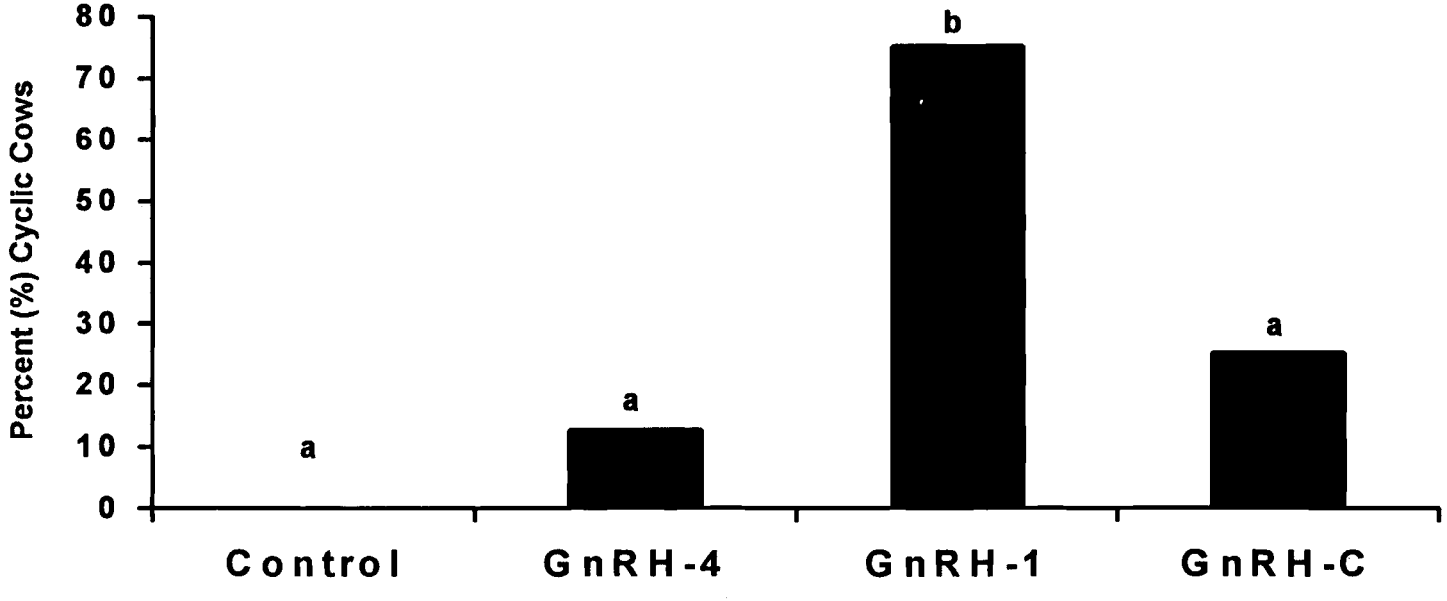

Figure 1. Percentage of cyclic cows after administration of 2.0 μg of exongenous gonadotropin-releasing hormone given as one injection every 4-hours (GnRH-4), hourly injections (GnRH-1), or as a continuous infusion (GnRH-C). Columns with different superscripts differ $P < .01$.

