NUTRITIONALLY INDUCED ANOVULATION IN THE BOVINE: OVARIAN AND ENDOCRINE EVENTS

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NUTRITIONALLY INDUCED ANOVULATION IN THE BOVINE: OVARIAN AND ENDOCRINE EVENTS

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CHAPTER I

INTRODUCTION

Nutritional management is a major controlling factor of reproductive efficiency in many types of domestic livestock, including cattle. Since domestic ruminants in many parts of the world are grazed on pasture and are bred by natural service, the quality and quantity of available feed are crucial for successful mating and subsequent fertilization. Pasture, however, is not always able to supply the required amount of feed for breeding stock. Consequently, grazing animals encounter qualitative and quantitative deficiencies in the diet at some time each year, which can have marked effects on reproductive efficiency. Superimposed upon these short term seasonal variations in feed supply are the occasional prolonged periods of severe undernutrition during drought. In practice, energy conservation mechanisms in ruminants balance out fluctuations in dietary supply by storing body reserves during periods of feed surplus and mobilizing these body reserves during periods of feed scarcity. Even under the worst environmental conditions, cow-calf operations must be maintained and managed at the lowest cost possible. An understanding of the effects of nutrition on the reproductive performance of cattle during times of feed scarcity is of economic importance.

The development of a variety of experimental models and approaches is necessary to unravel the mechanisms interfacing nutrition with reproduction. Models in which

nutrition is reduced are probably the most often used because of their well-established depression of reproduction. This allows the study of specific differences from the well-nourished individual and provides the opportunity to identify somatically-derived metabolic signals that might affect the hypothalamo-pituitary-ovarian axis. Another approach often used is to reduce the availability of nutrients functionally, not by reducing intake, but by reducing utilization. Reproductive function has been studied in animals with ad libitum feed in which fatty acid oxidation and/or glycolysis was inhibited.

Extensive undernutrition results in loss of body weight and body condition and cessation of normal estrous cycles in cattle (Richards et al., 1989). Level of nutrition and body condition appear to be the most important factors determining the duration of the postpartum anestrous interval in cattle (Wettemann, 1980). Even though age and breed are major factors determining the time of puberty, nutrition and BCS in heifers are also important (Kinder et al., 1995). The mechanism by which feed restriction induces anestrus, delays puberty and lengthens the interval from calving to conception in cattle has only been partially elucidated. Restriction of feed intake decreases release of GnRH from the hypothalamus (Rasby et al., 1991) which results in reduced secretion of gonadotropins (Beal et al., 1978) and decreased follicular growth.

Two experiments were conducted to elucidate the mechanisms through which reduced nutrition and loss of body energy reserves induced cessation of ovarian cycles and how realimentation of nutritionally anovulatory heifers results in resumption of ovulatory cycles. The specific objectives were: 1) to evaluate follicular growth and determine the concentrations of luteinizing hormone (LH), follicular stimulating hormone (FSH) and growth hormone (GH) in serum and progesterone, estradiol, insulin-like growth factor-I

(IGF-I), insulin, glucose and nonesterified fatty acids (NEFA) in plasma during the last two cycles (the last ovulatory and the subsequent anovulatory cycle) before the onset of nutritionally induced anovulation in beef heifers, and 2) to monitor sequential changes in follicular growth and concentrations of LH, FSH, estradiol, IGF-I, insulin, glucose and NEFA during the transition from nutritionally induced anovulation to resumption of ovulation after realimentation at two different rates of gain.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Reproductive efficiency in farm animals is influenced by nutritional status, particularly body energy reserves. Cows losing weight and body condition during the breeding season have decreased pregnancy rates compared with cows maintaining weight and body condition (Wiltbank et al., 1962). In addition, restriction of feed intake increases the interval from calving to conception in beef cows (Wetteman et al., 1980; Bishop et al., 1994). Energy intake influences age at puberty in heifers (Kinder et al., 1995) and undernutrition is associated with reduced follicular development in prepuberal heifers (Bergfeld et al., 1994), postpartum cows (Perry et al., 1991; Stagg et al., 1995) and cyclic heifers (Murphy et al., 1991; Rhodes et al., 1995).

The effects of undernutrition on reproduction may be mediated through changes in gonadotropin secretion, gonadal function or both. Direct effects of nutrition on the hypothalamus, pituitary and ovary are all possibilities (Keisler and Lucy, 1996). Feed restriction suppresses the frequency of LH release (Day et al., 1986; Richards et al., 1989; Kinder et al., 1995) and increases LH secretion in response to GnRH (Rasby et al., 1992). A high frequency of LH pulses (1 pulse/h) is required for final maturation of preovulatory

follicles, and this might be the mechanism by which feed restriction affects the final stages of follicular growth (Schillo, 1992; Fortune, 1994)

The mechanisms by which feed restriction is perceived by different reproductive tissues, may include changes in availability of metabolic hormones like insulin, GH and IGF-I and/or metabolites like glucose and NEFA. Growth factors that are involved in development and differentiation of follicles are IGF-I, transforming growth factor- α (TGF-α), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), inhibin and activin (Skinner,1992; Findlay, 1993; Monget and Monniaux, 1995; Spicer and Echternkamp, 1995). Alterations in systemic or local production of these factors might be a mechanism by which nutrition influences reproduction independent or in addition to alterations in gonadotropin secretion.

Bovine Folliculogenesis

Growth of follicles

Folliculogenesis is controlled by complex relationships between the hypothalamo-pituitary-ovarian feedback system, and growth factors (or cytokines) either of gonadal or extragonadal origin. Follicular growth is a process during which follicles acquire a number of properties such as multiple layers of granulosa cells, differentiation of connective tissue cells to thecal cells, antrum formation, acquisition of FSH receptors in granulosa cells and LH receptors in thecal cells, expression of aromatase activity and acquisition of LH receptors in granulosa cells. Progressive acquisition of these properties is an essential prerequisite for further development. Failure to acquire these properties at

the correct time and sequence will lead to deterioration of follicles through atresia (Ginther et al., 1996).

Folliculogenesis in sheep is a lengthy process, as it requires about 180 days for a follicle to grow from its primordial state (100 μm) to ovulatory size of greater than 7mm (Cahill and Mauleon, 1981). In cattle, the interval between the primordial and antrum stage has not been estimated. Antrum formation occurs at a follicular diameter of 0.4-0.8 mm, and it takes 40 days for follicles to develop from this stage to the ovulatory stage (Lussier et al., 1987). Follicular development in cattle is characterized by a marked hierarchy in the antral follicle population with a large number (20-30) of gonadotropin-responsive follicles (3-4 mm), a few (2-6) gonadotropin-dependent follicles (>4-5 mm) and one ovulatory follicle (> 10 mm). An ovulatory follicle is a large highly vascularized follicle which is estrogenic and has a full complement of granulosa cells containing LH receptors (Ireland and Roche, 1983). Although capable of ovulation, the ovulatory follicle will do so only if it can induce a preovulatory surge of LH (Price and Webb, 1989). If not, the ovulatory follicle will become atretic and another follicle recruited from the gonadotropin-responsive pool will develop to replace it (Driancourt et al., 1988). Development of ultrasound scanning in cattle and sheep, has confirmed earlier histological reports (Rajakoski, 1960) that development of large follicles occurs during the luteal phase (Webb and Gauld, 1985; Sirois and Fortune, 1988). In other mono-ovulatory animals, such as primates, follicular growth takes place only during the follicular phase and not during the luteal phase.

In cattle exhibiting estrous cycles, regular waves of ovulatory follicular

development are interrupted by the process of ovulation. Within10-12 h after initiation of luteal regression, a preovulatory follicle which is derived from either gonadotropic-dependent or ovulatory follicles emerges, and under the influence of increased LH stimulation secretes sufficient estradiol to induce the preovulatory surge of LH within 48-60 h (Ginther et al., 1996). Ovulation of the dominant follicle results in reinitiation of follicular development from the recruitable pool of gonadotropin-responsive follicles due to increased secretion of FSH on the day of estrus. (Adams et al., 1992).

Preantral follicles

Structure and development

In all mammals, ovaries develop as two swellings, termed genital ridges, along the ventral side of the mesonephros. Germ cells, originating from the yolk sac, then migrate to the genital ridge. Once they have arrived in the indifferent gonad, these primordial germ cells, like gonadal somatic cells, proliferate by mitosis. Subsequently, in the genetically female gonad, they become enclosed into cords that are located in the cortex, and are then referred to as oogonia. In these cords, groups of oogonia are surrounded by somatic cells (Hirshfield, 1991).

Mitosis of germ cells is generally completed before birth. In cows, germ cell proliferation occurs to 7.5 months of gestation (Van den Hurk et al., 1995). During this mitotic period, the number of germ cells in bovine ovaries reach approximately 2 million per fetus, of which the vast majority (90-99%) degenerate by the time the calf is born and

only few oocytes (less than 1% of the available non-degenerated oocytes at birth) maybe ovulated during the reproductive lifespan (Erickson 1966).

On completion of mitotic proliferation, oogonia enter meiosis. These germ cells then become arrested in the diplotene stage of prophase of the first meiotic division, and are termed oocytes. These oocytes, together with their surrounding cord cells, are sloughed from a cortical cord, which results in the formation of primordial follicles. Primordial follicles possess an oocyte surrounded by a single granulosa layer consisting of 21-29 flattened somatic (granulosa) cells in cattle (Van Wezel and Rodgers, 1996). The granulosa layer is encompassed by a basement membrane.

In pigs and ruminants, most or all primordial follicles are formed during fetal life but in rodents and rabbits they appear during the early neonatal period (Marion and Gier, 1971; Hirshfield, 1991). The diameter of bovine primordial follicles vary from 30 to 50 μm (Figueiredo et al., 1993). Once the population of primordial follicles are established, groups of follicles gradually leave the resting pool and begin to grow. Each day, 5-6 follicles begin to grow from the pool of primordial follicles in cows (Scaramuzzi et al., 1980). As primordial follicles gradually acquire a cuboidal layer of granulosa cells, they become intermediary primary follicle and then primary follicles. In the bovine, primary follicles have a diameter of 40 to 60 μm and their oocyte is surrounded by 27 to 58 granulosa cells (Van Wezel and Rodgers, 1996). Moreover, elongated vimentin-positive granulosa cells appear in follicles during the transition of primordial to primary follicles (Van den Hurk et al., 1995) indicating that granulosa cells increase in number during the primary follicle stage. Organization of vimentin filaments in bundles is associated with increased phosphorylation and appears in cells before mitosis. At least in primates,

transformation of primordial follicles into primary follicles and the subsequent growth of follicles may occur at any age from 7.5 months of gestation until the end of the reproductive life (Gougeon, 1996).

Proliferative activity of granulosa cells results in the formation of a multilayered granulosa around the oocyte. Follicles with more than one layer of granulosa cells are called secondary follicles. Secondary follicles in cows may have up to 6 layers of granulosa cells reaching a maximum diameter of approximately 150 μm (Lussier et al., 1987). The time necessary to double the number of granulosa cells in cows and pigs varies with size of follicle. In pigs, a primary follicle needs 84 d to reach the antral stage, while another 21 d is required to reach ovulatory status (Morbeck et al., 1992). The growth rate of bovine preantral follicles is also much slower than that of antral follicles greater than 0.5 mm in diameter. During the follicular phase of estrous cycles, antral follicles grow more than 8 mm within 2 d, due to accumulation of follicular fluid in antrum (Lussier et al., 1987).

During the early growth stage of a secondary follicle, connective tissue cells are arranged parallel to the basement membrane underneath the granulosa to form a thecal layer. Meanwhile the zona pellucida is formed between the growing oocyte and the innermost granulosa layer. At the end of this stage, the thecal layer stratifies and differentiates into thecal externa and thecal interna cells. Thecal externa cells do not differ from cells of the undifferentiated original thecal cells. The thecal interna cell layer is constituted by large epitheloid cells and a capillary network. In secondary follicles, oocytes reach a diameter of 60 μm in cows and 90 μm in sows. In contrast to laboratory species, where oocytes generally stop growing and acquire competence to resume meiosis

at the end of the preantral stage, diameters of oocytes continue to increase during the antral stage till about 150 μm in ruminants (cows, goats, sheeps) and 130 μm in pigs (Telfer, 1996).

Endocrine, paracrine and autocrine factors controlling development

Regulation of primordial follicle activation and subsequent preantral growth is not well understood. Follicle stimulating hormone (FSH) has a minor role, in vivo, in development of preantral follicles in rats (Richards, 1980). In contrast, FSH is involved in proliferation and differentiation of preantral granulosa cells in vitro and thus in growth and in vitro development of preantral follicles of many species, including cows (Hulshof et al., 1993). Binding of FSH and gene expression of the FSH receptor in granulosa cells of bovine primary, secondary and antral follicles and oocytes of primordial follicles supports the role of FSH on growth of preantral follicles (Wandji et al., 1992). Apparently, basal concentrations of FSH are necessary for the development of small follicles. Adequate neovascularization of small ovarian regions with primordial follicles may be essential for sufficient FSH to activate primordial follicles (Wandji et al., 1996).

Estrogens are endocrine and intrafollicular autocrine mitogenic compounds (Tonetta and DiZerega, 1989). Estrogens are synthesized in granulosa cells of antral follicles in mammals, and also in thecal cells of sows (Foxcroft and Hunter, 1985). Estradiol synergizes with FSH to enhance granulosa cell proliferation and antrum formation in cultured rat (Tonetta and DiZerega, 1989) and porcine large preantral follicles (Hirao et al., 1994). In contrast, estradiol did not affect the proliferative activity of granulosa cells in cultured bovine primary and secondary follicles but enhanced their

size (Hulshof et al., 1994). Adequate neovascularization may supply sufficient hormones to trigger the development of small preantral follicles in vivo.

Intraovarian peptides exert autocrine and paracrine communication within follicles. Activins, growth factors and vasoactive intestinal peptide (VIP) may be important regulating factors for preantral follicle development. Activin and inhibin are proteins, which modulate follicle development in an autocrine or paracrine manner (Robertson et al., 1988). Activin promotes growth of cultured bovine primary and secondary follicles (Hulshof et al., 1994) and activin receptors are present in both oocytes and granulosa cells of preantral follicles. The specific α-subunit of inhibin (Van den Hurk and Dijkstra, 1992) and its mRNA have been detected in antral follicles of various mammals (Hopko-Ireland and Ireland, 1994). However the presence of inhibin does not necessarily indicate an essential role of inhibin in preantral follicle development.

Epidermal growth factor (EGF), basic fibroblast-like growth factor (bFGF), transforming growth factors (TGFs), vascular epithelial growth factor (VEGF) and nerve growth factors (NGFs) may influence preantral folliculogenesis. Epidermal growth factor is involved in proliferation of granulosa cells from primary and secondary follicles in pigs (Morbeck et al., 1993), hamsters (Roy, 1993) and cows (Wandji et al., 1996), while EGF receptors were demonstrated in granulosa cells from feline secondary and antral follicles (Goritz et al., 1996) but not in bovine preantral follicles (Wandji et al., 1992). Basic fibroblast growth factor stimulates the growth of bovine preantral follicles (Wandji et al., 1996), probably via granulosa cell multiplication (Nuttinck et al., 1993). Binding sites for bFGF were detected in granulosa cells from bovine primary and secondary follicles (Wandji et al., 1992), and the peptide was immunohistochemically demonstrated in

oocytes from primordial and primary follicles (Van Wezel et al., 1995). In granulosa cells of hamster preantral follicles, TGF_{β} stimulates and TGF_{α} inhibits FSH-stimulated DNA synthesis (Roy, 1993). However in bovine preantral follicles, TGF_{β} reduces the survival rate of preantral follicles (Wandji et al., 1996). Apart from an effect of IGF-I on expression of the LH-receptor gene in the thecal cells of large preantral follicles in the rat (Magoffin and Weitsman, 1994), effects of IGFs, have only been found on antral follicles.

Growth factors may not only act directly on follicles, but also indirectly via other ovarian components like blood vessels. Vascular epithelial growth factor (VEGF) and bFGF have been implicated in angiogenesis during folliculogenesis (Gordon et al., 1996) and NGF may be involved in follicular innervation (Dissen et al., 1993). Formation of bovine primary follicles is accompanied by rapid growth of VIP and neuropeptide-Y (NPY) containing nerves (Hulshof et al.,1994). These results are in agreement with reports on the participation of sympathetic innervation in the control of follicular development (Dissen et al., 1993). It is possible that growth factors may serve as chemotactic compounds for neovascularization or neoinnervation of small follicles, which provide hormonal and neuropeptidergic compounds as well as cytokines and nutritional components that initiate and maintain follicular growth.

Antral follicles

Morphological description of antral follicular development

Formation of the antrum initiates the final phase of folliculogenesis. Appearance of an antral cavity starts with development of small fluid-filled cavities between granulosa

cells that aggregate to form the antrum. Upon formation of the antrum, cell proliferation progressively ceases while development of characteristics that provide functional maturation is observed (Hirshfield, 1991).

Antrum formation in cows takes place at a diameter of 0.4-0.8 mm. Furthermore, 42 d are required for a follicle to grow from the antral stage to the preovulatory stage (Lussier et al., 1987). Growth of antral follicles can be separated into two phases. Early growth of antral follicles (< 2.5 mm) results from an increase in number of granulosa and thecal interna cells, while in follicles larger than 2.5 mm, follicular growth can be attributed mainly to antrum development rather than multiplication of granulosa cells (Lussier et al., 1987).

In most mammals, shortly after the follicle acquires a single antral cavity, granulosa cells that border the basement membrane lose their cuboidal shape and assume a columnar appearance, while other granulosa cells remain cuboidal (Gougeon, 1993). Follicular growth is slow in small antral follicles (< 0.7 mm) but increases rapidly in follicles of 0.7-3.7 mm (Lussier et al., 1987). At this period, follicles are growing more rapidly than at any other time during development mainly due to an increase in mitotic index and a decrease in duration of mitosis (Hirshfield, 1991). Later in the growth phase (> 4 mm), the overall rate of granulosa cell proliferation progressively diminishes and occurs only in the region of the follicle that borders the antrum. In preovulatory follicles, cells divide at a very slow rate and growth occurs by means of antrum expansion through extensive accumulation of fluid formed by filtration of thecal blood (Gougeon, 1996). There is a direct correlation between the development of human preovulatory follicles and their blood supply (Balakier and Stromel, 1994). From the midfollicular phase, preovulatory

follicles become highly vascularized, with the area of the theca interna occupied by blood vessels that are twice as large as in any other follicle within the same ovary (Zeleznik et al., 1981). This may occur as a result of active endothelial cell proliferation in the theca interna throughout the follicular phase (McClure et al., 1994). In addition, thecal cells of fully mature porcine follicles destined to ovulate synthesize angiogenic factors which stimulate proliferation and migration of endothelial cells (Makris et al., 1984). Extensive vascularization and therefore adequate nutrient supply might be the means by which selected follicle(s) that are destined to ovulate escape atresia.

Follicular dynamics in cattle

By the early 1960's it was indicated that follicular growth in cattle occurs in waves (Rajakoski, 1960). Follicular growth proceeds through progressive and integrated stages of follicular recruitment, selection and dominance. During this process one or two dominant anovulatory follicles develop before the ovulatory follicle, resulting in two or three follicular waves in the majority of bovine estrous cycles (Sirois and Fortune, 1988; Ginther et al., 1989). Recruitment is a process where a group of antral follicles begins to grow at approximately the same time under sufficient secretion of gonadotropins to permit progress towards ovulation. Selection is a process by which a single follicle from the recruited group avoids atresia and becomes competent to ovulate. Dominance is the means by which the selected follicle prohibits recruitment of a new group of follicles Fortune, 1994). Growing phase of a follicle is the interval between the day that it begins to grow and the day that it ceases progressive increase in diameter. Static phase of a follicle is the interval between the last day of the growing phase and the first day that the

follicle begins a gradual decrease in diameter. The dominant follicle is the largest and the first subordinate follicle is the second largest of follicles that originate from the same recruited group of follicles (Savio et al., 1988).

Follicular waves are first detectable as 4-5 mm follicles on approximately d 0 (day of ovulation) and d 10 for two-wave cycles and on d 0, 9 and 16 for three-wave cycles (Savio et al., 1988). In cattle, the first follicular wave begins with emergence of a cohort of follicles from which a single follicle continues to grow while others undergo atresia. The periovulatory surge of FSH appears to be critical for recruitment of follicles (Adams et al., 1992) and variability in the responsiveness to gonadotropic stimulation among follicles likely determines which follicles continue to grow (selection) from the recruited pool. The selected follicle exerts its dominance through inhibition of recruitment of additional follicles (Savio et al., 1993a). Elimination (cauterization) of dominant follicles on d 3 (late growing or early static phase of the largest subordinate follicle) delayed the onset of regression of the largest subordinate follicle and hastened the emergence of the next wave (Ko et al., 1991). However, the largest subordinate follicle in four of six heifers appeared to grow after it had started decreasing in diameter, and in some, became the dominant follicle of the next wave. These findings suggest that selection against subordinate follicles is not a sudden event, but rather that regression is the result of prolonged suppression by the dominant follicle. Ginther et al. (1996) indicated that selected dominant follicles have a size advantage over first subordinate follicles around the time of deviation (the time that the greatest difference in growth rates between the two largest follicles is observed). Occasionally, a future subordinate follicle is initially larger compared with the future dominant follicle, however, it grows at a slower rate so that it is

not the first to reach the decisive stage. The exact mechanisms by which the dominant follicle inhibits the recruitment of a new wave of follicles are not well understood. It has been suggested that the dominant follicle may secrete hormonal factors which regulate gonadotropin dependent growth and differentiation of ovarian follicles (Ireland, 1987). Following selection, the dominant follicle remains active until approximately d 10-11 of the estrous cycle (Ginther et al., 1989a). At this time dominance of the first wave follicle is arrested since emergence of the second follicular wave has already begun. Under normal circumstances, the first dominant follicle regresses and initiation of the second follicular wave results in growth of the second active dominant follicle. Often, maturation of the second dominant follicle is associated with luteal regression and therefore this follicle is ovulated after luteolysis. Alternatively, the second dominant follicle may undergo atresia and a third follicular wave is initiated (Ginther et al., 1989d). Cows with three follicular waves tend to have longer inter-estrous intervals and longer CL life spans compared with cows with two follicular waves (Fortune, 1993).

Antral follicles greater than 2 mm in diameter can be detected by ultrasonography (Pierson and Ginther, 1984). During the first part of estrous cycles (d 1-5), the number of follicles with diameters of 3-5 mm (class 1) decrease while the number of follicles with diameters of 6-9 mm (class 2) increase (Lucy et al., 1992b). The increase in number of class 2 follicles represents growth of follicles out of class 1 and into class 2. On approximately d 5-6 of the estrous cycle, the average number of class 3 follicles ($\geq$ 10 mm) increases to one as the selected follicle continues to grow, while the number of class 2 follicles decreases since follicles that do not become dominant decrease in size and become atretic (Lucy et al., 1992b). The number of class 2 follicles remains small until d

10-12 of the cycle, likely as a result of the inhibitory effect of the first-wave dominant follicle on recruitment of a new cohort of follicles.

Gonadotropins and intrafollicular steroids associated with follicle selection and dominance

Cellular mechanisms regulating selection, growth divergence, growth termination and eventual regression of nonovulatory follicles are not well understood. However, a primary role of gonadotropins in regulation of follicular development has been well established (Ireland, 1987; Adams et al., 1992a). As stated earlier, under the influence of increasing plasma concentrations of FSH, a cohort of follicles begin to grow and a follicular wave emerges 1-2 d after the FSH surge (Adams et al., 1992a; Sunderland et al., 1994). During the subsequent decrease in concentrations of FSH, the dominant follicle of the wave continues development while subordinate follicles cease to grow and eventually regress. Dominant follicles normally reache a diameter of 11-14 mm or greater approximately 7 d after follicular wave emergence, and in nonovulatory waves will stop growing and ultimately undergo atresia (Ginther et al., 1989a).

The ability of the dominant follicle to continue growth under reduced FSH concentrations suggests that cellular responsiveness to FSH may be altered during follicular development. Exogenous FSH treatment for 2 d at the approximate time of follicular deviation (the time that the dominant follicle appears to grow faster than the subordinate follicles) delayed the deviation in growth between dominant and subordinate follicles and delayed regression of subordinates follicles (Adams et al., 1993). The same treatment given after deviation did not alter follicular growth. Therefore, differences in growth rate at the time of follicular deviation may be due to differences between dominant

and subordinate follicles in number of FSH receptors or decreased dependency of dominant follicles on FSH.

Diminished physiological capabilities of subordinate follicles such as decreased granulosa cell numbers, LH and FSH receptor concentrations and estradiol concentrations occur at follicular deviation. Badinga et al. (1992) indicated that follicular fluid estradiol concentration and granulosa cell aromatase activity are reduced in subordinate follicles compared with dominant follicles on d 5 (initial stages of selection). Moreover, the ability of granulosa cell gonadotropin receptors to bind FSH and hCG was reduced in estrogen-inactive follicles compared with estrogen-active follicles on d 4 and 6 (Ireland and Roche, 1983). Thus by the end of growth divergence (the time that the dominant follicle is clearly bigger than subordinate follicles; approximately d 6), a number of physiological differences between dominant and subordinate follicles become apparent.

At the end of follicular divergence, subordinate follicles cease to grow, whereas the dominant follicle continues to grow until approximately d 7 (Ginther et al., 1989b). The period of continued growth of dominant follicles is associated temporally with low concentrations of FSH in serum (Adams et al., 1992a; Stock and Fortune, 1993). It has been postulated that acquisition of LH receptors by granulosa and thecal cells of dominant follicles supports their growth under basal (luteal phase) concentrations of LH despite minimal concentrations of FSH at the time of follicular divergence (Fortune et al., 1991; Savio et al., 1993). Granulosa cells acquired LH receptors between d 2 and 4 after wave emergence (Xu et al., 1995; Bodensteiner et al., 1996). However, number of LH receptors are greater in granulosa cells of dominant follicles compared with subordinate follicles only during the late growing phase (d 8-9) and not during the early growing phase

(d 4-6), whereas LH receptors in thecal cells were greater in dominant follicles compared with subordinate follicles in both early and late growing phase (Wandji et al., 1992; Stewart et al., 1996). In addition, chronic treatment of heifers with a GnRH agonist reduced pulsatile LH secretion and maximum diameter of the largest follicle was less than 9 mm (Gong et al., 1995) indicating that LH is required for development of dominant follicles during and beyond the time of divergence. Increased LH pulse frequency is associated with maintenance of dominant follicles (Savio et al., 1993a; Stock and Fortune, 1993).

Minimal LH pulse frequency is associated with maximal progesterone concentrations during the luteal phase of bovine estrous cycles and leads to regression of dominant follicles (Savio et al., 1993a; Savio et al., 1993b). Moreover, the reduction in mean concentrations and pulse frequency of LH after the first trimester of pregnancy in cattle is associated with a reduction in maximum diameter of dominant follicles (Ginther et al., 1996) indicating a casual relationship between LH and dominant follicles. These results are consistent with the assumption that as concentrations of FSH decrease, the dominant follicle assures itself of continued development by acquiring LH receptors in granulosa and thecal cells, whereas subordinate follicles may lack this ability.

The extend to which estradiol directly regulates granulosa cell function and follicular development is not fully understood. Estradiol is obligatory for granulosa cell differentiation in rats (Richards, 1980). Synergistic actions of estradiol and FSH cause an increase in number of LH receptor binding sites on granulosa and thecal cells and increase activity of the adenylate cyclase system in rats (Knecht et al., 1984; Wang and Geenwald, 1993). Decreased androgen availability or decreased capability for steroid biosynthesis

may be an integral component in the mechanisms of follicular divergence, growth termination and follicular atresia (Badinga et al., 1992).

Concentrations of androstenedione during the early growing phase were greater in dominant follicles compared with subordinates and were highly correlated with the number of LH receptors in thecal cells and concentrations of estradiol (Stewart et al., 1996). In addition, estradiol concentrations and not aromatase activity in follicular fluid of d 8 dominant follicles were less compared with d 5 dominant follicles (Badinga et al., 1992). The apparent discrepancy between aromatase activity and estradiol concentrations in d 8 dominant follicles may be due to a lack of androgens precursors. Androgen synthesis by thecal cells is highly dependent on the secretion of LH (Merz et al., 1981; McNatty et al., 1984). Thus, a reduction in androgen availability for aromatization may occur when LH pulse frequency declines toward the middle of the estrous cycle due to maximal progesterone concentrations leading to regression of the dominant follicle in the first wave. Acquisition of LH receptors by thecal cells during the early growing phase of dominant follicles may be prerequisite for follicular selection and dominance.

Concentrations of FSH in blood are reduced during the time of follicular divergence. However, the amount of FSH that reaches the dominant follicle might not be reduced. In monkeys, during the time of selection, dominant follicles have greater vascularization than subordinates (Zeleznic et al., 1981). Production of angiogenic factors by dominant follicles, such as bFGF, angiotensin-II (Ang II) and cytokines, may account for the increase in thecal vascularization (Bryant et al., 1988; Katz et al., 1992). However changes in vascularization might be the result of selection and not the driving force. It is possible that a change in FSH receptor function or peptides actively synthesized by the

dominant follicle may play a basic protective role by enhancing sensitivity of granulosa cells to FSH. Granulosa cell aromatase activity is at least 10 times more sensitive to FSH in dominant follicles than in small follicles despite no difference in the number of FSH receptors (Harlow et al., 1986). Rat granulosa cells isolated from preovulatory follicles exhibited greater FSH-responsive adenylate cyclase than granulosa cells isolated from smaller antral follicles (Richards et al., 1995). Thus, an increase in angiogenesis and sensitivity of granulosa and thecal cells to LH and FSH may explain the maintenance of preovulatory follicles (or dominant follicles) when circulating concentrations of FSH are decreasing.

Involvement of systemic and intraovarian growth factors in antral follicular development

Increasing evidence (Hirshfield, 1991; Monget and Monniaux, 1995; Spicer and Ecthernkamp, 1995) suggests that growth factors modulate folliculogenesis. Growth factors originate from the periphery or are produced by granulosa and thecal cells, and in cooperation with gonadotropins modulate proliferation, survival and differentiation of follicular cells.

Growth factors can be classified into distinct and separate families based mainly on their structure and biological activity: the IGF family, which include IGF-I and insulin-like growth factor II (IGF-II); the EGF family which include EGF and TGFα; the FGF family; the platelet derived growth factor (PDGF) family; the TGFβ family which includes TGFβ_1, TGFβ_2, TGFβ_3, inhibin and activin; and the haematopoietic growth factor family. The role of growth factors has not been well elucidated because they belong to complex

systems made up of factors, their receptors and binding proteins (Monget and Monniaux, 1995).

Insulin-like Growth Factor I In ruminants, as in other species, IGF-I stimulates both proliferation and differentiation of granulosa cells in vitro. In sheep, IGF-I stimulates proliferation of granulosa cells from small (1-3 mm) but not from large (>5 mm) follicles. In contrast, IGF-I stimulates secretion of progesterone by granulosa from large, but not small follicles (Monniaux and Pisselet, 1992). Thus, IGF-I stimulates either proliferation or differentiation of granulosa cells depending on the stage of development of the follicle.

In cattle, follicular fluid concentrations of IGF-I are not correlated with follicular diameter (Stanko et al., 1994). Concentrations of IGF-I in dominant and small and large subordinate follicles are similar (Stewart et al., 1996). Furthermore, intrafollicular concentrations of IGF-I and estradiol are not correlated (Echternkamp et al., 1990; Spicer et al., 1991). Concentrations of IGF-I in preovulatory estrogen-active follicles and small and large estrogen-inactive follicles were not different (Spicer et al., 1988; Spicer et al., 1991).

The effects of IGF-I are mediated through IGF type I receptors. Number of IGF-I binding sites (presumably receptors) in both granulosa and thecal cells is greater in large dominant follicles compared with small follicles, and granulosa cells contain more IGF-I binding sites than thecal cells (Stewart et al., 1996). In addition, the number of IGF-I receptors decrease during atresia in small antral follicles (Wandji et al., 1992a). In contrast, no change in number of follicular IGF-I receptors occurred during growth of antral follicles between 0.8 and 8 mm (Monget et al., 1989).

In cattle and other domestic species, IGF-I stimulates granulosa cell proliferation and increases FSH-induced steroidogenesis by granulosa cells in vitro (Veldhuis et al., 1985; Spicer et al., 1993). Similarly, IGF-I-induced proliferation of granulosa cells was enhanced by LH and FSH only in small (<5 mm) but not large (<10 mm) follicles (Gong et al., 1993). In contrast, IGF-I, even in high doses, had no effect or inhibited FSH-induced estradiol production by bovine granulosa cells obtained from small follicles, but slightly enhanced FSH-induced estradiol production by granulosa cells from large follicles (Spicer et al., 1993).

Both basal progesterone and FSH-induced progesterone production by bovine granulosa cells in vitro is enhanced by IGF-I (Veldhuis et al., 1985; Monniaux and Pisselet, 1992; Spicer et al., 1993). In addition, IGF-I stimulates progesterone production by bovine thecal cells (Roberts and Skinner, 1990; Stewart et al., 1995). Basal androstenedione production is not affected by IGF-I, but LH-induced androstenedione production in bovine thecal cells is greatly enhanced by IGF-I (Stewart et al., 1995). The synergism between LH and IGF-I on androstenedione production is mediated by increased numbers of LH receptors in thecal cells induced by IGF-I (Stewart et al., 1995). Reduced concentrations of LH and IGF-I in plasma of undernourished cattle are likely to inhibit thecal cell differentiation and diminish concentrations of aromatizable androstenedione (Richards et al., 1995).

Insulin-like Growth Factor II Granulosa, thecal, stroma and luteal cells of most species contain mRNA for IGF-II (Murphy et al., 1987; Samaras et al., 1994; Spicer et al., 1995). Intrafollicular concentrations of IGF-II are greater in small than in large bovine

and ovine antral follicles (Monget et al., 1993; Spicer et al., 1995). Insulin-like growth factor-II has a negative effect on estradiol production by granulosa cells as it inhibits insulin-stimulated estradiol production by granulosa cells of both small and large bovine follicles (Spicer et al., 1994). In contrast, IGF-II stimulates progesterone production in cultured bovine granulosa cells, however to a lesser extend than IGF-I (Stewart et al., 1994).

Insulin-like Growth Factor Binding Proteins Under normal physiological conditions, IGFs in fluids are bound to a family of homologous proteins termed insulin-like growth factor binding proteins (IGFBP). Binding proteins have a determinant role in regulating growth factor action by acting as carriers and as reservoirs of growth factors in the extracellular matrix and at the cell surface, modulating bioavailability of their ligands. Follicular fluid of sheep and cattle contain IGFBP-3 (42-44 kDa), IGFBP-2 (35kDa), IGFBP-5 (28.5-32 kDa) and IGFBP-4 (24 kDa) (Echternkamp et al., 1994; Besnard et al., 1996; Stewart et al., 1996). During growth of bovine and ovine antral follicles, intrafollicular concentrations of IGFBP-2, -5 and -4 progressively decrease and are undetectable in preovulatory follicles, where concentrations of IGFBP-3 increase slightly (Monget et al., 1993; Echternkamp et al., 1994). In contrast, during atresia of ovine follicles, intrafollicular concentrations of IGFBP-2, -4 and -5 increase and IGFBP-3 decreases slightly (Monget et al., 1993).

In situ hybridization experiments with sheep granulosa cells indicate the expression, mainly of IGFBP-2 whereas IGFBP-4 and -5 are expressed in thecal cells (Besnard et al., 1996). During terminal follicular growth, gene expression of IGFBP-2

decreases but no change occurs in the expression of IGFBP-4 and -5, whereas gene expression of these proteins increases in atretic cells. In sheep, rats and pigs, expression of IGFBP-3 is minimal or undetectable in follicular cells suggesting that IGFBP-3 is mainly derived from the circulation (Hodgkinson et al., 1989; Monget et al., 1996).

Disappearance of IGFBP-2, -4 and -5 from preovulatory follicles cannot be completely explained by changes in local synthesis. Specific intrafollicular proteinases are probably involved in the degradation of IGFBPs. During terminal growth of ovine antral follicles, proteolytic activity degrading IGFBP-2 -4 and -5 increases (Besnard et al., 1996). During atresia, proteolytic activity degrading IGFBP-2 -4 and -5 decreases and proteolytic activity degrading IGFBP-3 increases (Besnard et al., 1996). Thus, during terminal development of large antral follicles, the decrease in intrafollicular concentrations of IGFBP-2, -4 and -5 due to reduced synthesis and increased degradation, results in increased bioavailability of IGFs. Increased bioavailability of IGFs is likely to be the mechanism by which the action of gonadotropins is amplified in dominant follicles.

Epidermal Growth Factor Family and basic Fibroblast Growth Factor Presence of mRNA for bFGF has not be detected by northern blot analysis in the bovine ovary (Stirling et al., 1991). However, binding sites for bFGF were demonstrated in bovine granulosa and thecal cells from antral follicles (Wandji et al., 1992). Expression of mRNA for TGF-α was detected in bovine thecal but not granulosa cells (Skinner and Coffey, 1988). Immunoreactivity to TGF-α is maximal in thecal cells of small antral follicles but declines in large preovulatory follicles (Lobb et al., 1989). The number of EGF receptors, which mediate the action of EGF and TGF-α, are greater in small healthy antral bovine

follicles compared with atretic small follicles (Wandji et al., 1992). In cultured bovine granulosa cells, EGF, TGF-α and bFGF stimulate proliferation but inhibit steroidogenesis and inhibin production (Franchimomt et al., 1986; Skinner and Coffey, 1988; Vernon and Spicer, 1994), suggesting that they may act in vivo by enhancing growth and delaying terminal maturation.

The Transforming Growth Factor-beta family Granulosa cells of bovine follicles express mRNAs encoding the inhibin subunits α, βA and βB (Hopko-Ireland and Ireland, 1994). In cattle, expression of βB-subunit does not change during follicular development, while expression of α- and βA-subunits increase during terminal development of antral follicles and decrease in atretic follicles (Hopko-Ireland and Ireland, 1994). Intrafollicular concentrations of the α-subunit of inhibin increase with follicular size in healthy antral follicles and decrease with atresia (Henderson et al., 1984). Similarly, large ovine estrogen-active follicles secrete maximal concentrations of immunologically active inhibin in ovarian venous blood, whereas secretion is less by atretic or small follicles (Campell et al., 1991). Interpretation of these results is difficult because each subunit is synthesized in a precursor form and several combinations of precursor and mature α, βA and βB subunits occur in follicular fluid and serum (Groome et al., 1995). Seven to nine different molecular forms of immunologically and biologically active inhibin have been detected in bovine follicular fluid (Good et al., 1995; Sunderland et al., 1996).

Immunization of ruminants against the inhibin α-subunit stimulates growth of antral follicles and increases ovulation rate by a mechanism involving an increase in concentrations of FSH in plasma (Mann et al., 1993). In addition to well defined

inhibitory effects on FSH secretion, inhibin enhances androstenedione secretion by bovine thecal cells in vitro (Wrathall and Knight, 1995). Thus, inhibin secreted by granulosa cells may enhance the supply of aromatizable androgen for the production of follicular estrogen. In contrast, activin inhibits progesterone production in both granulosa and thecal cells (Shukovski et al., 1991; Shukovski et al., 1993). Activin may prevent premature luteinization of follicular cells in the late stages of antral follicular development (Findlay, 1995). In vivo, the paracrine effects of inhibin and related peptides on follicular cells are probably determined by the relative proportion of intrafollicular activin, the different forms of inhibin and amounts of binding proteins, especially follistatin (Shukovski et al., 1991; Wrathall and Knight, 1995). Follistatin is a monomeric glycosylated protein that is the major high affinity binding protein for activin, and it binds inhibin with lower affinity (Findlay, 1995).

In ruminants, TGF-β inhibits cell proliferation and enhances steroidogenesis (Skinner et al., 1987; Roberts and Skinner, 1991), thus, it might sustain terminal follicular maturation. However, discrepancies in results obtained with different species and/or different culture systems indicate that the role of TGF-β in folliculogenesis is more complex.

Growth factors may have an important regulatory role in initiation of follicular waves, selection of dominant follicles and terminal maturation of preovulatory follicles. Factors such as bFGF, EGF and IGF in combination with FSH may control growth and proliferation of cells from small antral follicles and are probably involved in the initiation of follicular waves. In addition, IGFs may have a role in the process of selection of dominant follicles. The greater responsiveness of dominant follicles to FSH compared

with subordinates during dominance and terminal follicular growth is associated with increased concentrations of estradiol and increased bioavailability of IGFs in dominant follicles. Moreover, greater intrafollicular concentrations of inhibin in dominant follicles compared with subordinate follicles may result in greater concentrations of aromatizable androgen and estradiol in dominant than subordinates (Wrathal and Knight, 1995).