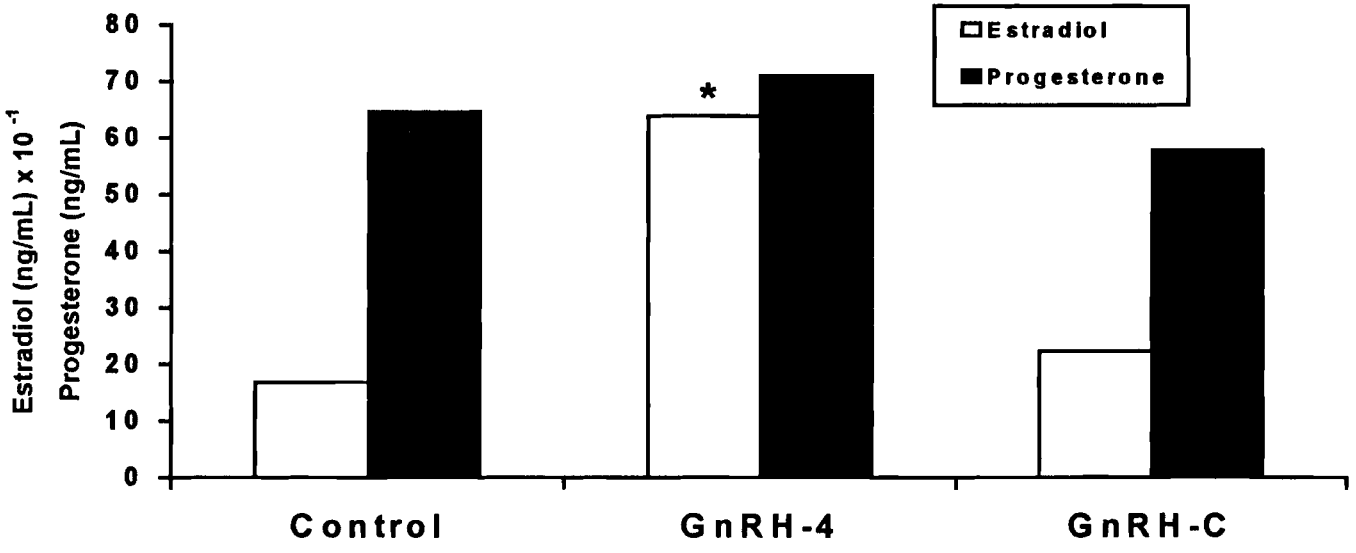

Figure 2. Concentrations (ng/mL) of estradiol and progesterone in follicular fluid of nutritionally anestrous cows after administration of 2.0 μg of exogenous gonadotropin-releasing hormone given as one injection every 4-hours (GnRH-4) or as a continuous infusion (GnRH-C).

*P < .07 versus control.

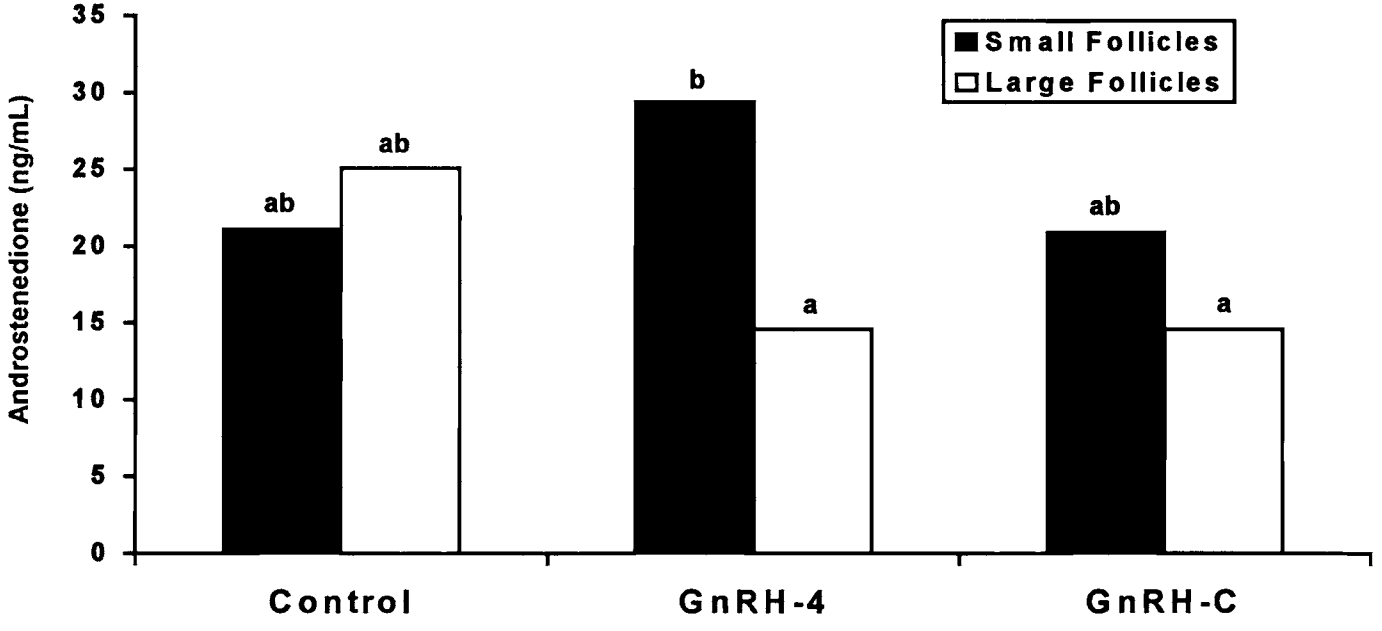

Figure 3. Concentrations (ng/mL) of androstenedione in follicular fluid of small (≤ 4.9 mm) and large (≥ 5 mm) follicles of nutritionally anestrous cows after administration of 2.0 μg of exogenous gonadotropin-releasing hormone given as one injection every 4-hours (GnRH-4) or as a continuous infusion (GnRH-C).

Columns with different superscripts differ $P < .05$.