Effects of metabolic hormones and energy substrates on the female reproductive system

Introduction

Feed intake and associated effects on metabolic rate may be a mechanism regulating reproductive function (Kennedy and Mitra, 1963). Nutrition has a profound effect on concentrations of energy substrates and metabolic hormones in blood which in turn have the potential to affect the reproductive axis. Fluctuations in blood hormones and substrates during the postprandial and postabsorptive periods may provide signals to the brain that link metabolic status to reproductive system. In support of this hypothesis, administration of metabolites such as glucose and amino acids elicited significant increases in LH secretion in castrated monkeys in the absence of any change in body weight (Steiner et al., 1983; Cameron et al., 1985). Similarly, LH pulse frequency increased dramatically within 48 h of realimentation in ovariectomized feed-restricted lambs (Foster et al., 1989) and feed restricted cows, without change in body weight (McCann and Hansel, 1986). However severe short term nutritional deprivation in steers did not alter concentrations of LH in serum (Ojeda et al., 1996). Adipose tissue reserves, certain excitatory amino acids such as tyrosine, aspartate and glutamate, metabolic hormones such as IGF-I and insulin

and energy substrates such as glucose, NEFA and VFA may provide signals that influence reproductive function. Realimentation of nutritionally anestrous young female pigs occurred in the presence of a significant weight gain but little alternation in backfat thickness (Armstrong and Britt, 1987). Metabolic hormones, energy substrates and/or excitatory amino acids rather than adiposity may influence reproductive function. An alternative hypothesis suggests that availability of total body energy, of which energy stored in adipose tissue is an important component, rather than a specific substrate , metabolite, and/or metabolic hormone, modulates reproductive performance (Bronson and Manning, 1991; Schillo et al., 1992).

Body condition score in cows is associated with onset of nutritionally induced anestrus (Richards et al., 1989) and duration of postpartum anestrous interval (Wright et al.,1987; Selk et al., 1988; Bishop et al., 1994). Infusion of deoxy-2-glucose (2DG), an inhibitor of glucose utilization, in ovariectomized lambs had no effect on LH secretion, but infusion of both 2DG and methyl palmoxirate (inhibitor of fatty acid oxidation) suppressed secretion of LH (Hileman et al., 1991). Acute feed restriction blocked estrous cycles immediately in lean hamsters but not until several estrous cycles in obese hamsters (Schneider and Wade, 1989), indicating that the amount of adipose tissue is critical to reproductive function.

Growth hormone-IGF axis

Secretion of growth hormone (GH) by the anterior pituitary occurs in pulses (episodic release). The physiological significance of such pulsatility is not known. In humans, there is evidence that growth disorders may be associated with altered pulsatility

of GH (Blatt et al., 1984), and onset of puberty in humans is associated with changes in pulsatile secretion of GH (Frohman and Jansson, 1986). The secretion of GH is regulated by a dual system of hypothalamic hormones: GH-releasing factor (GRF) stimulates and somatostatin (SRIF) inhibits GH release. The release of these hypothalamic hormones is influenced by neurotransmitters and extrahypothalamic factors (Malven, 1993). Growth hormone releasing factor has been isolated and characterized as a 40-44 amino acid peptide from human pancreatic tumors that caused symptoms of acromegaly (Guillemin et al., 1982). Synthetic preparations of GRF possess full biological activity and specifically stimulate the release of GH in cultures of anterior pituitary cells in vivo (Jansson et al., 1984; Moseley et al., 1985). Active immunization against GRF effectively decreases serum GH in swine and cattle (Armstrong and Benoit, 1996).

The initial event in the generation of a GH pulse is diminished release of SRIF (Cowan et al., 1984) which is followed by a greater release of GRF. The pulse is then terminated by increased SRIF release (Plotsky and Vale, 1985). Negative feedback operates within the somatotropic axis and a reduction in concentrations of IGF-I in blood results in increased secretion of GH and vice versa (Berelovitz et al., 1981).

Restricted nutrient intake increases concentrations of GH in blood of ewes (Thomas, 1994), pigs and cows (Armstrong and Benoit, 1996). In rodents, feed restriction causes decreased concentrations of GH in blood (Ross and Bucchanan, 1990). In sheep, feed restriction does not influence concentrations, pulse frequency or pulse amplitude of GRF in portal vessels of the pituitary (Thomas et al., 1994), however portal concentrations of SRIF were reduced to half as compared with control animals. Other factors such as reduced concentrations of IGF-I in plasma (Berelovitz et al., 1981) or

decreased metabolic clearance might also contribute to increased concentrations of GH during feed restriction (Trenkle, 1976; Lapierre et al., 1992).

Despite increased concentrations of GH in undernourished animals, serum IGF-I is reduced, suggesting that tissues are insensitive to GH. Undernutrition of growing steers was associated with a marked reduction in GH binding to hepatic membranes (Breier et al., 1988). Well-fed animals had both high and low affinity growth hormone receptors (GHr) while undernourished animals had only low affinity GHr. In rats fed a protein deficient diet, the decline in GHr was less severe compared with rats fed energy deficient diets. However concentrations of IGF-I in plasma were similar and minimal in rats fed either protein or energy deficient diets (Thissen et al 1994). These results indicate that GHr activity rather than number may be involved in the uncoupling of the GH-IGF-I axis during feed restriction.

Growth hormone expresses a variety of metabolic effects that include anticatabolic actions on carbohydrate metabolism (O'Sullivan et al., 1989) and fat mobilization by increasing lipolysis and decrease lipogenesis (Wallis, 1988). Resistance to GH in underfed animals represents a mechanism for preferential utilization of mobilized substrates to maintain homeostasis and provide metabolic fuels for reproductive function (Hileman et al., 1991). The anabolic actions of GH may be mediated by IGF synthesis. The insulin-like actions of the IGFs (IGF-I and IGF-II) include glucose transport and oxidation, glycogen synthesis, lipogenesis and protein synthesis (Clemmons and Van Wyk, 1981). The catabolic actions of GH may be modulated by the responses of insulin and other hormones to metabolic changes. Concentrations of insulin in plasma contribute to the

regulation of GH receptors in the liver by stimulating expression of mRNA for the GHR modulating the ability of GH to stimulate hepatic IGF-I production (Thissen et al., 1994).

Unlike GH, concentrations of IGF-I in blood are depressed during feed restriction in domestic animals (Granger et al., 1989; Armstrong et al., 1996). Concentrations of IGF-I are positively correlated with growth rate in pigs, and low rates of gain and a tendency for fat deposition are associated with low GH concentrations (McCusker et al., 1985). During undernutrition low insulin concentrations may reduce IGF-I production from hepatic cells, thereby allowing the catabolic actions of GH to dominate. In support of this, insulin-deficient diabetic rats have reduced concentrations of IGF-I in serum and decreased GH binding in the liver compared with normal rats (Tollet et al., 1990; Boni-Schnetzer et al., 1991). In addition, insulin reduces turnover rate of study state IGF-I mRNA even in absence of GH (Johnson et al., 1989), and potentiates the stimulatory effect of GH on IGF-I production in hepatic cell cultures (Houston and O'Neil, 1991).

Treatment of lactating cows with bovine somatotropin (bST) increases intramammary concentrations of IGF-I (Glimm et al., 1988). Administration of bST to lactating cows increases concentration of IGF-I in the milk, but the change is minor and concentrations remain within the range of values detected in milk from untreated cows (Collier et al., 1991). Concentrations of IGF-I in blood and the response to intravenous infusions of GH in cattle are reduced during periods of restricted protein and/or energy intake (Brier et al., 1988; Ronge and Blum, 1989; Granger et al., 1989; Armstrong et al., 1993).

The majority of IGFs in extracellular fluids are bound to soluble, high affinity binding proteins (IGFBP). Approximately 90 % of IGF-I in serum is bound to IGFBP-3

and an acid-labile subunit to form a complex of 150-kDa (Baxter and Martin, 1989). This complex does not to cross the capillary endothelium prolonging the half-life of IGF-I in blood (Binoux and Hossenlopp, 1988). Binding of IGF-I to IGFBP-3 reduces the rate of metabolic clearance of IGF-I (Binoux and Hossenlopp, 1988). A small percentage (3-6 %) of IGF-I in serum is also bound to IGFBP-1 and -2 in smaller complexes (30-40 kDa) that can cross the capillary endothelium (Bar et al., 1990) and these proteins may be involved in delivery of IGF-I to tissues.

Feed intake and GH regulate concentrations of at least some IGFBPs. In sheep, increased plasma concentrations of IGFBP-3 after birth are associated with a marked increase in GH receptors (Butler and Gluckman, 1986). Goats, pigs, humans and cows treated with bGH have increased concentrations of IGFBP-3 and decreased concentrations of IGFBP-2 (Davis et al., 1989; Walton and Etherton, 1989; Rechler and Nissley, 1990; Cohick et al., 1992;). The GH-dependent increase in IGFBP-3 may be mediated by IGF-I (Clemmons et al., 1989). Deprivation of feed decreases plasma concentrations of IGFBP-3 and increased IGFBP-1 and -2 in pigs (McClusker et al., 1991). Plasma concentrations of IGFBP-2 increase in feed restricted cattle (Vandehaar et al., 1995) and postpartum dairy cows (Sharma et al., 1994). Concentrations of IGFBP-3 are greater and concentrations of IGFBP-2 are less in cyclic compared with anestrous cows (Roberts et al., 1994). In diabetic pigs, IGF-I is decreased and IGFBP-1 is increased (White et al., 1993), and in humans, IGFBP-1 is suppressed by insulin (Suikkari et al., 1988) and increased with reduced intake (Cotterill et al., 1988). During starvation when GH resistance and hypoinsulinaemia develops, the proportion of IGFBP-1 and -2 relative to IGFBP-3 increases. Hodgkinson et al. (1987) found that in sheep IGFBP-1 and -2 have

much shorter half-lifes (~ 30 min) compared with IGFBP-3 (~600 min), and binding of IGF-I to IGFBP-1 and -2 increases the clearance of IGF-I from the circulation. Therefore during starvation, IGF-I is reduced by two mechanisms: through reduced secretion as a consequence of GH resistance and through increased metabolic clearance.

Although GH is an excellent indicator of metabolic status, it is unlikely that it mediates the effects of underfeeding on reproduction. Intravenous infusion of FFA in ovariectomized underfed ewes reduced concentrations of GH but had no effect on LH concentrations (Estienne et al., 1990). In addition, exogenous FSH is required to detect an increase in the number of follicles in cows treated with bST (Gong et al., 1993). Even though GH receptors have been identified in follicles, the number is minimal (Lucy et al., 1993), and the substantial increase in circulatory GH during underfeeding rules against a role of GH in mediating the effects of feed restriction on reproductive tissues.

The effects of GH on reproduction are probably mediated through IGF-I. Decreased concentrations of IGF-I are associated with delayed puberty in cattle (Granger et al., 1989) and increased postpartum anestrous intervals (Rutter et al., 1989; Nugent et al., 1993). Concentrations of IGF-I can directly affect both hypothalamic and pituitary function in rats and ewes (Chandrasekher and Bartke, 1993; Funston et al., 1995). The IGF-I receptor (Type-I receptor) is distributed throughout the brain and anterior pituitary in rats (Bach and Bondy, 1992), but it is concentrated in the median eminence of the hypothalamus (Lesniac et al., 1988). Although IGF-I secreted by the liver can reach the pituitary through the circulation, it is also synthesized and secreted in the pituitary (Sara and Hall, 1990; Daughaday et al., 1989), and both sources may contribute to modulate LH release. In vitro studies have demonstrated that IGF-I can stimulate the secretion of

gonadotropins (Kanematsu et al., 1991), increase anterior pituitary LH response to GnRH (Soldani et al., 1995), and increase GnRH secretion from nuclei in the median eminence (Hiney et al., 1994) in rats. Administration of GH to dwarf mice (GH-deficient mice) increases concentrations of IGF-I in blood which in turn alters LH secretion, by modulating responsiveness of the pituitary to GnRH and steroids (Chandrashekar and Bartke, 1993). In addition, administration of rhIGF-I to adolescent female monkeys reduces the interval from menarche to first ovulation by decreasing the hypersensitivity of pituitary gonadotropes to estradiol negative feedback inhibition (Wilson,1995).

Although IGF-I may modulate the hypothalamo-pituitary axis, most of its effects on reproduction are mediated through ovarian follicles and particularly granulosa cell differentiation and proliferation, FSH-stimulated steroidogenesis by granulosa, and LH-stimulated steroidogenesis by thecal cells (Spicer and Echternkamp, 1995).

Insulin

Although the role of insulin in carbohydrate and lipid metabolism has been well established, its effects on reproductive tissues are not well known. Feed restriction results in reduced plasma concentrations of insulin in all domestic species (McCann and Hansel, 1986; Richards et al., 1989). The possibility that insulin modulates GnRH neuronal activity in response to changes in metabolic status is supported by evidence that insulin can act within the brain to modulate the function of neurons. Insulin binding sites have been identified in the central nervous system (CNS), mainly in endothelium of the microvessels in circumventricular organs such the median eminence, in nerve terminals arising form cells of the arcuate nucleus, and the mediobasal hypothalamus (Van Houten

and Posner, 1979; Baskin et al., 1983). The endothelial cells in these areas mediate transcytosis of insulin through the blood-brain barrier via a receptor-mediated transport system (Schwartz et al., 1991).

Insulin stimulates GnRH release from hypothalamic explant cultures of median eminence of rats (Arias et al., 1992). Hypothalamic GT-1 cells (transformed cell line), which secrete GnRH, have insulin receptors and insulin exhibits mitogenic effects (Olson et al., 1995). In addition, insulin facilitates the transport of tryptophane and tyrosine across the blood-brain barrier (Fenstrom, 1983). These amino acids are precursors of monoamine neurotransmitters. Induced diabetes in rats decreased the content of GnRH in the hypothalamus and LH in the pituitary (Besteti et al., 1989; Valdes et al., 1991) and insulin replacement therapy in diabetic rats restored ovulations and cyclicity (Katayama et al., 1984). However, the role of insulin in mediating the effects of feed intake on LH secretion has been disputed. Intracerebraventricular infusion of insulin in growth restricted ovariectomized ewes did not alter LH secretion (Hileman et al., 1993) and suppression of postmeal insulin secretion by diazoxide in previously underfed rats did not prevent LH secretion (Williams et al., 1996).

It is likely that insulin concentrations in plasma must be maintained above a threshold for normal hypothalamo-pituitary function in cattle. Similarly, physiological insulin concentrations are probably required for normal follicular steroidogenesis. Receptors for insulin are in granulosa cells of cattle and pigs (Otani et al., 1985; Spicer et al., 1994). In vitro studies in swine, sheep and cattle have revealed that insulin stimulates proliferation of granulosa cells and enhances luteal cell steroidogenesis (Baranao and Hammond, 1984; Spicer et al., 1993; Moniaux et al., 1994), however with less potency

than IGF-I. In contrast, insulin is a more potent stimulator of FSH-induced estradiol production by bovine granulosa cells compared with IGF-I (Spicer et al., 1994), and insulin infusion during a superovulatory regime in cattle increased intrafollicular concentrations of estradiol in large follicles by fivefold (Simpson et al., 1994). Insulin has no effect on basal androstenedione production by bovine thecal cells, but greatly enhances LH-induced androstenedione production (Stewart et al., 1996).

Administration of insulin to pigs reduced follicular atresia and increased ovulation rate without altering concentrations of gonadotropins (Matamoros et al., 1991; Cox et al., 1994). Induced diabetes in pigs decreased follicle diameter, intrafollicular concentrations of estradiol and IGF-I in follicles of all diameters, and increased intrafollicular IGFBP-2 and secretion of IGFBP-1 to the media (Edwards et al., 1996). Induced diabetes in pigs increased serum IGFBP-2 and decrease serum IGF-I, and insulin replacement restored IGF-I concentrations (White et al., 1993). Insulin modulates the stimulatory effects of GH on IGF-I production by regulating expression of the GHR as previously mentioned. Therefore, in addition to direct effects on ovarian function, insulin can influence other reproductive tissues through modulation of IGF-I production by liver .

Glucose

In contrast to monogastric species where an increase in plasma glucose is observed after consumption of a meal, microbial fermentation of dietary glucose into volatile fatty acids (VFAs) in ruminants normally results in less than 10% of the total glucose requirement absorbed from the gut (Trenkle, 1981). In monogastric species, plasma concentrations of both insulin and glucagon normally increase during the postprandial

period. In ruminants, plasma concentrations of glucose during the postprandial period may initially decrease, but then increase as a consequence of enhanced gluconeogenesis from propionate (Bassett, 1974).

Glucose availability in brain tissues may modulate several function of CNS since 25-30% of an oral glucose supply in humans is utilized by the brain while only 15% is utilized by muscles and adipose tissue (Felig et al., 1975). Infusion of 5-thioglucose (inhibitor of glucose oxidation) directly into the fourth ventricle elicited food intake (Ritter et al., 1981). Induction of feeding behavior by intravenous infusion of 2DG in rats was abolished by lesions of the area postrema and the adjacent nucleus tractus solitarious (Schneider and Zhu, 1994). Projections from neuronal cell bodies located at the area postrema and nucleus tractus solitarious innervate structures of the brain such as the paraventricular nucleus and the lateral hypothalamus, both of which are implicated in the regulation of feeding and reproduction by modulating secretion of GnRH (Gross et al., 1990; Murahashi et al., 1996). Thus, signals about glucose availability may affect GnRH secretion.

Glucose concentrations were positively associated with feed intake and LH pulse frequency in prepuberal heifers fed at two different levels of nutrition (Yelich et al., 1996). Injection of 2DG before or during the estrous cycle of beef heifers prevented estrus and CL formation (McClure et al., 1978). Peripheral infusion of 2DG in seasonal anestrous ewes delayed and reduced estradiol-induced LH surges, but did not alter the response to exogenous GnRH (Crump et al., 1982), indicating that reduction in glucose availability reduces secretion of GnRH and not pituitary sensitivity to GnRH. Similarly, insulin-induced hypoglycemia significantly reduced the incidence of an estradiol-induced LH

surge in anestrous ewes (Crump and Rodway, 1986) and phlorizin-induced decrease in glucose concentrations compromised the increase in glucose, insulin and LH that normally follow early weaning in postpartum beef cows (Rutter and Manns, 1987). Glucose infusion in lactating anestrous beef cows increased pulse frequency and concentrations of LH in serum during treatment with GnRH (Garmendia, 1986). Intracerebraventricular infusion of 2DG to gonadectomized male lambs, at doses that did not affect peripheral concentrations of glucose, suppressed concentrations of LH in serum (Bucholtz et al., 1996). However most studies indicate that a physiological range of glucose concentrations is required for normal LH secretion which does not necessarily mean that glucose is stimulatory for LH secretion. Concentrations of glucose in plasma of postpartum cows are not predictive of luteal activity (Vizcarra et al., 1996). Glucose infusion in postpartum cows with good BCS and nonlactating well-fed cows did not alter LH secretion (McCoughey et al., 1988; Rutter et al., 1989) and infusion of 2DG in well-fed ewes had no effect on LH secretion (Hileman et al., 1991).

Although a reduction in the availability of glucose or other metabolic fuels usually results in suppressed LH secretion, FSH secretion is not affected. Treatment of ovariectomized ewes with high dose of 2DG reduced concentrations of LH in serum but did not influence concentrations of FSH in serum (Funston et al., 1995). Concentrations of glucose in plasma can affect the reproductive axis either directly by influencing fuel availability within the CNS, or indirectly by causing alternations in metabolic hormones like insulin which in turn may provide a signal to the reproductive axis.

Nonesterified fatty acids (NEFA)

During periods of negative energy balance lipolysis occurs which results in the release of glycerol and free fatty acids. Glycerol serves as a glycogenic precursor and free fatty acids (FFAs) are used as an energy source in adipose tissue and muscle, thereby reducing glucose utilization to provide sufficient amounts for the CNS (Riis and Grummer, 1969; Lindsay and Setchell, 1976).

Concentrations of lipid metabolites in blood may act as signals of the metabolic status to the liver and brain. Hepatic oxidation of glycerol and 3-hydroxybutyrate alters the firing rate of hepatic vagal afferent nerves that provide information to the CNS (Langhans et al., 1986; Novin, 1985). Increased NEFA concentrations are associated with increased circulating GH (Armstrong et al, 1993) and short term peripheral infusion of free fatty acids into ovariectomized lambs suppressed pulsatile GH release but did not alter pulsatile LH release (Estiene et al.,1990). During periods that cows are on a high plane of nutrition and do not mobilize fat, NEFA concentrations in plasma are positively correlated with BCS, but during periods of reduced feed intake cows mobilize fat, and NEFA concentrations in plasma are negatively correlated with changes in BW and BCS (Garmendia, 1984; Vizcarra et al., 1996), however concentrations of NEFA in postpartum beef cows are not predictive of luteal activity (Vizcarra et al., 1996). Concentrations of NEFA in plasma of feed restricted beef cows increased before changes in serum concentrations of LH could be detected (Richards et al., 1989). Therefore it is unlikely that NEFA directly affect the hypothalamo-pituitary axis but concentrations of NEFA in plasma are an indicator of metabolic status in all mammals including ruminants. In

contrast, a role for NEFA in control of ovarian function can not be excluded. In cultures of mouse Leydig cells, NEFA and particularly oleic acid negatively affects LH-induced testosterone production by inhibiting cholesterol esterase and cholesterol utilization (Meikle et al.,1996). A similar role of NEFA on LH-induced androstenedione production by theca cells is possible.

Nutritional modulation of the hypothalamo-pituitary-ovarian axis

Gonadotropin secretion

Reduced feed intake decreases pulsatile LH release in rats (Bronson, 1988), humans (Wheeler et al., 1983), monkeys (Cameron et al., 1985), ewes (Foster and Olster, 1985) and beef cattle (Day et al., 1986; Imakawa et al., 1986; Richards et al., 1989; Kurz et al., 1990). Sensitivity of the pituitary to GnRH during periods of reduced nutrient intake may or may not be compromised, depending mainly on the animal species as well as the intensity of feed restriction. Pituitary responsiveness to GnRH is decreased in malnourished women and men (Vigersky et al., 1977; Klibanski et al., 1981). In growth restricted lambs anterior pituitary function was not compromised by undernutrition since physiological doses of GnRH (2.5-5 ng / kg body weight) readily induce LH and FSH secretion (Ebling et al 1990). In adult female sheep, pituitary responsiveness to GnRH was not altered after 14 wk of reduced feed intake; in fact, pituitary response was increased, rather than decreased, compared with ad libitum fed females (Haresign, 1981). In adult female pigs, pituitary responsiveness to GnRH is also increased following chronic energy restriction, even though LH pulse frequency is dramatically reduced (Britt et al.,

1988). Intravenous infusion of estradiol in lambs maintained at high or low feed intake elicited similar preovulatory like surges of LH (McShane and Keisler, 1991). Cows fed restricted diets released more LH in response to exogenous GnRH as compared with moderate or fat cows (Beal et al., 1978; Whisnant et al., 1985; Rasby et al., 1991) and pulsatile infusion of GnRH initiated luteal activity in nutritionally anestrous beef cows (Bishop and Wettemann, 1993; Vizcarra et al., 1997). In addition, a preovulatory-like surge of LH was observed 24 h after estradiol injection in 2 out of 3 nutritionally anestrous beef cows (Richards et al., 1991). These results indicate that pituitary responsiveness to GnRH is not impaired by feed restriction in cows.

During lactational anestrus, a decrease in pituitary responsiveness does not seem to be the primary cause of infertility. Although pituitary responsiveness to GnRH is reduced in the early postpartum period in cows (Lamming, 1978; Schallenberger et al., 1978) and ewes (Jenkin et al., 1977), this is only temporary. Pituitary responsiveness to exogenous administration of GnRH in lactating dairy cows has recovered by 10-20 days after calving to a level comparable to the luteal phase of the cycle (Schams et al., 1978; Webb et al., 1980). In lactating ewes, a similar recovery of pituitary responsiveness to exogenous GnRH occurred as early as 21 days postpartum (Wright et al., 1981). Since there is no change in either affinity or number of GnRH receptors in the pituitary of postpartum beef cows (Moss et al., 1985; Leung et al., 1986), reduced secretion of LH is presumably due to a reduction in the hypothalamic release of GnRH. In postpartum beef cows, suckling can delay the onset of estrus for a variable time up to 150 days. Typically, time to the onset of estrus would be 25-30 days for milked cows and 55-70 days for suckled cows (Williams, 1990). Increased in pulsatility of LH secretion occurs more rapidly in milked

cows (around d 10 postpartum; Schams et al., 1978) than in suckled cows (around d 50 postpartum; Shively and Williams, 1989). The major increase in LH pulsatile secretion occurs in the few days preceding the first ovulation postpartum, and suckling prolongs the nonpulsatile period (Shively and Williams, 1989).

In prepuberal animals, during periods of reduced nutrition, pituitary function is compromised in some species but not in others. In both young growth-restricted rats and lambs, pituitary responsiveness to GnRH is not altered by feed restriction (Bronson, 1988; Foster et al., 1989). In contrast, in prepuberal cows and pigs, the response to GnRH is reduced during periods of reduced feed intake (Day et al., 1986; Booth, 1990).

Concentration of LH and FSH in the pituitary is unaltered by feed restriction in rats (Bronson, 1988) and sheep (Landefeld et al., 1989). Also, anterior pituitary weight and LH content in nutritionally anestrous beef cows are similar to those observed in cyclic beef cows (Beal et al., 1978; Carruthers et al., 1980; Rasby et al., 1991; Vizcarra et al., 1997). Continuous or hourly intravenous infusion of GnRH for 13 d to anestrous cows resulted in a reduction in pituitary LH content, and ovulation occurred in 6 out of 8 cows that received 1 pulse/h (Vizcarra et al., 1997).

Decreased GnRH secretion during feed restriction may result in reduced biosynthesis of pituitary gonadotropins. Hypothalamic-pituitary disconnection (HPD) of OVX sheep resulted in a rapid decrease in concentrations of LH in serum and a moderate reduction in concentrations of FSH in serum, and these responses were associated with decreased mRNAs for the gonadotropin subunits in the pituitary (Hamernik et al., 1986; Mercer et al., 1988; Mercer et al., 1989). Concentrations of mRNA for gonadotropin subunits were restored after hourly intravenous infusion of GnRH. Biosynthesis of

gonadotropin subunits (mRNAs for the α and LHβ) and concentration of LH in serum and pituitary in growth restricted lambs increased after two weeks of ad libitum feeding (Landefeld et al., 1989). Hourly intravenous infusion of GnRH in chronically underfed OVX ewes restored serum concentrations of LH and pituitary mRNA content for gonadotropin subunits (Kile et al., 1991). These results suggest that GnRH is not only critical for stimulating gonadotropin secretion but also regulates gene expression. A reduction in GnRH neuronal activity during feed restriction may result in decreased secretion and biosynthesis of LH. Thus, reduced secretion of gonadotropin in nutritionally anestrous cows does not alter pituitary content although biosynthesis is reduced.

Serum concentration of FSH in rats is affected by feed restriction only if the restriction is severe and greatly prolonged (Ronnekleiv et al., 1978; Sick and Bronson, 1986; Campell et al ., 1989). Feed restriction does not decrease the number of antral follicles (Meredith et al., 1986) and spermatogenesis proceeds in prepuberal rats provided that feed restriction is imposed after this process has been initiated (Hamilton and Bronson, 1985). Pulses of GnRH given at 30 min intervals in castrated-testosterone replaced male rats increased all three gonadotropin subunits, whereas pulses given at intervals of 120 min or longer increased only FSHβ mRNA (Dalkin et al., 1989). Furthermore, α and LH β subunit mRNAs were maximally increased by more frequent GnRH pulses (1 / 15–60 min) whereas FSHβ responded maximally to less frequent pulse frequencies (1 / 120 min) (Weis et al., 1990; Ishizaka et al., 1992). These results indicate that reduction in GnRH pulse frequency during feed restriction does not affect serum concentration of FSH in the rat. Similarly, less frequent pulses of exogenous GnRH to monkeys reduced concentrations of LH and increased concentration of FSH in serum

(Wildt et al., 1981). Pituitary content of FSH but not LH was reduced in nutritionally anestrous beef cows infused with GnRH at a slow rate of 1 pulse / 4 h, indicating that less frequent pulses of GnRH are required for FSH secretion compared with LH (Vizcarra et al., 1997). In addition, the onset of nutritionally induced anestrus in Bos Indicus heifers was associated with increased circulatory levels of FSH (Rhodes et al., 1996).

GnRH secretion

Decreased GnRH neuronal activity, rather than pituitary responsiveness, is the means by which nutritional deprivation causes reproductive dysfunction or delayed sexual maturation. This inference is made based on results of numerous studies in which concentrations of gonadotropins in serum of underfed animals can be increased by exogenous administration GnRH. Normal gonadotropin secretion was restored after exogenous GnRH administration to fasting male rhesus monkeys (Dubey et al., 1986), and to women with anorexia nervosa (Marshall and Kelch, 1979). In feed restricted prepuberal rat, sexual maturation can be initiated by administration of GnRH (Bronson, 1986). Pulsatile administration of GnRH resulted in luteal activity in nutritionally anestrous female pigs within 7 days (Armstrong and Britt, 1987).

There is little information regarding GnRH synthesis during feed deprivation. Feed restriction for 5 days in male rats, reduced the number of neurons containing mRNA for GnRH but not intracellular content in medial preoptic area (MPOA) and diagonal band of Broca (Gruenewald and Matsumoto, 1993). In contrast, hypothalamic content of mRNA for GnRH was not different between well fed and undernourished lambs (McShane et al., 1993). Hypothalamic content of GnRH is not altered in prepuberal growth restricted

female rats (Bronson, 1988). Content of GnRH in the median eminence of nutritionally restricted lambs was not different compared with well fed lambs (Ebling, 1990). Hypothalamic content of GnRH was actually increased in the adult male rats during acute undernutrition (Pirke and Spyra, 1981). Similarly, cows fed restricted diets had increased concentrations of GnRH in the median eminence (Rasby et al., 1992). In support of these findings, intravenous injections of NMA (N-methyl-d,l-aspartate) in nutritionally restricted lambs produced LH pulses similar to those induced by a physiological dose of GnRH (Ebling et al., 1990), indicating that GnRH release rather than synthesis is the limiting factor during feed restriction.

A possible neurotransmitter which may inhibit secretion of GnRH during feed restriction is neuropeptide-Y (NPY). This neuropeptide is widely distributed in the CNS, especially within the arcuate nucleus and the suprachiasmtic nucleus (Allen et al., 1983; Gray and Marley, 1986). Intracerebraventricular administration of NPY causes a decrease in LH secretion in gonadectomized male and female rats (Karla and Crowley,1984; Kerkerian et al., 1985; McDonald et al., 1985) and rabbits (Khorram et al., 1987), but increases LH secretion in intact or steroid-treated animals (Karla and Crowley, 1984). Intracerebraventricular administration of NPY to rats and sheep induced feeding behavior (McDonald, 1988; Miner et al., 1989), and in sheep reduced LH secretion (Malven et al., 1992). Feed restriction increased concentrations of NPY in the arcuate and paraventricular nuclei of rat hypothalami (Sahu et al., 1988) and cerebrospinal fluid (CSF) of sheep (McShane et al., 1992). Feed restricted lambs had greater hypothalamic concentrations of mRNA for NPY compared with well fed lambs (Ober and Malven,

1992; McShane et al., 1993). Thus, increased concentrations of NPY in the hypothalamus during reduced feed intake may inhibit GnRH release.

Gonadal function

Metabolic hormones and growth factors have important actions at the ovary to either attenuate gonadotropic stimulation or to amplify the response to other stimuli. However there is little direct evidence to indicate the relative importance of these local mechanisms in mediating the effects of nutrition and body energy reserves on reproduction. Administration of bST increases the number of medium size follicles in cows without any changes in serum concentrations of gonadotropins (Gong et al., 1991; Lucy et al., 1993). Greater concentrations of IGF-I in plasma have been observed in cattle and sheep selected for increased twining rate (Echternkamp et al., 1990; Spicer et al., 1993). Administration of glucose and branched chain amino acids to sheep, increased ovulation rate without any alternations in serum concentrations of gonadotropins (Downing and Scaramuzzi, 1991). However the mechanisms controlling ovulation rate are independent of those determining if a single follicle will ovulate. Direct actions of nutrition at the ovary are probably more important in multiple ovulators such as pigs, compared with mono-ovulatory cows.

Reduced nutrient intake in cyclic (Murphy et al., 1991) and prepuberal (Bergfeld et al., 1994) beef heifers decreased persistence and maximum size of the dominant follicle and tended to increase the incidence of three wave-cycles (Murphy et al., 1991). A linear reduction in persistence, maximum size of dominant and ovulatory follicles and corpora lutea, with decreasing body weight and condition score occurred in feed restricted beef

heifers, whereas a linear increase in persistence, growth rate and maximum size of dominant follicles was observed during realimentation of nutritionally anestrous beef heifers (Rhodes et al., 1995). Restricted energy intake in postpartum beef cows reduced the size of dominant follicles and the number of large estrogen-active follicles and increased the persistence of small subordinate follicles indicating reduced dominance by the larger follicle (Perry et al., 1991). Negative energy balance in the postpartum dairy cows was associated with decreased maximum diameter of dominant follicles and increased number of medium size follicles (Lucy et al., 1991). Nevertheless, it should be noted that alterations in follicular growth and function are probably mediated through alterations in gonadotropin secretion since luteal activity can be induced in nutritionally anestrous beef cows and pigs by exogenous administration of GnRH. In addition, ovaries of growth restricted lambs are capable of secreting estradiol and inducing a preovulatory-like surge of LH and ovulation after exogenous administration of LH at similar levels required to induce ovulation in seasonally anestrous but well fed prepuberal lambs.

Concentrations of estradiol in plasma decrease during the late follicular phase before the onset of nutritionally induced anestrus in Bos indicus heifers (Rhodes et al., 1996). Reduced nutrient intake for 10 weeks resulted in decreased intrafollicular concentrations of estradiol (Spicer et al., 1991) in healthy follicles. In addition, well fed prepuberal heifers had greater concentrations of estradiol in plasma compared with feed restricted heifers (Bergfeld et al., 1994).

Feed restriction does not alter estrous cycle length (Murphy et al., 1991; Rhodes et al 1996). Conflicting evidence exist about the effects of underfeeding on progesterone concentrations. Feed restriction increases (Donaldson et al., 1970; McCann and Hansel,

1986), decreases (Hill et al., 1970; Gombe and Hansel, 1973; Imakawa et al., 1986; Villa-Godoy et al., 1990;) or has no effect (;Spitzer et al., 1978; Shrick et al., 1990; Murhpy et al., 1991; Rhodes et al., 1996;) on peripheral concentrations of progesterone. Differences in severity of undernutrition and sampling periods may account for the differences in progesterone concentrations. However, cows fed restricted diets had decreased CL weights (Rasby et al., 1991) and a linear decrease in the maximum CL size is associate with decreasing body weight and condition (Rhodes et al., 1996). Since concentrations of receptors for LH in bovine CL are not decreased with feed restriction (Schrick et al., 1992), reduced secretion of LH during food deprivation is probably the primary cause for reduced CL function.

The influence of nutrition and body energy reserves on reproductive function is mediated primarily through changes in LH secretion. Whether alteration of body condition in cattle has direct effects on the ovary is unknown. The mechanisms by which nutrition and body energy reserves influence the GnRH pulse generator have not been elucidated. Alterations in metabolic hormones, energy substrates and fat depots may provide the signals for reduced GnRH secretion during restriction of energy intake. Evidence exist to support that changes in concentrations of IGF-I, insulin and glucose may affect the hypothalamo-pituitary-ovarian axis. Ruminants maintain cyclicity after extended periods of reduced feed intake. Ovarian function, secretion of hormones and concentrations of energy substrates in blood of cattle immediately before nutritionally induced anovulation and in the follicular waves immediately before resumption of ovulation after realimentation have not been evaluated. Follicular growth in cycling cattle has been extensively studied since the development of ultrasounding techniques.

Endocrine regulation of follicular growth preceding nutritionally induced anovulation and resumption of ovulation after realimentation has not been elucidated.

CHAPTER III

NUTRITIONALLY INDUCED ANOVULATION IN BEEF HEIFERS: OVARIAN AND ENDOCRINE FUNCTION PRECEDING CESSATION OF OVULATION

Abstract: Eighteen cyclic Angus x Hereford heifers with moderate to good body condition (BCS of 5.4 $\pm$ 0.2 and BW of 378 $\pm$ 15 kg) were used to determine endocrine, and ovarian function preceding nutritionally induced cessation of ovarian cycles. In two replications (fall of 1994 and fall of 1995), a total of six heifers were fed to maintain BCS (M group) and growth, while 12 heifers were fed a restricted diet (R group) to lose 1 % of their BW/wk. At the initiation of the study, estrous cycles of all heifers were synchronized by treatment with $PGF_{2\alpha}$ (Lutalyse) followed by a second treatment 11 d later. Starting on d 13 of the induced cycle, heifers were given $PGF_{2\alpha}$ every 16 d thereafter to synchronize and maintain 16 d estrous cycles. Transrectal ultrasonography was performed daily every second cycle to monitor the ovaries from d 8 until ovulation (d 1 of the subsequent cycle). When heifers had lost 12 % of their initial body weight, ultrasonography was performed every cycle until R heifers became anovulatory. Size and growth rate of the ovulatory follicle and maximum corpus luteum size were determined during the last ovulatory cycle (cycle -2) and the subsequent anovulatory cycle (cycle -1). Concentrations of LH, FSH and GH were quantified in serum samples collected every 10 min for 8 h on d 2 and d 15 (48 h post-$PGF_{2\alpha}$) during cycles -2 and -1. Estradiol,

progesterone, glucose, insulin, IGF-I and NEFA were quantified in daily plasma samples from d 8 until d 16 during cycles -2 and -1. Restricted heifers became anovulatory at a BW of 298 ± 3 kg and a BCS of 3.8 ± 0.1, after losing 22 ± 2 % of their initial BW. Maintenance heifers had larger ovulatory follicles than R heifers (15.7 ± .8 mm and 10.4 ± .7, respectively, $P < .0001$) and greater growth rate of the ovulatory follicles (1.4 ± .1 mm/d and .87 ± .1 mm/d, respectively; $P < .0001$). There was a treatment x cycle effect ($P < .1$) for maximum CL size. Maintenance heifers had greater maximum CL size (19.7 ± .6 and 20.2 ± 1 mm for cycles -2 and -1, respectively) than R heifers (15.5 ± .4 and 14.1 ± .7 mm for cycles -2 and -1, respectively). There was a treatment x day effect ($P < .05$) on progesterone concentrations. Maintenance heifers had greater progesterone concentrations compared with R heifers during both cycles -2 and -1. There was a treatment x cycle x day effect ($P < .001$) for concentrations of estradiol. Maintenance heifers had greater estradiol concentrations compared with R heifers during cycle -1, but not during cycle -2. The preovulatory increase in estradiol after induced luteolysis in R heifers occurred only in cycle -2 but not during cycle -1. A treatment x cycle x day effect ($P < .05$) influenced LH concentrations. During cycle -2, LH concentrations were similar for M and R heifers, but during cycle -1, M heifers had greater LH concentrations on both d 2 and d 15. There was a treatment x day effect ($P < .001$) on LH pulse frequency, and M heifers had greater LH pulse frequency only on d 15 of the cycle but not on d 2, compared with R heifers. A treatment x cycle effect ($P < .001$) influenced LH pulse amplitude, and M heifers had a greater pulse amplitude than R heifers during cycle -1 but not during cycle -2. There was a treatment x cycle x day effect ($P < .005$) on concentrations, pulse frequency and pulse amplitude of FSH, and R heifers had greater

concentrations, pulse frequency and pulse amplitude of FSH compared with M heifers only on d 15 of cycle -1. Restricted heifers had greater concentrations and pulse amplitude of GH compared with M heifers ($P < .0001$) however pulse frequency was similar for M and R heifers. There was a treatment x cycle effect ($P < .05$) on IGF-I concentrations, and M heifers had greater IGF-I concentrations compared with R heifers. Maintenance heifers had greater glucose and insulin concentrations than R heifers ($P < .01$). There was a treatment x cycle effect ($P < .05$) on NEFA concentrations, and R heifers had greater NEFA concentrations compared with M heifers. We conclude that growth rate and size of the ovulatory follicle, max CL size and concentrations of LH in serum and progesterone, estradiol, IGF-I, insulin and glucose in plasma are reduced, and GH and FSH in serum and NEFA in plasma are increased before the onset of nutritionally induced anovulation in beef heifers.

Key Words: Gonadotropins, Heifers, Nutrition, Ovarian Function, Steroids

Introduction

Nutrition is a major factor determining reproductive efficiency of beef cattle. Reduced nutrient intake delays the onset of puberty in heifers (Yelich et al., 1996) and increases the postpartum interval to first conception in beef cows (Dunn and Kaltenbach, 1980; Wettemann et al., 1980; Selk et al., 1988). Prolonged restriction of dietary energy in cattle results in loss of body weight and body condition, and cessation of estrous cycles (Richards et al., 1989).

Restriction of feed intake in beef cattle suppresses secretion of LH (Day et al., 1986; Richards et al., 1989; Yelich et al., 1996). This effect is probably mediated by

reduced GnRH secretion since thin cows have greater concentration of gonadotropin releasing hormone (GnRH) in the hypothalamic stalk median eminence (Rasby et al., 1992) and exogenous administration of GnRH to nutritionally anestrous cows induces luteal activity (Bishop and Wettemann, 1993; Vizcarra et al., 1997). Feed restriction increases (Rhodes et al., 1996) or has no effect (Wright et al., 1990; Stagg et al., 1995) on FSH concentrations in serum preceding nutritionally induced anovulation.