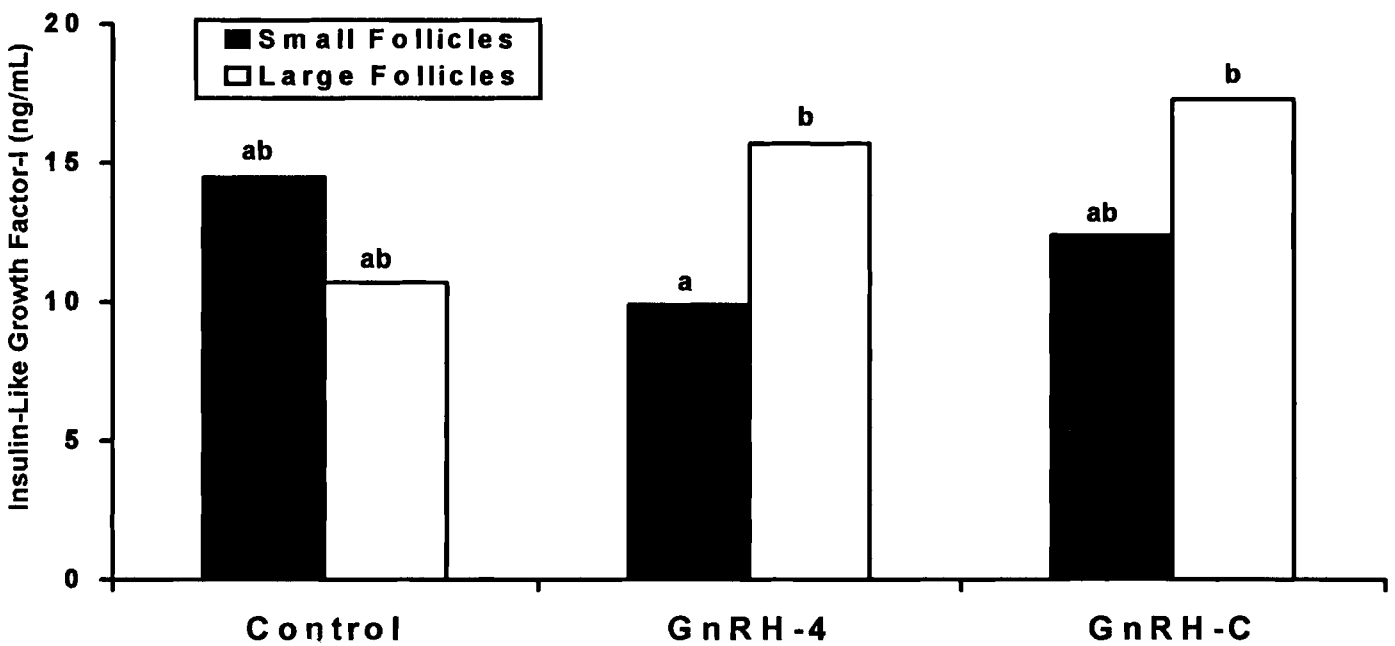

Figure 4. Concentrations (ng/mL) of insulin-like growth factor-I in follicular fluid of small (≤ 4.9 mm) and large (≥ 5.0 mm) follicles in nutritionally anestrous cows after administration of 2.0 μg of exogenous gonadotropin-releasing hormone given as one injection every 4-hours (GnRH-4) or as a continuous infusion (GnRH-C).

Columns with different superscripts differ $P < .05$.

Table 2. Numbers of small (≤ 4.9 mm) and large (≥ 5 mm) follicles, and ovarian and uterine weights in nutritionally anestrous cows.

	Treatment[a]			
	Control	GnRH-4	GnRH-C	**Mean**
Number of Follicles				
Small (1 to 4.9 mm)	36.5 ± 8.7	38.8 ± 9.4	36.8 ± 10.1	**37.4 ± 9.4**
Large (≥ 5 mm)	2.5 ± .7	3.4 ± .8	3.2 ± .8	**3.0 ± .8**
Ovarian Weight (g)				
Right Ovary	7.9 ± .7	6.3 ± .5	7.8 ± 1.1	**7.3 ± .8**
Left Ovary	6.4 ± .5	7.2 ± 1.0	6.1 ± .6	**6.6 ± .7**
Total	14.3 ± 1.3	13.5 ± 1.4	14.0 ± 1.5	**13.9 ± 1.4**
Uterine Weight (g)	335 ± 25	304 ± 25	278 ± 27	**306 ± 26**

[a]Treatments were as follows: Control (saline), GnRH-4 (1 pulse/4 hr), GnRH-C (continuous infusion).