Reduced nutrient intake in cyclic (Murphy et al., 1991) and prepuberal (Bergfeld et al., 1994) beef heifers decreased persistence and maximum size of dominant follicles. A linear reduction in persistence, maximum size of dominant and ovulatory follicles, and corpora lutea (CL) size with decreasing body weight and condition score was observed when feed was restricted for beef heifers (Rhodes et al., 1995). Similarly, reduced energy intake by postpartum beef cows decreased the size of the dominant follicle and the number of large estrogen-active follicles and increased the persistence of small subordinate follicles (Perry et al., 1991).

Feed restriction dramatically reduces IGF-I and increases GH concentrations in blood of cattle (Breier et al., 1986; Granger et al., 1989; Yelich et al., 1996) and concentrations of glucose, IGF-I and insulin are decreased and concentrations of NEFA increased in plasma of nutritionally anestrous beef cows (Richards et al., 1989b; Richards et al., 1991). Alterations in metabolic hormones such as GH, insulin and IGF-I, and blood metabolites such as glucose and NEFA, are indicative of energy availability and may provide short- or long-term signals that mediate the effects of undernutrition on the hypothalamo-pituitary-ovarian axis.

The objectives of this experiment were to evaluate follicular growth and concentrations of LH, FSH and GH in serum and progesterone, estradiol, IGF-I, insulin, glucose and NEFA in plasma during the last two cycles before the onset of nutritionally induced anovulation in beef heifers.

Materials and Methods

Eighteen cyclic Angus x Hereford heifers with moderate to good body condition (BCS = 5.4 $\pm$ 0.2; 1 = emaciated, 9 = obese, Wagner et al., 1989; BW = 378 $\pm$ 15 kg) were used. In two replications (fall of 1994 and fall of 1995), a total of 6 heifers were fed to maintain BCS (M group), while 12 heifers were fed a restricted diet (R group) to lose 1 % of their BW/wk (Table 1). Body weight and BCS were recorded every 2 and 4 wk, respectively. At the initiation of the study, estrous cycles of all heifers were synchronized with an injection of $PGF_{2\alpha}$ (Lutalyse, 25 mg; The Upjohn Company, Kalamazoo, MI) followed with a second injection 11 d later. Starting on d 13 of the induced cycle, heifers were given PGF_2a every 16 d for 11 to 17 cycles to synchronize and maintain 16 d estrous cycles until they became anovulatory. Transrectal ultrasonography was performed daily with an Aloka 500V ultrasound scanner equipped with a 7.5-MHz transducer (Corometrics Medical Systems, Wallingford, CT) every second cycle to monitor ovaries from d 8 of the cycle until ovulation (d 1 of the subsequent cycle). During ultrasound scanning, approximate position and size of follicles and CL in both ovaries were sketched. Scans of the ovaries were also recorded on a video camera recording tape and viewed later to draw complete ovarian maps recording all follicles $\geq$ 4 mm and CL. Reference points on the ovaries included the poles, the hilus and CL (Ginther et al., 1989). Size of

follicles and CL were calculated as the mean of the longest and shortest diameters. The size of CL with fluid-filled cavities was estimated by subtracting the diameter of the cavity from the diameter of the entire CL (Savio et al., 1988). Day of emergence of the ovulatory follicle was defined as the day before the first day that the ovulatory follicle could be individually identified. Growth rate of the ovulatory follicle was estimated as the increase in diameter from the day of emergence to the maximum diameter divided by days of growth. When heifers lost 12 % of their initial body weight, ultrasonography was performed every cycle until R heifers became anovulatory. During the cycles that ultrasonography was performed, blood samples were collected every 10 min for 8 h on d 2 (1 d after ovulation) and d 15 (48 h post-$PGF_2\alpha$). The day before sampling, a polyvinyl cannula (Bolab Inc., BB 317-V/10, inside diameter .062 inches, outside diameter .082 inches, Lake Havasu City, Arizona) was inserted into a jugular vein of each heifer, and animals were confined to stalls. Blood samples were allowed to clot for 24 h at 4^0 C, centrifuged at 2,800 g for 30 min and serum was stored at -20^0 C until analyzed. In the same cycles, a preprantial blood sample was collected each day from d 8 until ovulation via tail venipuncture in 15 mL tubes containing EDTA (.1 ml of a 15 % solution). Tubes were placed on ice and centrifuged within 1 h at 2,800 g for 15 min, and plasma was stored at -20 C^0 until analyzed. Size and growth rate of the ovulatory follicle and maximum CL size were determined during the last ovulatory cycle (cycle -2) and the subsequent anovulatory cycle (cycle -1) in R heifers and ovarian and endocrine function were evaluated on the same days in M heifers. Concentrations of LH, FSH and GH were determined in serum collected every 10 min for 8 h on d 2 and d 15 during cycles -2 and-1. Concentrations of estradiol, glucose, insulin, IGF-I and NEFA were quantified in daily

plasma samples collected from d 8 until d 16 and progesterone was quantified in daily samples from d 8 until d 15 during cycles -2 and -1.

Progesterone concentrations in plasma were quantified with a solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997). Intra- and interassay coefficients of variation (n = 6 assays) were 3 % and 6 %, respectively. Estradiol-17β concentrations in plasma were quantified by RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intra- and interassay coefficients of variation (n = 6 assays) were 11 % and 14 %, respectively. Concentrations of LH in serum were quantified by RIA (Bishop and Wettemann, 1993) with NIH LH-B9 for standards and intra- and interassay coefficients of variation (n = 24 assays) were 8 % and 16 %, respectively. Concentrations of FSH in serum were quantified by RIA (Vizcarra et al., 1997) with USDA-bFSH-I-2 for standards and intra- and interassay coefficients of variation (n = 24 assays) were 3 % and 9 %, respectively. Concentrations of GH in serum were quantified by RIA (Yelich et al., 1995) with NIH-GH-B17 for standards and intra- and interassay coefficients of variation (n = 24 assays) were 5 % and 15 %, respectively. Concentrations of IGF-I in plasma were quantified by RIA (Echternkamp et al., 1990) after acid ethanol extraction. Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used for standards and intra- and interassay coefficients of variation (n = 6 assays) were 3 % and 9 %, respectively. Concentrations of insulin in plasma were quantified by solid phase RIA for human insulin (Coat-A-Count Insulin kit, Diagnostic Products Corp., Los Angeles, CA) using bovine pancreatic insulin for standards (28.6 USP units / mg, Sigma Chem. Co., St Louis, MO) and .2 mL sample volume. Sensitivity of the assay was 0.05 ng / mL plasma and addition

of .8, 1.6 and 3.2 ng of insulin in 1 mL of plasma resulted in recovery of 97 %, 109 % and 108 %, respectively (n = 4). When .05, .10, .15 and .20 mL of plasma were assayed, concentrations were parallel to the standard curve. Intra- and interassay coefficients of variations (n = 6 assays) were 4 % and 8 %, respectively. Concentrations of glucose in plasma were determined by an enzymatic colorimetric procedure (Sigma, No.510, Sigma Chemical Co., St. Louis, MO) and intra- and interassay coefficients of variations (n = 6 assays) were 4 % and 9 %, respectively. Concentrations of NEFA in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C, Wako Chemicals Inc., Dallas, TX) with modification (McCutcheon and Bauman, 1986). Intra- and interassay coefficients of variation (n = 6 assays) were 8 % and 14 %, respectively.

Pulse frequency and amplitude of LH, FSH and GH were determined using the pulsar program (Merriam and Wachter, 1982). The G values used for LH were G1=99, G2=3.25, G3=2.75, G4=2.25, G5=1.75; for FSH, G values were G1=99, G2=3.5, G3=3, G4=2.5 G5=99; and for GH, G values were G1=99, G2=4.5, G3=4, G4=3.5, G5=3. The G values are chosen to serve as criteria to determine if variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variations in concentrations. Pulsar has an empirically derived set of G values [G(1-5) of 3.8, 2.6, 1.9, 1.5 and 1.2, respectively) calibrated at the P=.01 level for human LH concentrations. Often G1 value is set at 99 to exclude a one sample increase in concentration to be identified as a pulse, which by our definition of pulses can not occur considering the frequency at which samples are collected. Sometimes G5 was also set to 99 to avoid the false positive determination of a small increase followed by a return to baseline as a pulse.

Depending on how conservative you want to be in identifying increase in hormone concentrations as pulses, you determine the G values.

Split plot ANOVA were used to determine treatment effects on day of emergence, growth rate and maximum size of the ovulatory follicle (or the anovulatory dominant follicle of the second wave in R heifers during cycle -1) and maximum CL size during the last two cycles before anovulation. Treatment (M and R), replication (rep) and treatment x rep were the main plot, and cycle (-2 and -1), treatment x cycle, rep x cycle and treatment x rep x cycle were the subplot. Mean square error (MSE) for heifer within treatment x rep was used as the error term for the main plot effects. Split-split plot ANOVA were used to determine treatment effects on concentrations, pulse frequency and amplitude of LH, FSH and GH. Treatment, rep and treatment x rep was the main plot, cycle, treatment x cycle, rep x cycle and treatment x rep x cycle x was the subplot and day (d 2 and d 15), treatment x day, cycle x day, rep x day, treatment x cycle x day, treatment x rep x day, rep x cycle x day and treatment x cycle x rep x day was the subsubplot. The MSE for heifer within treatment x rep was used as the error term for the main plot effects. Heifer within treatment x cycle x rep was the error term for the subplot. The residual MSE was used as the error term for the subsubplot. Tukey-Kramer's procedure (unequal cell size) for pairwise comparisons was used to compare treatment means (SAS, 1990). Multivariate analyses of variance for repeated measures were used to determine treatment effects on progesterone, estradiol, IGF-I, insulin, glucose, and NEFA concentrations during the last two cycles before anovulation. Concentrations of hormones and metabolites from d 8 through d 16 (d 8 to d 15 for progesterone) during the last two cycles before anovulation were the repeated response variable (within-subject factors).

The between-subject factors were treatment, rep and treatment x rep in the main plot, and cycle, treatment x cycle, rep x cycle and treatment x rep x cycle were the subplot. Because interactions of treatment with rep were either not significant or were due to differences in the magnitude of the response and not in direction, data from the two replicates were combined and rep was removed from the model. If interactions with day were significant and sampling was performed on more than two days, polynomial response curves of appropriate order were fit and tested for homogeneity of regression (Snedecor and Cochran, 1968) to evaluate treatment effects. In any other case, Tukey-Kramer's procedure was used to compare means.

Results

Reduced nutrient intake resulted in loss of BW in R heifers and ovulation ceased 32 ± 3 wk after initiation of feed restriction. Maintenance heifers gained $.43 \pm .05$ kg/d and R heifers lost $.38 \pm .07$ kg/d (Table 2). During treatment R heifers lost $22 \pm 2\%$ and M heifers gained $19 \pm 2\%$ of their initial BW, and at anovulation R heifers had less ($P < .001$) BCS ($3.8 \pm .3$) compared with M heifers ($5.3 \pm .3$).

Nutritional treatment did not influence the interval from treatment with $PGF_{2\alpha}$ to ovulation. During cycle -3, nine R and four M heifers ovulated 4 d after $PGF_{2\alpha}$ and three R and two M heifers ovulated 5 d after treatment. During cycle -2, ten R and five M heifers ovulated 4 d after $PGF_{2\alpha}$ and two R and one M heifers ovulated 5 d after $PGF_{2\alpha}$. During cycle -1, all M heifers ovulated 4 d after $PGF_{2\alpha}$ treatment and none of the R heifers ovulated. Day of emergence of ovulatory follicle (anovulatory dominant follicle of the second wave in R heifers during cycle -1) during cycle -2 and cycle-1 was not influenced

by treatment (P>.1). Ovulatory follicles emerged on d 10.6 ± 1.2 of the estrous cycle for heifers on both treatments and were identified at a diameter of ≥4 mm (Table 3). Maintenance heifers had larger (P<.0001) ovulatory follicles (15.7 ± .9 mm) compared with R heifers (10.4 ± .9 mm) during the last two cycles before anovulation. Growth rate of the ovulatory follicle was greater (P<.001) for M (1.4 ± .1 mm/d) than R heifers (.87 ± .1 mm/d) in both cycles -2 and -1. Growth rate and size of the ovulatory follicle (or dominant follicle of the second wave in the anovulatory cycle) were not different between cycle -2 and -1 in R heifers. In both M and R heifers, CL were maximal in size on d 13 of the cycle but there was a treatment x cycle interaction (P<.05) for maximum CL size. Maintenance heifers had a greater maximum CL size (19.7 ± .6 and 20.4 ± 1.0 mm for cycle -2 and -1, respectively) compared with R heifers (15.5 ± .4 and 14.1 ± .7 mm for cycle -2 and -1, respectively).

There was a treatment x day of cycle effect (P<.05) for progesterone concentrations during the last two cycles before anovulation (Figure 1), but progesterone concentrations were similar between cycle -2 and -1 in both R and M heifers. Concentrations of progesterone for the two treatments were best described by quintic regression equations (Figure 1). Analysis of heterogeneity of regression indicated that concentrations of progesterone for M heifers were different from those in R heifers (P<.01).

There was a treatment x cycle x day effect (P<.001) on concentrations of estradiol during the last two cycles before anovulation. During cycle -2, concentrations of estradiol in M and R heifers were best described by cubic regression equations (Figure 2). Analysis of heterogeneity of regression indicated that concentrations of estradiol during cycle -2

were similar (P>.1) for M and R heifers. During cycle -1, concentrations of estradiol in M and R heifers were best described by cubic regression equations (Figure 2) and analysis of heterogeneity of regression indicated that M heifers had greater estradiol concentrations than R heifers (P<.003). An increase in concentrations of estradiol after induced luteolysis occurred in R heifers during cycle -2 but not during cycle -1.

There was a treatment x cycle x day effect (P<.01) on LH concentrations. During cycle -2, concentrations of LH were similar for M and R heifers, however, during cycle -1, M heifers had greater (P<.05) LH concentrations on both d 2 and d 15 than R heifers (Table 4). There was a treatment x day effect (P<.0001) on LH pulse frequency and M heifers had more (P<.05) pulses of LH than R heifers on d 15 but not on d 2 during the last two cycles before anovulation (Table 4). There was a treatment x cycle effect (P<.001) on LH pulse amplitude and M heifers had greater pulse amplitude (P< .05) than R heifers during cycle -1 but not during cycle -2 (Table 4).

There was a treatment x cycle x day effect (P<.005) on concentrations, pulse frequency and pulse amplitude of FSH. Restricted heifers had greater (P<.05) concentration, pulse frequency and pulse amplitude of FSH compared with M heifers only on d 15 of cycle -1 (Table 5).

Cycle and day did not influence secretion of GH. Restricted heifers had greater concentrations (P<.0001) and greater (P<.0001) pulse amplitude of GH compared with R heifers during the last two cycles before anovulation (Table 6). Treatment did not influence the frequency of GH pulses.

There was a treatment x cycle effect (P<.05) on IGF-I concentrations. Day did not influence concentrations of IGF-I, so data were averaged across days. Maintenance

heifers had greater (P<.0001) IGF-I concentrations than R heifers in both cycles -2 and -1 (Table 7). Concentrations of IGF-I in restricted heifers during cycle -2 were greater compared with cycle -1.

Treatment influenced concentrations of glucose and insulin in plasma (P<.01). Cycle and day did not influence insulin and glucose concentrations. Concentrations of glucose and insulin were less in R heifers compared M heifers during both cycles -2 and -1 (Table 7).

There was a treatment x cycle effect (P<.05) for NEFA concentrations. Restricted heifers had greater (P<.001) concentrations of NEFA than M heifers in both cycles -2 and -1, and the concentrations of NEFA in R heifers during cycle -2 were greater compared with cycle -1 (Table 7).

Discussion

A reduction in feed intake by R heifers resulted in loss of BW and BCS and failure of ovulation at an average of 32 wk after initiation of feed restriction. At the time of anovulation, R heifers had lost 22% of their initial BW and 30% of their initial BCS. During the same period M heifers had gained 19% of their initial BW and maintained their BCS. Richards et al. (1989) found that beef cows became anestrous 26 wk after initiation of feed restriction. In that study, cows had lost 24% and 36% of their initial BW and BCS, respectively. Similarly, Rhodes et al. (1996) found that beef heifers became anovulatory 23 wk after initiation of feed restriction and this was accompanied by a 19% loss of initial BW.

Feed restriction reduced follicular growth rate and maximum size of the ovulatory follicle during the last two cycles before anovulation. Reduced nutrient intake of cyclic (Murphy et al., 1991) and prepubertal (Bergfeld et al., 1994) beef heifers also resulted in decreased maximum size of the dominant follicle. A linear reduction in the maximum size of the dominant and ovulatory follicles occurred when BW and BCS were decreased during feed restriction of beef heifers (Rhodes et al., 1995). Similarly, reduced energy intake in postpartum beef cows (Perry et al., 1991) and negative energy balance in postpartum dairy cows (Lucy et al., 1991) were associated with decreased maximum diameter of dominant follicles. In our study, the maximum size of the dominant follicles was less in R than M heifers during both cycle -2 and -1. However, growth rate and maximum size of the dominant follicle in R heifers were not different between cycle -2 (the last ovulatory) and cycle -1 (the anovulatory cycle). Therefore a reduction in size alone does not determine the ability of a dominant follicle to ovulate.

Concentration of estradiol in plasma of M and R heifers during cycle -2 were similar, even though there was a substantial (34 %) decrease in the size of the ovulatory follicle in R heifers compared with M heifers. Suprisingly this difference in follicle size was similar to the difference in BW (i.e., 33%) between M and R heifers at anovulation. Although the maximum size of the dominant follicle was similar during cycles -2 and -1 within R heifers, concentrations of estradiol were less during cycle -1 vs -2 and a preovulatory increase in estradiol did not occur during the anovulatory cycle. The rate of metabolic clearance of estradiol-17β in ewes was not affected by dietary intake, however the rate of metabolic clearance of metabolites of estradiol-17β and particularly estradiol-17α sulfate was reduced in feed restricted ewes indicating that the overall estrogenic

activity may be increased due to feed restriction (Adams et al., 1994). These observations indicate that a reduction in the maximum size of the dominant ovulatory follicle in feed restricted cycling heifers is not always associated with reduced peripheral estradiol concentrations. Rhodes et al. (1996) found a reduction in concentrations of estradiol in plasma of feed restricted beef heifers in the days following luteolysis during the last estrous cycle before anovulation, but concentrations of estradiol during the last ovulatory cycle were not reported. Prepuberal heifers that were adequately fed had greater estradiol concentrations compared with feed restricted heifers, and concentrations were associated with size of the dominant follicles (Bergfeld et al. 1994). They suggested that greater concentrations of estradiol in adequately fed heifers could be the result of greater concentrations of LH in blood.

Concentration and pulse amplitude of LH were reduced in R heifers during cycle -1 but not during cycle -2, while pulse frequency of LH was reduced in R heifers during the late follicular phase compared with M heifers in both cycles -2 and -1. Reduction in LH pulse frequency may result in decreased maximum size of the dominant follicle, while the reduction in LH concentrations results in decreased estradiol concentrations and failure of ovulation. Suppression of pulsatile LH secretion in cattle with a GnRH agonist resulted in a substantial decrease in the maximum size of the dominant follicle (Gong et al., 1995). Reduction in the size of dominant follicles was observed in feed restricted postpartum beef cows compared with adequate fed cows and this was associated with reduced LH pulse frequency (Perry 1991). The relative large increase in the size of dominant follicles in the month preceding puberty (Burgled et al., 1994) in beef heifers was attributed to increased LH pulse frequency prior to puberty (Kinder et al., 1995).

Reduced feed intake decreases pulsatile LH release in rats (Bronson), humans (Wheeler et al., 1983), monkeys (Cameron et al., 1985), lambs (Foster and Olster, 1985) heifers (Day et al., 1986) and cows (Richards et al., 1989). In ewes, cows and sows, pituitary responsiveness to GnRH during periods of reduced nutrient intake is not compromised. In growth restricted lambs, anterior pituitary function was not altered by undernutrition since physiological doses of GnRH induce LH and FSH secretion (Ebling et al., 1990). Intravenous infusion of estradiol in lambs maintained at high or low feed intakes elicited similar preovulatory-like surges of LH (McShane and Keisler, 1991). In addition, a preovulatory-like surge of LH was observed 24 h after estradiol injection in 2 of 3 nutritionally anestrous beef cows (Richards et al., 1991). Pituitary responsiveness to GnRH was not influenced in adult female sheep after 14 wk of reduced feed intake; in fact, pituitary response was increased, rather than decreased, compared with ad libitum fed females (Haresign, 1981). Similarly, thin cows that had been fed restricted diets released more LH in response to exogenous GnRH as compared with moderate or fat cows (Beal et al., 1978; Whisnant et al., 1985; Rasby et al., 1991). Pituitary responsiveness to GnRH is increased in the adult female pig following chronic energy restriction even though LH pulse frequency is dramatically reduced (Britt et al., 1988). Pulsatile infusion of GnRH initiated luteal activity in nutritionally anestrous pigs within 7 d (Britt et al., 1987) and in nutritionally anestrous beef cows within 13 d (Bishop and Wettemann, 1993; Vizcarra et al., 1997).

Feed restriction for 5 d in male rats, reduced the number of neurons containing mRNA for GnRH but intracellular content in medial preoptic area and diagonal band of Broca was not affected (Gruenewald and Matsumoto, 1993). In contrast, hypothalamic

content of mRNA for GnRH was similar for well fed and undernourished lambs (McShane et al., 1993). Hypothalamic content of GnRH is not altered in prepubertal growth restricted female rats (Bronson, 1988), and increases in the adult male rats during acute undernutrition (Pirke and Spyra, 1981). Content of GnRH in the median eminence of nutritionally restricted lambs was not different compared with well fed lambs (Ebling, 1990). However, when cows were fed restricted diets and became anestrous, concentrations of GnRH in the median eminence were increased compared with cows fed adequate diets (Rasby et al., 1992). Intravenous injections of NMA (N-methyl-d,l-aspartate) in nutritionally restricted lambs produced LH pulses similar to those induced by a physiological dose of GnRH (Ebling et al., 1990) indicating that GnRH release rather than synthesis is the limiting factor during feed restriction. Reduced GnRH secretion during feed restriction may decrease synthesis of pituitary gonadotropins. The rapid decrease in serum LH after hypothalamic-pituitary disconnection in ewes was associated with decreased mRNAs for the gonadotropin subunits in the pituitary, and exogenous administration of GnRH restored concentrations of mRNA for the gonadotropin subunits in the pituitary (Hamernik et al., 1986; Mercer et al., 1988; Mercer et al., 1989). Synthesis of gonadotropin subunits (mRNAs for α and LHβ) and peripheral LH concentrations increased after growth restricted lambs received ad libitum feed for two weeks (Landefeld et al., 1989). Intravenous infusion of GnRH restores secretion and mRNA for gonadotropins in ovariectomized ewes after prolonged periods of feed restriction (Kile et al., 1991). Thus, GnRH is not only essential for gonadotropin secretion but also regulates gene expression and biosynthesis of LH. Vizcarra et al. (1997) found that anterior pituitary weights and LH content in nutritionally anestrous beef

cows were similar with those previously observed in cyclic beef cows. In addition, feed restriction did not affect pituitary LH and FSH content in rats (Bronson, 1988) and sheeps (Landefeld et al., 1989). Pituitary content of LH may not be altered after chronic feed restriction because less LH is secreted. In our study, the reduction in LH pulse frequency during cycle -2 was followed by a reduction in LH pulse amplitude and mean concentrations during cycle -1.

Although LH secretion is reduced before the onset of nutritionally induced anovulation, FSH secretion increased during the late follicular phase of the anovulatory cycle (cycle -1) in R heifers compared with M heifers, and this was associated with reduced concentrations of estradiol in plasma. In rats, FSH is affected by feed restriction only if the restriction is severe and greatly prolonged (Campell et al ., 1989; Sick and Bronson, 1986; Ronnekleiv et al., 1978), and feed restriction does not decrease the number of antral follicles (Meredith et al., 1986). Less frequent exogenous GnRH pulses to monkeys reduced concentrations of LH and increased concentration of FSH in serum (Wildt et al., 1981). Pituitary content of FSH but not LH was reduced in nutritionally anestrous beef cows infused with GnRH at a slow rate of 1 pulse / 4 h, indicating that less frequent pulses of GnRH are required for FSH secretion compared with LH (Vizcarra et al., 1997). The onset of nutritionally induced anestrus in Bos Indicus beef heifers was associated with increased concentrations of FSH in serum (Rhodes et al., 1996). Stagg et al. (1995) found that concentrations of FSH were similar during normal estrous cycles, nutritionally induced anestrus and resumption of cyclicity after realimentation of nutritionally anestrous heifers. Emergence of a follicular wave is preceded by a surge of FSH which is coincident with the cessation of growth of a dominant nonovulatory follicle

or ovulation (Adams et al. 1992). Increased FSH concentrations preceding emergence of a follicular wave have been attributed to declining concentrations of inhibitory substances (estradiol, inhibin or other proteins) originating from the dominant follicle of the previous wave (Ginther et al., 1996). During the first anovulatory cycle in the present study, arrest of the dominant follicle due to insufficient LH support in R heifers might have trigger the increased FSH concentrations and emergence of a new wave.

Feed restriction resulted in reduced maximum size of CL by 21 to 31 % during the last two cycles before the onset of anovulation, and this was associated with reduced concentrations of progesterone in plasma. Various levels of feed restriction increases (Donaldson et al., 1970; McCann and Hansel, 1986), decreases (Hill et al., 1970; Gombe and Hansel, 1973; Imakawa et al., 1986; Villa-Godoy et al., 1990) or has no effect (Spitzer et al., 1978; Shrick et al., 1990; Murhpy et al., 1991; Rhodes et al., 1996) on peripheral concentrations of progesterone. Differences in severity of undernutrition and sampling periods may account for the differences in progesterone concentrations reported in previous studies. Cows fed restricted diets had decreased CL weights (Rasby et al., 1991) and a linear decrease in maximum CL size was associated with decreasing body weight and condition (Rhodes et al., 1996). Since receptors for LH in bovine CL are not decreased with feed restriction (Schrick et al., 1992), reduced follicle ovulatory size and/or pulse frequency of LH during food deprivation is probably the primary cause for reduced CL function.

Concentrations of NEFA in plasma are inversely related to feed intake or energy balance in ruminants (Peters, 1986; Lucy et al., 1991). Reduced feed intake in cows is associated with increased concentrations of NEFA (Richards et al., 1989) as a result of

increased lipogenesis and fatty acid release from adipocytes. Concentrations of NEFA were greater in R compared with M heifers before the onset of nutritionally induced anovulation. Circulating lipid metabolites may act as a signal of metabolic status to the liver and/or brain. Hepatic oxidation of glycerol and 3-hydroxybutyrate alters the firing rate of hepatic vagal afferent nerves that provide information to the central nervous system CNS (Novin, 1985; Langhans et al., 1986). However, concentrations of NEFA in plasma increased in feed restricted beef cows before changes in LH concentrations could be detected (Richards et al., 1989), and short term infusion of free fatty acids to ovariectomized lambs did not alter pulsatile LH release (Estiene et al.,1990). Therefore it is unlikely that NEFA directly affect the hypothalamo-pituitary axis to regulate secretion of gonadotropins. A negative effect of increased NEFA concentrations on ovarian function can not be excluded. Nonesterified fatty acids and particularly oleic acid negatively affects LH-induced testosterone production in mouse Leydig cells in vitro by inhibiting cholesterol esterase and cholesterol utilization (Meikle et al.,1996). A similar role of NEFA on LH-induced androstenedione production by thecal cells is possible. Concentrations of NEFA in R heifers during cycle -1 were less compared with cycle -2 which is indicative of fat depletion or reduced metabolic rate in chronically underfed heifers. Feed restriction reduces resting metabolic rate in heifers and steers (Lapierre et al., 1992; Yambayamba et al., 1996).

Increased NEFA concentrations in plasma of feed restricted cycling heifers are associated with increased concentrations of GH (Armstrong et al., 1993). Concentrations and pulse amplitude of GH were greater in R heifers compared with M heifers but pulse frequency was not affected by treatment. Increased GH concentrations during dietary

restriction have been attributed to increased pulse amplitude (Brier et al., 1986; Houseknecht et al., 1988; Yelich et al., 1996) or increased pulse frequency (Villa-Godoy et al., 1990; Armstrong et al., 1993). Restricted nutrient intake increases GH concentrations in sheep (Thomas, 1994), pigs and cattle (Armstrong and Benoit, 1996), although in rodents the opposite occurs (Ross and Bucchanan, 1990). Restriction of feed intake to sheep did not alter concentrations, pulse frequency and amplitude of GRF in pituitary portal vessels, but concentrations of SRIF were reduced by 50 % compared with control animals (Thomas et al., 1994). Other factors such as reduced plasma IGF-I (Berelovitz et al., 1981) or decreased metabolic clearance might contribute to increased concentrations of GH during feed deprivation (Trenkle, 1976; Lapierre et al., 1992).

Despite increased GH secretion, concentrations of IGF-I was markedly reduced indicating that the liver is insensitive to GH in undernourished animals. Peripheral concentrations of IGF-I and the response to intravenous injections of GH in cattle are reduced during periods of restricted protein and/or energy intake (Brier et al., 1988; Ronge and Blum, 1989; Granger et al., 1989; Armstrong et al., 1993). In contrast with GH, the insulin-like effects of IGF-I include enhanced glycogenesis and lipogenesis. It has been suggested that increased concentrations of GH and decreased concentrations of IGF-I and insulin in feed restricted animals provides a mechanism for preferential utilization of mobilized substrates to maintain homeostasis and provide metabolic fuels for reproductive function (Hileman et al., 1991). Undernutrition of growing steers was associated with a marked reduction in GH binding to hepatic membranes, and well fed steers had both high and low affinity hepatic growth hormone receptors (GHr) while the undernourished animals had only low affinity hepatic GHR (Breier et al., 1988). In contrast, Hayden et

al., (1992) found that concentrations of hepatic GHR in steers was not affected by dietary restriction, but the amount of GH receptors within liver was decreased because of reduced liver mass during feed restriction.

Growth hormone treatment stimulates follicular growth in pigs (Spicer et al., 1990) and cows (De La Sota et al., 1991) and GRF treatment increased the size of large follicles in heifers (Spicer and Enright, 1991). Growth hormone also influences granulosa cell function in vitro in cows (Langhout et al., 1991; Stewart et al., 1996) and pigs (Hsu and Hammond, 1987), however, it is highly unlikely that GH mediates the effects of underfeeding on reproduction. Intravenous infusion of free fatty acids in ovariectomized underfed ewes reduced concentrations of GH in serum but had no effect on LH concentrations (Estienne et al., 1990). In addition, exogenous FSH is required to facilitate the increase in number of follicles in cows treated with bST (Gong et al., 1993). Even though GH receptors have been identified in follicles, the number is minimal (Lucy et al., 1993), and the substantial increase in circulatory GH during underfeeding rules against a role of GH in mediating the effects of feed restriction in reproductive tissues.

Substantial reduction in IGF-I concentrations during cycle -2 in R heifers was not associated with reduced peripheral estradiol concentrations but was associated with reduced maximum diameter of the ovulatory follicles and maximum CL size. In agreement with our findings, Lucy et al. (1996) found that in miniature Brahman cows, which have low IGF-I concentrations due to a GH-receptor defect, the maximum size of dominant follicles and CL are reduced without any difference in peripheral estradiol and LH concentrations. Short term fasting or chronic feed restriction do not affect intrafollicular concentrations of IGF-I in follicles that are less than seven mm in diameter (Spicer et al.,

1992; Kirby et al., 1993). The effect of underfeeding on concentrations of biologically active IGF-I in reproductive tissues need further study since feed restriction alters concentrations of IGFBPs (Vandehaar et al., 1995; Roberts et al., 1994).

In agreement with our results, feed restriction reduces plasma concentration of insulin in heifers (Harrison and Randel, 1986; McCann and Hansel, 1986; Wiley et al., 1991) and cows (Richards et al., 1989; Grimard et al., 1995). Insulin stimulates GnRH release from explant cultures of median eminence of rats (Arias et al., 1992). Hypothalamic GT-1 cells (transformed cell line), which secrete GnRH, have insulin receptors and insulin exhibits mitogenic effects (Olson et al., 1995). Induced diabetes in rats decreased the content of GnRH in the hypothalamus and LH in the pituitary (Besteti et al., 1989; Valdes et al., 1991) and insulin replacement therapy in diabetic rats restored ovulations and cyclicity (Katayama et al., 1984). However, the role of insulin in mediating the effects of feed intake on LH secretion has recently been disputed. Intracerebraventricular infusion of insulin in growth restricted ovariectomized ewes did not alter LH secretion (Hileman et al., 1993) and suppression of postmeal insulin secretion by diazoxide in previously underfed rats did not prevent LH secretion (Williams et al., 1996). It is likely that insulin concentrations in plasma must be maintained above a threshold for normal hypothalamo-pituitary function and maintenance of cyclicity in cattle.

Physiological insulin concentrations are probably required for normal follicular steroidogenesis. Receptors for insulin are in granulosa cells of cattle and pigs (Otani et al., 1985; Spicer et al., 1994). Insulin is a potent stimulator of FSH-induced estradiol production by bovine granulosa cells (Spicer et al., 1994), and insulin infusion during a superovulatory regime in cattle increased intrafollicular concentrations of estradiol in large

follicles by fivefold (Simpson et al., 1994). Surprisingly, a 50% reduction in peripheral insulin concentrations during cycle -2 in the present study was not associated with reduced peripheral estradiol concentrations. Streptozocin-induced diabetes in pigs resulted in decreased follicular diameter but peripheral estradiol concentrations and in vitro estradiol production were not compromised (Edwards et al., 1996). Spicer et al. (1994) found that IGF-I inhibited insulin-induced estradiol production by granulosa cells from both small and large bovine follicles, indicating that IGF-I can act as an insulin antagonists. The 7- to 10-fold reduction in peripheral IGF-I concentrations observed during cycle -2 in R heifers may have counteracted the 40-50% reduction in peripheral insulin concentrations resulting in unaltered estradiol concentrations in the last ovulatory cycle.

Chronic feed restriction in ruminants and loss in BW and/or BCS are associated with decreased glucose concentrations (McCann and Hansel, 1986; Richards et al., 1989; Rutter and Manns 1991). Glucose concentrations were less in R than M heifers during the last two cycles before the onset of anovulation. Injection of 2-deoxyglucose (2DG; inhibitor of glucose utilization) before or during the estrous cycle in cyclic beef heifers prevented estrus and CL formation (McClure et al., 1978). Glucose concentrations were positively associated with feed intake and LH pulse frequency in prepubertal heifers fed at two different levels of nutrition (Yelich et al., 1996). Glucose infusion in lactating anestrous beef cows increased pulse frequency and concentrations of LH in serum during treatment with GnRH (Garmendia, 1986). Intracerebraventricular infusion of 2DG to gonadectomized male lambs, at doses that did not affect peripheral concentrations of glucose, suppressed concentrations of LH in serum (Bucholtz et al., 1996). However concentrations of glucose in plasma of postpartum cows are not predictive of luteal

activity (Vizcarra et al., 1996). Glucose infusion in postpartum cows with good BCS and nonlactating well-fed cows did not alter LH secretion (McCoughey et al., 1988; Rutter et al., 1989) and infusion of 2DG in well-fed ewes had no effect on LH secretion (Hileman et al., 1991). It is possible that the effect of glucose on LH secretion depends on BCS of cattle and total energy availibility.

Implications

Reduction in LH concentrations preceding the onset of nutritionally induced anovulation, as a result of reduced body energy reserves, is associated with reduced estradiol concentrations and failure of ovulation. Reductions in LH pulse frequency, IGF-I and insulin concentrations after chronic feed restriction in beef heifers are associated with reduced growth and maximum diameter of ovulatory follicles, but are not directly associated with reduced peripheral estradiol concentrations and the ability of a follicle to ovulate. Chronically underfed heifers continue estrous cycles and normal ovulations before they become anovulatory. Further research is required to determine whether reduced follicular growth of chronically underfed but ovulatory heifers compromises fertilization rate.

Table 1. Composition of diets

Item	Maintenance	Restricted
Ingredients, as fed %		
Corn distillers grain	-	-
Rolled corn	37.5	-
Cottonseed hulls	21.7	-
Alfalfa pellets	32.5	-
Prairie hay	-	96
Cane molasses	3.0	-
SBM	5.0	3
Limestone 38%	-	.6
Salt	.3	.3
Zinc oxide	-	.002
Vitamin A-30,000	-	.04
Vitamin E--50%	-	.04
Calculated values as fed		
Kg	4.5	4.0
DM %	88.9	89.8
Total NEm, Mcal	6.7	3.7
Total NEg, Mcal	3.6	1.7
CP %	12.2	6.7

Table 2. Least squares means for BW and body condition score[a] (BCS) at nutritionally induced anovulation

	Treatment		
Criteria	Maintenance	Restricted	MSE
Initial BW, kg	374[b]	382[b]	354
BW at anovulation, kg	447[b]	298[c]	281
Change in BW, %	+19%[b]	-22%[c]	7
Initial BCS	5.3[b]	5.4[b]	.2
BCS at anovulation	5.2[b]	3.8[c]	.2

[a] 1 = emaciated, 9 = obese.
[b, c] Means within a row without a common superscript differ (P<.0001).

Table 3. Least square means for size of the ovulatory follicle, day of emergence, growth rate of the ovulatory follicle and maximum CL size during the last two cycles before anovulation

	Treatment				
	Maintenance		Restricted		
Criteria	Cycle -2	Cycle -1	Cycle -2	Cycle -1	MSE
Ovulatory follicle, mm	15.8[a]	15.5[a]	10.5[b]	10.2[b]	.9
Day of emergence	10.9[a]	11.1[a]	10.8[a]	10.4[a]	.7
Follicle growth rate, mm/d	1.40[a]	1.40[a]	.89[b]	.85[b]	.01
Maximum CL size, mm	19.7[a]	20.4[a]	15.5[b]	14.1[c]	1.70

[a, b, c] Means within a row without a common superscript differ (P<.05).

Table 4. Least squares means for concentration, pulse frequency and pulse amplitude of LH during the last two cycles before the onset of nutritionally induced anovulation

	Treatment								
	Maintenance				Restricted				
	Cycle -2		Cycle -1		Cycle -2		Cycle -1		
Criteria	d 2	d 15	d 2	d 15	d 2	d 15	d 2	d 15	MSE
Concentration (ng/mL)	5.7[a]	7.9[b]	5.6[a]	8.1[b]	5.5[a]	7.0[b]	3.9[c]	4.1[c]	1.8
Pulse frequency (pulses/8 h)	3.7[a]	7.0[b]	3.6[a]	6.9[b]	3.5[a]	4.6[a]	3.6[a]	4.2[a]	2.2
Pulse amplitude (ng/mL)	2.8[a]	2.7[a]	2.9[a]	2.9[a]	3.1[a]	3.1[a]	1.5[b]	1.2[b]	.6

[a, b, c, d] Means within a row without a common superscript differ ($P<.05$)

Table 5. Least squares means for concentration, pulse frequency and pulse amplitude of FSH during the last two cycles before the onset of nutritionally induced anovulation

	Treatment								
	Maintenance				Restricted				
	Cycle -2		Cycle -1		Cycle -2		Cycle -1		
Criteria	d 2	d 15	d 2	d 15	d 2	d 15	d 2	d 15	MSE
Concentration (ng/mL)	.47[a]	.22[b]	.53[a]	.23[b]	.46[a]	.18[b]	.55[a]	.59[a]	.03
Pulse frequency (pulses/8 h)	1.5[a]	1.8[a]	2.0[a]	1.5[a]	1.7[a]	1.8[a]	2.3[a]	4.6[b]	.7
Pulse, amplitude (ng/mL)	.10[a]	.08[a]	.14[a]	.09[a]	.10[a]	.07[a]	.12[a]	.26[b]	.01

[a, b] Means within a row without a common superscript differ (P<.05).

Table 6. Least squares means for concentration, pulse frequency and pulse amplitude of GH during the last two cycles before the onset of nutritionally induced anovulation

	Treatment		
Criteria	Maintenance	Restricted	MSE
Concentration of GH, (ng/mL)	12.1[a]	38.7[b]	54.8
Pulse frequency of GH, (pulses/8 h)	5.0[a]	4.8[a]	2.0
Pulse amplitude of GH (ng/mL)	17.6[a]	36.3[b]	52.4

[a,b] Means within a row lacking a common superscript differ (P<.05).