Table 3. Effect of reproductive status, season, and follicle size on concentrations (ng/mL) of estradiol, progesterone, androstenedione, and IGF-I in ovarian follicular fluid of nutritionally anestrous cows treated with or without gonadotropin-releasing hormone (GnRH).

Treatment[a]	Status	Season[b]	No. Cows	Size[c]	n[d]	E_2	P_4	A_4	IGF-I
Control	Acyclic[e]	1	4	Small	8	1.75	98.4	21.0	13.3
				Large	7	2.14	29.0	37.1	11.9
		2	4	Small	8	1.08	36.9	19.9	15.4
				Large	12	1.84	76.3	16.6	10.3
GnRH-4	Acyclic	1	3	Small	8	11.57	48.9	28.8	11.4
				Large	7	13.81	98.4	15.6	18.8
		2	4	Small	8	1.86	42.6	30.1	8.4
				Large	14	2.68	96.2	12.6	13.1
	Cyclic	1	1	Small	2	2.11, .42	101.3	10.6	10.1, 7.5
				Large	2	52.78, 17.88	58.4, 11.4	19.3, 1.1	12.5
		2	0	Small	0	-	-	-	-
				Large	0	-	-	-	-
GnRH-1	Acyclic	1	1	Small	2	7.03, 1.60	22.1, 24.1	22.3, 10.2	10.5, 10.5
				Large	1	4.25	100.1	163.2	6.3
		2	1	Small	1	.76	28.3	24.6	5.8
				Large	1	2.35	26.9	-	-
	Cyclic	1	3	Small	9	5.46	15.8	36.5	34.6
				Large	11	8.85	58.8	6.5	25.9
		2	3	Small	5	1.18	102.3	45.5	17.6
				Large	6	3.16	110.8	81.3	12.4
GnRH-C	Acyclic	1	3	Small	9	1.97	32.9	19.4	12.5
				Large	7	4.66	83.3	12.3	14.6
		2	3	Small	6	.62	59.6	22.8	12.5
				Large	6	2.80	60.8	15.6	19.5
	Cyclic	1	1	Small	1	.57	146.5	29.2	-
				Large	5	.58	195.7	22.1	22.2
		2	1	Small	2	.82, .73	50.2, 46.9	49.4, 60.3	29.1, 19.6
				Large	4	3.62	107.6	30.0	23.2
Pooled SE						2.38	42.3	15.8	4.5

[a]Treatments were as follows: Control (saline), GnRH-4 (1 pulse/ 4 hr), GnRH-1 (1 pulse/hr), and GnRH-C (continuous infusion).

[b]Season 1 = cows on treatment October through May; Season 2 = cows on treatment June through August.

[c]Small = $\leq$ 4.9 mm; Large = $\geq$ 5.0 mm.

[d]Number of samples

[e]All contol cows acyclic.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The largest contributor to reproductive inefficiency of beef cows is animals that are noncyclic during the breeding season. When cattle are subjected to conditions that alter their reproductive cycles and subsequent pregnancy, proficiency and profitability are lost. Many factors influence the reproductive status of an animal. Nutritional management, however, appears to be one of the most important elements in controlling the reproductive process. The mechanism(s) by which nutrition causes reproductive failure has not been well defined, but it has been reported that severe nutrient restriction and loss of body energy reserves can cause a reduction in the size of dominant follicles and ultimately result in anestrus in normally cycling cows (Richards et al., 1989; Murphy et al., 1991; Bishop and Wettemann, 1993; for review see, Randel, 1990).

Thirty-two crossbred cows were fed a limited hay diet until nutritional anestrous was induced. Once the cows became anestrus, they were assigned to treatment groups where they received varying doses of GnRH. At the conclusion of the two wk treatment period cows were slaughtered and the ovaries were removed. Ovaries were weighed and follicles were measured. Follicular fluid was collected from follicles for determination of concentrations of estradiol, androstenedione, progesterone, and IGF-I.

Pulsatile administration of exogenous GnRH had an effect on the percentage of cows that ovulated, and on the difference between right and left ovarian weight ($P < .01$). It did not, however, affect the number of small (≤ 4.9 mm) or large (≥ 5 mm) follicles present on the ovary. Concentrations of estradiol tended ($P < .07$) to be increased by GnRH treatment in those cows that did not ovulate although, androstenedione, progesterone, and IGF-I concentrations were unaffected.

The tendency for intravenous pulsatile infusion of GnRH to increase the concentrations of estradiol in follicular fluid and induce the onset of ovarian luteal activity in nutritionally anestrous cows indicates that pulsatile secretion of GnRH is necessary for the stimulation of follicular growth and ovulation in cattle.

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VITA

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