Table 7. Least square means for concentrations of glucose, insulin, IGF-I and NEFA during the last two cycles before the onset of nutritionally induced anovulation

	Treatment				
	Maintenance		Restricted		
Criteria	Cycle -2	Cycle -1	Cycle -2	Cycle -1	MSE
Glucose, mg %	70.1[a]	72.9[a]	60.5[b]	56.9[b]	17.8
Insulin, ng/mL	1.8[a]	1.9[a]	.9[b]	.8[b]	.2
IGF-I, ng/mL	91.4[a]	96.8[a]	19.5[b]	11.2[c]	31.7
NEFA, mEq/L[d]	197[a]	213[a]	645[b]	536[c]	4060

[a, b, c] Means within a row lacking a common superscript differ (P<.05).
[d] mEq of palmitate

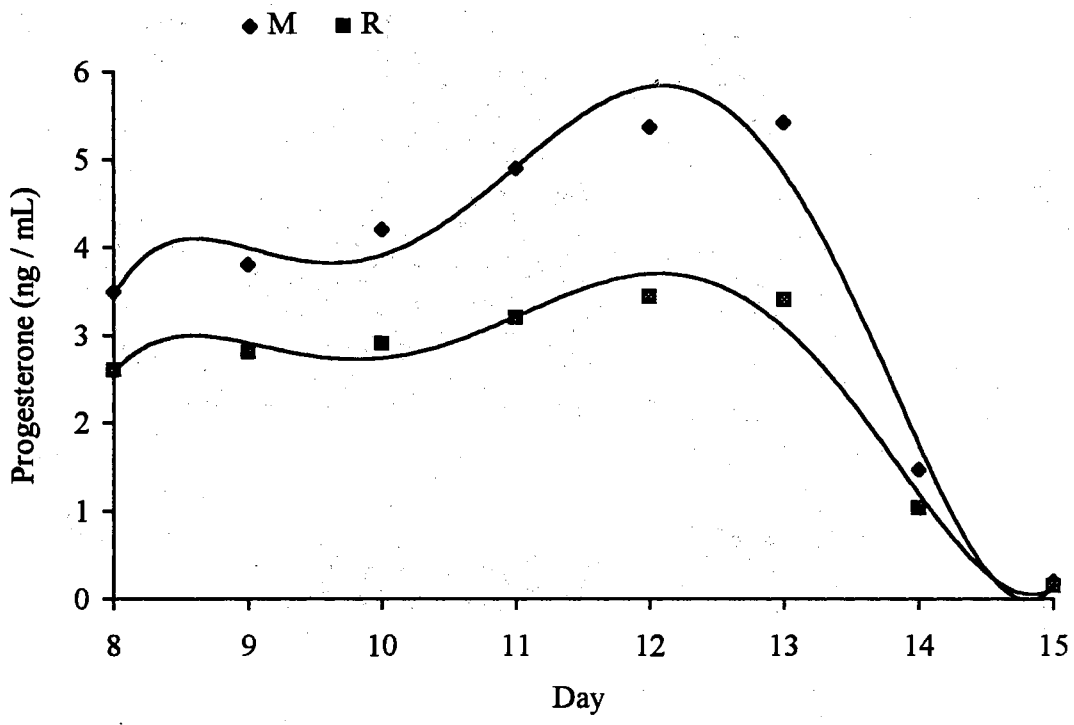

Figure 1. Least squares means (MSE =2.58) and least squares regression lines for progesterone concentrations during the last two cycles before the onset of nutritionally induced anovulation (PGF_{2a} was given on d 13).

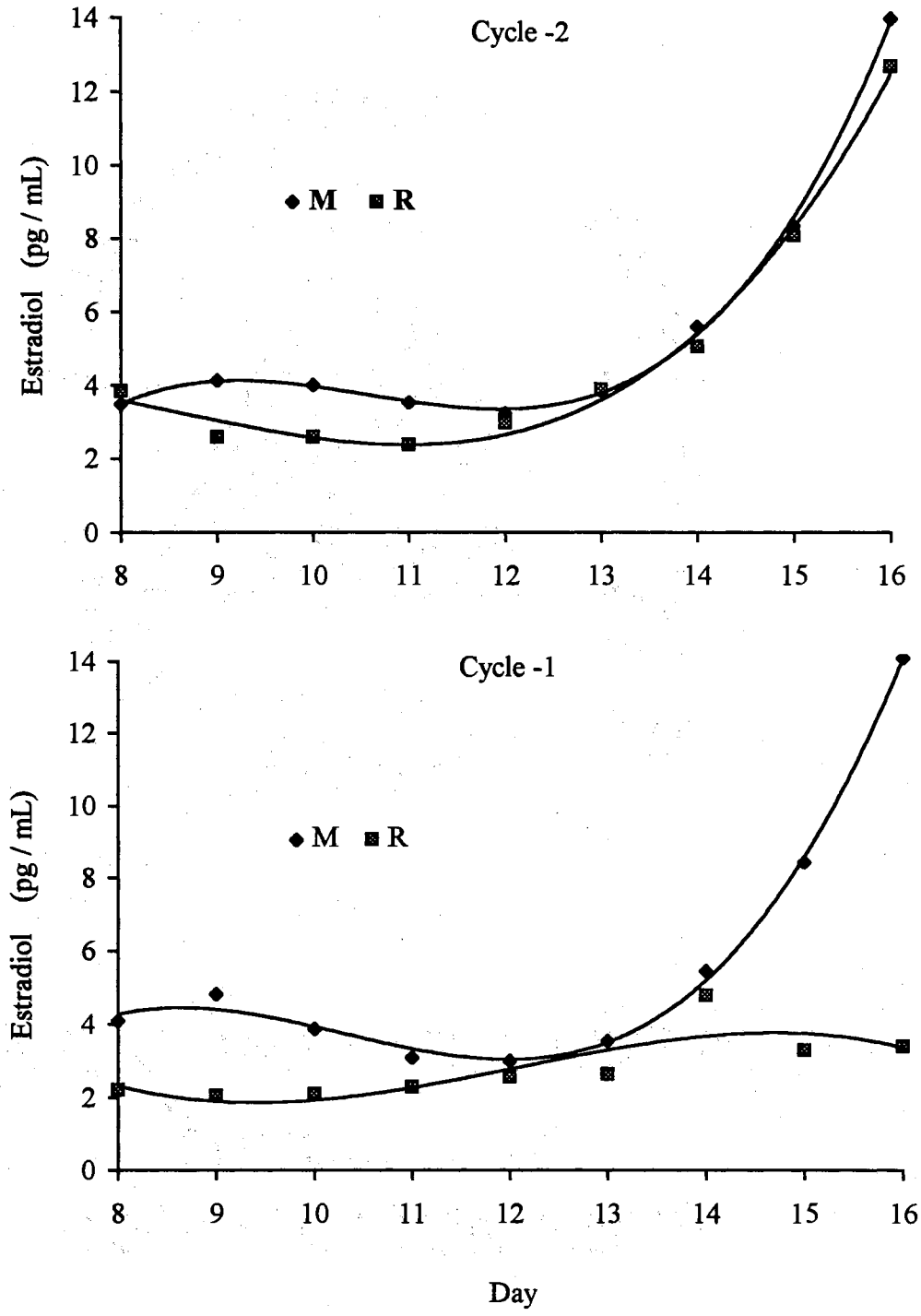

Figure 2. Least squares means (MSE = 4.63) and least squares regression lines for estradiol concentrations during the last two cycles before the onset of nutritionally induced anovulation.

CHAPTER IV

NUTRITIONALLY INDUCED ANOVULATION IN BEEF HEIFERS: OVARIAN AND ENDOCRINE FUNCTION DURING REALIMENTATION AND RESUMPTION OF OVULATION

Abstract: Twelve nutritionally induced anovulatory beef heifers with a BW of 298 ± 3 kg and body condition score (BCS) of 3.8 ± .1 and six cyclic heifers with a BW of 453 ± 10 kg and BCS of 5.2 ± .2 were used to evaluate follicular growth and concentrations of hormones and metabolites during anovulation and resumption of ovulation. During each of two replications, six anovulatory heifers were randomly assigned to one of two groups and fed to gain .6 (LGAIN) or 1.5 kg/d (HGAIN). Three cyclic heifers in each replication were fed a maintenance diet (M) and estrous cycles were synchronized with injections of $PGF_2\alpha$ (25 mg of Lutalyse; Upjohn) to a length of 16 d. Transrectal ultrasonography was performed and blood samples were collected daily during anovulation and after realimentation until resumption of ovulations. Follicles ≥4 mm in diameter were measured in one follicular wave before realimentation (Wan) and in two waves (W-2, W-1) immediately before the first ovulation or luteinization (W0). Concentrations of LH, and FSH in serum and estradiol, IGF-I, insulin, glucose and NEFA in plasma were determined in samples obtained the last 5 d of the growing phase of the dominant or ovulatory follicle

in anovulatory and cyclic heifers, respectively, during Wan, W-2, W-1 and W0 in anovulatory heifers and coincident days in cyclic heifers. Resumption of ovulation after realimentation occurred earlier (P<.05) in HGAIN than LGAIN (57 and 80 d, respectively). At first ovulation, BW was greater (P<.05) for HGAIN than LGAIN (387 and 343 kg, respectively) but BCS at first ovulation was not influenced by treatment. Follicular growth was similar for HGAIN and LGAIN, so data were combined. Maximum diameter of the dominant follicle (DF) increased between anovulation and resumption of ovulation (9.2, 11.7, 13.2 and 15.3 mm for Wan, W-2, W-1 and W0, respectively; P<.0001). Growth rate of the DF was increased linearly with waves (.9, 1.2, 1.5 and 1.9 mm/d for Wan, W-2, W-1 and W0, respectively; P<.0001). The regression rate of the DF was also influenced by wave (-1.0, -1.2 and -1.5 mm/d for Wan, W-2 and W-1, respectively; P<.001), persistence of the DF increased during realimentation (14.0, 17.2 and 17.4 d for Wan, W-2 and W-1, respectively; P<.001). Maximum diameter of the largest subordinate follicle (SF) increased during realimentation (7.3, 8.3, 8.2 and 8.2 for Wan, W-2, W-1 and W0, respectively; P<.05), and growth rate of SF was also influenced by wave (.8, 1.2, 1.4 and 1.7 mm/d for Wan, W-2, W-1 and W0, respectively; P<.0001). Regression rate and persistence of SF were influenced by wave (-.7, -1.1, -1.4 and -1.8 mm/d for Wan, W-2, W-1 and W0, respectively; P<.0001) and (11.2, 9.3, 7.7 and 6.0 d for Wan, W-2, W-1 and W0, respectively; P<.0001). At W0, nine heifers ovulated and two developed a luteinized follicle. All nine heifers that ovulated had a short cycle (10.5 $\pm$ .9 d) and the other two heifers ovulated 9 and 10 d after the luteinized follicle was first detected. The subsequent cycle was normal (> 17 d) for all heifers. There was a treatment (cyclic vs anovulatory) x wave x day effect (P<.01) on LH concentrations, and

concentrations were similar (P>.1) for HGAIN and LGAIN heifers and gradually increased during realimentation (P<.05). During W0, concentrations of LH were similar for M and realimented heifers. Concentrations of FSH were not influenced by treatment or wave (P>.1), however day affected (P<.0001) FSH concentrations. There was a treatment x wave x day effect (P<.05) on estradiol concentrations. Concentrations of estradiol were similar for HGAIN and LGAIN heifers and increased during realimentation. The preovulatory increase of estradiol during W0 was less in realimented heifers compared with M heifers. There was a treatment x wave effect (P<.0001) on IGF-I concentrations. Concentrations of IGF-I in HGAIN and LGAIN heifers gradually increased during realimentation (P<.05) but were less than concentrations of IGF-I in M heifers. Glucose and insulin concentrations in realimented heifers were less than those in M heifers during Wan, but during W-2, W-1 and W0 there were no differences between M and realimented heifers (treatment x wave, P<.001). There was a treatment x wave effect on NEFA concentrations (P<.0001). Realimented heifers had greater (P<.05) NEFA concentrations than M heifers during anovulation. During realimentation, NEFA concentrations were less (P<.05) in realimented compared with M heifers, however, an increase was observed during W-2, W-1 and W0. We conclude that anovulatory heifers that have greater daily gain during realimentation have a shorter interval to ovulation. Increased diameter, growth rate and persistence of the DF and reduced persistence of the larger SF are associated with increased concentrations of LH, estradiol and IGF-I during the transition from nutritionally induced anovulation to resumption of ovulatory cycles.

Key words: Heifer, Nutritional Anovulation, Follicular Growth, LH, Estradiol,

Introduction

Reproductive performance of beef cows is associated with BCS (Richards et al., 1986; Selk et al., 1988). Body condition at calving influences the duration of the postpartum anestrous period (Bishop et al., 1994) and increases in BW and BCS are required to cause resumption of cyclicity after induction of nutritionally induced anestrus (Louw et al., 1988; Richards et al., 1989).

Reduced energy intake delays the development of dominant follicles in prepuberal heifers (Bergfeld et al., 1994) and postpartum cows (Ryan et al., 1994; Stagg et al., 1995). Loss of BW and BCS result in reduced growth rate and size of ovulatory follicles before the onset of nutritionally induced anestrus in beef heifers (chapter III). A linear increase in persistence, growth rate and maximum size of dominant follicles occurs during realimentation of nutritionally anestrous beef heifers (Rhodes et al., 1995).

The mechanisms whereby undernutrition causes anestrus, and realimentation results in resumption of cyclicity in cattle have not been determined. Feed restriction suppresses secretion of LH in beef cows (Richards et al., 1989), cyclic heifers (Day et al., 1986) and prepuberal heifers (Yelich et al., 1996), and this effect is probably mediated by reduced GnRH secretion since exogenous administration of GnRH to nutritionally anestrous cows induces luteal activity (Bishop and Wettemann, 1993; Vizcarra et al., 1997). Alterations in concentrations of GH, insulin, IGF-I, glucose and NEFA in blood are indicative of energy availability and may provide short- or long-term signals that mediate the effects of nutrition on LH secretion. Follicular growth and gonadotropin concentrations in serum at particular stages of follicular waves during nutritionally induced

anovulation and resumption of ovulation after realimentation have not been documented. The objectives of this experiment were to evaluate the effect of two rates of gain during realimentation on time, BW and BCS at first ovulation after nutritionally induced anovulation and to evaluate follicular growth and concentration of LH, FSH, estradiol, IGF-I, insulin, glucose and NEFA in blood during the transition from anovulation to resumption of ovulation.

Materials and Methods

Animals and procedures

Twelve nutritionally induced anovulatory beef heifers with a BW of 298 $\pm$ 3 kg and a BCS of 3.8 $\pm$ .1, and six cyclic heifers with a BW of 453 $\pm$ 10 kg and a BCS of 5.2 $\pm$ .2 were used in two replications (commencing in July of 1995 and 1996) to determine follicular growth and concentrations of hormones and metabolites during anovulation and resumption of ovulatory cycles. Heifers were of similar age, and differences in BW and BCS were the result of a nutritional regimen to induce anovulation (chapter III). Ultrasonography was performed and plasma and serum samples were collected daily in anovulatory heifers until a complete follicular wave occurred. Twenty-two days were required to obtain at least one follicular wave for all anovulatory heifers. During anovulation, heifers were fed 5 kg of prairie hay per day. Thereafter, heifers were randomly assigned to one of two groups and fed a complete diet (Table 1) to gain .6 (LGAIN) or 1.5 kg/d (HGAIN). At the initiation of realimentation, ten days were required in order to gradually increase the amount of concentrate for each group. The

amount of feed provided each day was adjusted every second week to maintain the prescribed rate of gain. Shrunk BW (after 16 h withdrawal of feed and water) was obtained weekly and BCS (1 = emaciated; 9 = obese; Wagner et al., 1989) every two wk. One heifer of the HGAIN group was removed from the trial for health reasons. During realimentation, ultrasonography was performed and plasma and serum samples were collected daily until ovulation resulting in a normal estrous cycle (> 17 d). Cyclic heifers (M) were fed a maintenance diet (Table 1) and estrous cycles were synchronized by treatment with $PGF_2\alpha$ (25 mg of Lutalyse; Upjohn Kalamazoo, MI) followed by a second treatment 11 d later. Starting on d 13 of the induced cycle, heifers were given PGF_2a every 16 d thereafter to synchronize and maintain 16 d estrous cycles until realimented heifers resume ovulations. Ovaries of cyclic heifers were evaluated by ultrasonography and serum and plasma samples were collected daily from d 8 (d 1 = d of ovulation) until subsequent ovulation.

Ultrasonography and follicular data analyses

Transrectal ultrasonography was performed with an Aloka 500V ultrasound scanner equipped with a 7.5-MHz transducer (Corometrics Medical Systems, Wallingford, CT). During ultrasound scanning, approximate position and size of follicles and CL in both ovaries were sketched. Scans of the ovaries were also recorded on a video camera recording tape and viewed later to draw complete ovarian maps recording all follicles $\geq$ 4 mm. Reference points on the ovaries included the poles and the hilus (Ginther et al.,1989). Size of follicles was calculated as the mean of the longest and shortest diameters. Follicles $\geq$4 mm in diameter were sequentially identified and measured in one

follicular wave before realimentation (Wan), two waves (W-2, W-1) immediately before the first wave resulting in ovulation or luteinization (W0) and during W0. A dominant follicle (DF) was identified as luteinized when luteal tissue (thickened wall) surrounding the nonechogenic area (dark) of a follicle developed without previous ovulation (Farin et al., 1990). For each of the above follicular waves, diameters of the DF and first subordinate (SF) follicles were determined. Changes in diameter of DF and SF were used to determine growing, static and regressing phases (Ginther et al., 1989a), so that comparisons could be made amongst waves. Day of emergence of the DF was defined as the day before the first day that the ovulatory follicle could be individually identified. Growing phase was defined as the interval between the day of emergence and the day that the follicle ceased its progressive increase in diameter by .5 mm or more, and was characterized by growth rate in mm/d, and duration of the growing phase in days. Growth rate of the DF was estimated as the increase in diameter from the day of emergence to the maximum diameter divided by days of growth. Static phase was defined as the interval between the last day of the growing phase and the first day that the follicle began a progressive decrease in diameter by .5 mm or more, and was characterized by the number of days and the average diameter during the static phase. The regression phase was the interval from the last day of the static phase until the last day that the follicle could be identified on the ovary, and was characterized by the regression rate in mm/d and duration of the phase. The wave that resulted in ovulation or luteinization (W0) was characterized by growth rate and duration of the growing phase, duration of the static phase, diameter of the DF and SF, and regression rate and duration of regression phase of the SF.

The interval between anovulation and resumption of ovulation, BW and BCS at first ovulation were compared between HGAIN and LGAIN using ANOVA in a 2 x 2 factorial with rep and treatment (HGAIN and LGAIN). Follicular wave parameters were compared among reps, treatments and waves using a split plot ANOVA with rep, treatment and treatment x rep in the main plot and wave, rep x wave, treatment x wave and treatment x wave x rep in the subplot. Mean square error (MSE) of heifer within treatment x rep was used as the error term for the main plot effects. Interactions of treatment and wave with rep were either not significant or were due to differences in the magnitude of the response and not in direction, so data from the two replicates were combined and rep was removed from the model. Tukey-Kramer's test (SAS user's guide 1990) for pairwise comparisons was used to compare means.

Collection of blood samples and hormone analyses

Concentrations of LH and FSH in serum and estradiol-17β, insulin, IGF-I, glucose and NEFA in plasma were determined in samples during the last 5 d of the growing phase of the DF in anovulatory heifers and the ovulatory follicle in cyclic heifers, during one wave before realimentation (Wan), during two waves (W-2 and W-1) immediately before the first wave resulting in ovulation or luteinization (W0) and during W0 in anovulatory heifers and at coincident times in cycles of cyclic heifers. Blood samples were collected via tail venipuncture. For serum, samples were allowed to clot for 24 h at 4^{o} C and then centrifuged at 2,800 x g for 20 min. Plasma was obtained from blood collected in 15 mL tubes containing EDTA (.1 ml of a 15 % solution) and placed on ice and centrifuged within 1 h at 2,800 x g for 20 min. Estradiol-17β concentrations in plasma were

quantified by a RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intra- and interassay coefficients of variation (n = 4 assays) were 10 % and 14 %, respectively. Concentrations of LH in serum were quantified by RIA (Bishop and Wettemann, 1993), with NIH LH-B9 as the standard, and intra- and interassay coefficients of variation (n = 2 assays) were 9 % and 15 %, respectively. Concentrations of FSH in serum were quantified by RIA (Vizcarra et al., 1997), with USDA-bFSH-I-2 as the standard and intra- and interassay coefficients of variation (n = 2 assays) were 3 % and 7 %, respectively. Concentrations of IGF-I in plasma were quantified by RIA (Echternkamp et al., 1990) after an acid ethanol extraction. Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used as standard and intra- and interassay coefficients of variation (n = 2 assays) were 3 % and 16 %, respectively. Concentrations of insulin in plasma were quantified by a solid phase RIA (Chapter III) for human insulin (Coat-A-Count insulin kit, Diagnostic Products Corp., Los Angeles, CA) using bovine pancreatic insulin as the standard (28.6 USP units/mg, Sigma Chem. Co., St Louis, MO). Intra- and interassay coefficients of variations (n = 2 assays) were 4 % and 9 %, respectively. Concentrations of glucose in plasma were determined by an enzymatic colorimetric procedure (Sigma, No.510, Sigma Chemical Co., St. Louis, MO). Intra- and interassay coefficients of variations (n = 2 assays) were 4 % and 14 %, respectively. Concentrations of NEFA in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C, Wako Chemicals Inc., Dallas, TX) with modification (McCutcheon and Bauman, 1986). Intra- and interassay coefficients of variation (n = 2 assays) were 6 % and 11%, respectively. Multivariate analyses of variance for repeated measures was used to determine the effect of treatment (M, HGAIN,

LGAIN), wave (Wan, W-2, W-1 and W0) and day [0,-1, -2, -3, -4, where d 0 is the day that the DF had a maximum diameter in anovulatory heifers (HGAIN and LGAIN) and one day before ovulation in cyclic (M) heifers] on LH, FSH, estradiol, IGF-I, insulin, glucose, and NEFA concentrations. Concentrations of hormones and metabolites during days represented the repeated response variable (within-subject factors). The between-subject factors were treatment, rep, and treatment x rep in the main plot, and wave, treatment x wave, rep x wave and treatment x rep x wave in the subplot. Mean square error of heifer within treatment x rep was the error term for the factors in the main plot. The residual MSE was the error term for the factors in the subplot. Because interactions of treatment and wave with rep were either not significant or were due to differences in the magnitude of the response and not in direction, data from the two replicates were combined and rep was removed from the model. If interactions with day were significant, polynomial response curves of appropriate order were fit and tested for homogeneity of regression (Snedecor and Cochran, 1968). Tukey-Kramer's test (SAS user's Guide, 1990) for pairwise comparisons was used to compare means among treatments and waves.

Results

Follicular data

Increased nutrient intake of nutritionally anovulatory beef heifers resulted in increased BW and BCS and resumption of ovarian cycles. During W0 nine heifers ovulated and two developed a luteinized follicle. The interval between initiation of realimentation and first ovulation or luteinization (Table 2) was shorter for the HGAIN

than LGAIN heifers (P<.05). At first ovulation or luteinization, BCS was not significantly different between HGAIN and LGAIN heifers, however BW was greater (P<.05) for HGAIN compared with LGAIN heifers (Table 2).

Neither treatment (HGAIN and LGHAIN) or treatment x wave influenced follicular characteristics, so data for the treatments were combined. Wave influenced (P<.0001) the maximum diameter of DF, and size increased from anovulation to first ovulation or luteinization (Table 3). Wave also influenced growth rate of DF (P<.0001) which increased after realimentation but was not different between W-1 and W0 (Table 3). Duration of the growing phase of DF was greater (P<.05) in W-2, W-1 and W0 compared with Wan (Table 3), and duration of the static phase of DF was greater (P<.05) in W-2 and W-1 compared with Wan.

Static phase of DF was 2.5 $\pm$ .3 d before ovulation or luteinization during W0 (Table 3). A static phase did not occur in cycling heifers before ovulations. Wave increased (P<.001) the regression rate of DF during realimentation in W-2 and W-1 (Table 3). Duration of the regression phase of DF was greater (P<.05) in W-2 and W-1 compared with Wan (Table 3).

Wave influenced (P<.0001) growth and regression rates of SF and duration of growing, static and regression phases. Growth and regression rates of first SF gradually increased while the duration of growing, static and regression phases gradually decreased in successive waves during realimentation (Table 4). The maximum diameter of first SF during W-2, W-1 and W0 was greater (P<.05) compared with the maximum diameter of first SF during Wan (Table 4). Diameters of DF and SF during anovulation and resumption of ovulation after realimentation are depicted in figure 1.

During W0, nine heifers ovulated and two developed a luteinized follicle. All nine heifers that ovulated had a short cycle with an interovulatory interval of 10.5 ± .9 d. The other two heifers (one from the HGAIN and one from the LGAIN group) ovulated 9 and 10 d after the luteinized follicle was first detected. Concentrations of progesterone in plasma after ovulation or luteinization are depicted in figure 2. The subsequent interovulatory interval was of normal duration in all heifers and averaged 20.2 ± .4 d.

Hormones and Energy Metabolites

There was a treatment x wave x day effect (P<.01) on LH concentrations. Concentrations were best described by quadratic equations (Figure 3). Analyses of homogeneity of regression indicated that M heifers had greater (P<.05) concentrations of LH than HGAIN and LGAIN during Wan, W-2 and W-1. Concentrations of LH were not different between HGAIN and LGAIN heifers in any of the follicular waves (P>.1), and concentrations gradually increased after realimentation for both treatments. During W0, concentrations of LH were similar for M, HGAIN and LGAIN heifers.

Concentrations of FSH were not influenced (P>.1), by treatment or wave, but there was a day effect (P<.0001). Concentrations of FSH were best described by quadratic equations (Figure 4) and increased during the last five days of the growing phase of DF.

There was a treatment x wave x day effect (P<.05) on estradiol concentrations and concentrations were best described by quadratic equations (Figure 5). Analyses of homogeneity of regression indicated that M heifers had greater (P<.05) estradiol concentrations than HGAIN and LGAIN during all waves and concentrations were similar

in HGAIN and LGAIN heifers. A gradual increase in estradiol concentrations occurred after realimentation in HGAIN and LGAIN heifers. The magnitude of the preovulatory increase of estradiol during W0 was less in HGAIN and LGAIN heifers compared with M heifers. During W0, DF in realimented heifers ovulated or luteinized approximately 3 d after the maximum preovulatory increase of estradiol occurred. The maximum preovulatory increase of estradiol was coincident with the end of the growing phase. Cycling heifers ovulated 1 d after the maximum preovulatory increase of estradiol occurred (Figure 6).

Concentrations of IGF-I, glucose, insulin and NEFA were not influenced by day and there were no interaction with day, so concentrations within treatments and waves were averaged over days. There was a treatment x wave effect ($P<.0001$) on IGF-I concentrations. Concentrations of IGF-I in HGAIN and LGAIN heifers gradually increased after realimentation but were less ($P<.05$) than concentrations of IGF-I in M heifers during all waves (Figure 7). Concentrations of IGF-I were not significantly different between HGAIN and LGAIN heifers.

There was a treatment x wave effect ($P<.01$) on glucose and insulin concentrations (Figure 8 and 9). Concentrations of glucose and insulin were similar for HGAIN and LGAIN heifers. Concentrations of glucose and insulin in HGAIN and LGAIN heifers increased after realimentation, and concentrations were similar for M, HGAIN and LGAIN during W-2, W-1 and W0.

There was a treatment x wave effect ($P<.0001$) on NEFA concentrations (Figure 10). The HGAIN and LGAIN heifers had greater ($P<.05$) NEFA concentrations during Wan than M heifers. After realimentation, NEFA concentrations were less ($P<.05$) in

HGAIN and LGAIN compared with M heifers, however, concentrations of NEFA gradually increased during W-2, W-1 and W0 in both HGAIN and LGAIN heifers.

Discussion

Increased average daily gain in HGAIN resulted in a shorter interval between anovulation and resumption of ovulation after realimentation compared with LGAIN heifers. Although the interval was shorter, BW at ovulation was greater in HGAIN vs LGAIN heifers. At the first ovulation or luteinization, BCS was not different between HGAIN and LGAIN heifers. Even though BW measurements were obtained after 18 h without feed and water, part of the difference in BW of HGAIN and LGAIN heifers at first ovulation could be due to difference in fill. Protein accretion predominates over fat deposition during compensatory growth, especially in young animals weighting less than 350 kg (Rompala et al., 1985; Wright and Russel 1991; Hayden et al., 1993) and increased muscle growth in HGAIN than in LGAIN heifers may also account for some of the difference in BW between the two groups at ovulation.

Increased rates of gain in prepuberal beef heifers resulted in puberty at a younger age but at greater BW (Bergfeld et al., 1994) and BCS (Yelich et al., 1995). Increased rate of gain in postpartum beef cows resulted in a shorter interval from parturition to first ovulation but at a significantly greater BCS and similar BW (Stagg et al., 1995). In the present study, as in other studies (Louw et al., 1988; Richards et al., 1989; Rhodes et al., 1995), resumption of cyclicity after nutritionally induced anovulation occurred after an increase in BCS. Rhodes et al., (1995) found that the time of first ovulation after realimentation of nutritionally anestrous Bos Indicus heifers was not associated with a

critical BCS. Our study indicates that an increase in amount of fat is required for reoccurrence of estrous cycles after periods of anovulation.

Rate of gain during realimentation had no effect on any of follicular characteristics during the wave when ovulation occurred or the two preceding waves. In agreement with the present study, prepuberal beef heifers fed a low energy diet had delayed puberty compared with heifers fed a high energy but follicular characteristics were similar for the two groups at 30, 60, 90 and 120 d before puberty (Bergfeld et al., 1994). Similarly, follicular parameters were not affected by feed intake of postpartum beef cows, even though cows fed the high energy diet had a shorter postpartum interval to ovulation (Stagg et al., 1995). Realimentation of nutritionally anovulatory heifers in the present study resulted in a gradual increase in the maximum size, growth rate, regression rate and persistence of dominant follicles. Reduced nutrient intake in cyclic (Murphy et al., 1991) and prepuberal beef heifers (Bergfeld et al., 1994) decreased persistence and maximum size of dominant follicles and tended to increase the incidence of three wave-cycles in cyclic heifers (Murphy et al., 1991). Reduced feed intake resulted in a substantial reduction in growth rate and maximum size of ovulatory follicles before the onset of nutritionally induced anovulation (Chapter III). A linear reduction in persistence and maximum size of dominant and ovulatory follicles with decreasing body weight and condition score was observed in feed restricted beef heifers, whereas a linear increase in persistence, growth rate and maximum size of dominant follicles was observed during realimentation of nutritionally anestrous beef heifers (Rhodes et al., 1995). These findings indicate that decreased BCS and/or feed intake in cattle results in reduced growth and

persistence of dominant follicles and increased feed intake and BCS of undernourished cattle results in increased growth and persistence of dominant follicles.

In contrast with dominant follicles, persistence of largest subordinate follicles was substantially decreased after realimentation. Reduced energy intake in postpartum beef cows reduced the size of dominant follicles and the number of large estrogen-active follicles and increased the persistence of small subordinate follicles (Perry et al., 1991).

Negative energy balance in postpartum dairy cows was associated with increased number of medium size follicles and decreased maximum diameter of dominant follicles, while positive energy balance was associated with increased maximum diameter of dominant follicles and reduced growth of subordinates (Lucy et al., 1991). These studies and our results indicate that reduced energy intake and/or BCS increases persistence of subordinate follicles while increased energy intake and/or BCS of underfed cattle decreases persistence of subordinate follicles. The phenomenon of reduced dominance of the dominant follicle over the subordinate follicles during periods of underfeeding and reduced body energy reserves can be reversed with increased feed intake and BCS.

The gradual increase in growth rate, size and persistence of dominant follicles after realimentation was probably caused by increased LH secretion. The failure of ovulation during nutritionally induced anestrus results from inadequate secretion of LH, especially during the later part of the growing phase of dominant follicles. Furthermore, the preovulatory increases in LH in realimented heifers were similar to that observed in maintenance heifers with normal estrous cycles. However our results should be interpreted cautiously because evaluation of the magnitude of a preovulatory increase requires more frequent sampling.

The importance of LH for the final maturation of ovulatory follicles in cattle has been reviewed (Ginther et al., 1996). Concentrations of LH in this experiment were determined in daily samples, thus an inference about pulse frequency and amplitude of LH can not be made. Imakawa et al. (1986) found that realimentation of nutritionally anestrous beef heifers resulted in resumption of cyclicity after 50 d of initiation of refeeding and during this period pulse frequency, pulse amplitude and mean concentrations of LH gradually increased. Frequency of pulsatile LH secretion is positively associated with persistence and maximum diameter of dominant follicles in prepuberal heifers (Kinder et al., 1995), postpartum lactating cows (Grimard et al., 1995), in beef heifers chronically treated with a GnRH-agonist (Gong et al., 1995) and feed restricted cycling heifers (Chapter III). The gradual increase in LH concentrations after realimentation, and before resumption of ovulation, was associated with a gradual increase in estradiol production. Persistence of dominant follicles and ability to produce sufficient amounts of estradiol to induce preovulatory surges of LH depends on secretion of LH (Fortune, 1994). Hourly injections of LH to undernourished prepuberal lambs increased secretion of estradiol which in turn induced preovulatory surges of LH and ovulation (Mcshane and Keisler, 1991), and pulsatile infusion of GnRH in nutritionally anestrous beef cows increased peripheral estradiol concentrations and induced ovulations (Vizcarra et al., 1997). A gradual increase in estradiol concentrations occur during the last 50 d before the onset of puberty in beef heifers (Wolfe et al., 1989; Bergfeld et al., 1994) and this is associated with increased pulsatile secretion of LH. During the transition from seasonal anestrus to the breeding season in ewes, changes in responsiveness to estradiol negative feedback accounts for a gradual and parallel increase in concentrations of LH and estradiol (Karsch

et al., 1980). Maturation of the hypothalamus and changes in responsiveness to estradiol negative feedback result in increasing LH secretion and eventually attainment of puberty in beef heifers (reviewed by Kinder et al., 1995). However, changes in responsiveness to estradiol negative feedback cannot account for the gradual increase in LH secretion and resumption of cyclicity after realimentation of nutritionally anestrous beef heifers (Imakawa et al., 1986). Rather the mechanisms that control the GnRH pulse generator return to normal function during realimentation of nutritionally anestrous heifers and this allows a gradual increase in LH secretion which in turn increases ovarian output of estradiol and eventually resumption of ovulation.

Even though concentrations of LH in serum and size of ovulatory or luteinized follicles during W0 were similar among M, HGAIN and LGAIN heifers, the magnitude of the preovulatory increase in estradiol was less in HGAIN and LGAIN compared with M heifers. This observation indicates that size of a dominant follicle is not associated with its ability to produce estradiol. We also observed that dramatic reductions in estradiol secretion by dominant follicles preceding nutritionally induced anovulation was not associated with the size of dominant follicles (Chapter II). Reduced concentrations of estradiol preceding the first ovulation compared with subsequent ovulations, have been observed in postpartum beef cows (Perry et al., 1991; Stagg et al., 1995). In addition, intrafollicular concentrations of estradiol are less in preovulatory follicles of postpartum beef cattle destined to form short life-span CL compared to those destined to form normal life-span CL (Inskeep et al., 1988; Braden et al., 1989).

The reduced steroidogenic capabilities of first ovulatory follicles in postpartum cattle and of GnRH-induced preovulatory follicles in seasonal anestrous ewes has been

attributed to reduced LH receptors (Hunter et al., 1986; Inskeep et al., 1988; Braden et al., 1989). Reduction in LH receptors may account for reduced estradiol secretion by dominant follicles preceding first ovulation or luteinization in the present study. Reduced secretion of estradiol by preovulatory follicles was associated with short cycles in 9 out of 11 heifers. The other two heifers developed a luteinized follicle. The interval between detection of luteinization and subsequent ovulation was similar with the length of the short cycle observed in the 9 heifers. Short luteal phases have been observed in cattle and sheep during puberty, and following spontaneous and gonadotropin-induced postpartum ovulations at the start of the breeding season in anestrous ewes (for review see Garverick et al., 1992), and during resumption of cyclicity after realimentation of nutritionally anestrous beef heifers (Rhodes et al., 1995). Reduced concentrations of estradiol in plasma before the first ovulation after a period of anestrus results in decreased synthesis of uterine progesterone receptors, allowing premature synthesis of uterine oxytocin receptors and earlier release of $PGF_2\alpha$ (Zollers et al., 1993). This mechanism, and delayed ovulation after the preovulatory increase in estradiol in the present study, might explain the incidence of short cycles during resumption of ovulatory cycles after realimentation of nutritionally anestrous heifers. A similar static phase of dominant ovulatory follicles and delayed ovulation after the preovulatory increase in estradiol, have been observed preceding the first ovulation in prepuberal heifers (Evans et al., 1994).

Concentrations of FSH in serum of nutritionally anovulatory heifers and preceding resumption of ovulation after realimentation were similar when compared with cycling heifers fed maintenance diets. Feed restriction in rats does not decrease circulatory concentrations of FSH (Campell et al ., 1989; Sick and Bronson, 1986; Ronnekleiv et al.,

1978) and number of antral follicles (Meredith et al., 1986). Less frequent exogenous GnRH pulses to monkeys reduced concentrations of LH and increased concentration of FSH in serum (Wildt et al., 1981). Pituitary concentrations of FSH but not LH were reduced in nutritionally anestrous beef cows infused with one pulse of GnRH every 4 h, indicating that less frequent pulses of GnRH are required for secretion of FSH compared with LH (Vizcarra et al., 1997). The onset of nutritionally induced anovulation in beef heifers was associated with increased concentrations of FSH in serum (Chapter III) but this was probably due to reduced persistency of the dominant follicle and acceleration of events (reduced secretion of estradiol and/or other factors) leading to an FSH surge and initiation of a new follicular wave. Concentrations of FSH were not different between cycling and anovulatory heifers when compared at the same stage of follicular growth. Realimentation of nutritionally anovulatory heifers resulted in reduced persistency of the first subordinate follicle. Our results indicate that changes in FSH concentrations can not account for recovery of mechanisms of dominance after realimentation. In agreement with our results, Stagg et al. (1995) found that concentrations of FSH and secretion of FSH during normal estrous cycles, nutritionally induced anestrus and resumption of cyclicity after realimentation of nutritional anestrous heifers were similar. Factors in follicular fluid other than steroids and inhibin can suppress follicular development without affecting peripheral FSH concentrations (Law et al., 1992). Gradual increases in size of dominant follicles and concentrations of LH and estradiol after realimentation may result in increasing production of factors in follicular fluid and reestablishment of dominance.

Realimentation of nutritionally anovulatory heifers resulted in a gradual increase in concentrations of IGF-I in plasma. Peripheral concentrations of IGF-I are positively

associated with body condition and nutrient intake (Chapter III, Houseknecht et al., 1988; Richards et al., 1991; Yelich et al., 1996). Concentrations of IGF-I were not significantly different in HGAIN and LGAIN heifers. Even though HGAIN heifers ovulated earlier than LGAIN heifers, IGF-I concentrations in the two groups increased similarly preceding resumption of ovulation. This provides evidence that some effects of nutrition on reproductive tissues could be mediated by IGF-I. Decreased concentrations of IGF-I are associated with delayed puberty (Granger et al., 1989) and increased postpartum anestrous intervals (Rutter et al., 1989; Nugent et al., 1993) in beef cattle. In cattle and other domestic species, IGF-I mediates some of the effects of gonadotropins in vitro, especially FSH-stimulated estradiol production by granulosa cells, LH-induced androstenedione production by thecal cells, and granulosa cell proliferation and differentiation (reviewed by Spicer and Echternkamp, 1995). The substantial reduction in concentrations of IGF-I in plasma during the last ovulatory cycle before the onset of nutritionally induced anovulation was not associated with reduced peripheral estradiol concentrations but was associated with reduced maximum diameter of ovulatory follicles (chapter III). In the present study, the gradual increase in IGF-I concentrations after realimentation of nutritionally anestrous heifers was associated with a gradual increase in the size of the dominant follicle. The effect of underfeeding on the concentrations of biologically active IGF-I in reproductive tissues needs further study since feed restriction alters concentrations of IGFBPs (Roberts et al., 1994; Vandehaar et al., 1995;).

Concentrations IGF-I can directly affect both hypothalamic and pituitary function. Secretion of gonadotropins in cultures of rat pituitary cells can be enhanced by IGF-I (Kanematsu et al., 1991). In addition, IGF-I enhanced GnRH-stimulated LH secretion

from rat pituitary gonadotropes in vitro (Soldani et al., 1995) and secretion of GnRH from nuclei isolated from median eminence of rats (Hiney et al., 1994). Administration of GH to dwarf mice (GH-deficient mice) increased circulatory IGF-I which in turn altered LH secretion by modulating responsiveness of the pituitary to GnRH and steroids (Chandrashekar and Bartke, 1993). Administration of rhIGF-I to adolescent female monkeys reduced the interval from menarche to first ovulation by decreasing the hypersensitivity of pituitary gonadotropes to estradiol negative feedback (Wilson,1995). The effects of reduced concentrations of IGF-I on the hypothalamo-pituitary axis during nutritionally induced anestrus needs further study since IGF-I and IGFBPs have been detected in the hypothalamus and anterior pituitary gland of beef cattle (Funston et al., 1993).

Realimentation of nutritionally anestrous heifers resulted in increased insulin and glucose concentrations in plasma. Feed restriction and loss of BW and/or BCS in cattle are associated with reduced plasma concentrations of insulin and glucose (McCann and Hansel, 1986; Richards et al., 1989; Vizcarra et al., 1996; Chapter III).

Realimentation of steers that had been chronically underfed increased concentrations of insulin and glucose to concentrations similar to those in control steers within 30 d, and concentrations of IGF-I in realimented steers were similar to those in control steers by 60 d after initiation of refeeding. In our study, two follicular waves before the emergence of the ovulatory wave (25-30 d before the first ovulation or luteinization) concentrations of insulin and glucose in plasma of the HGAIN and LGAIN heifers were similar to those observed in cycling heifers fed maintenance diets. This indicates that insulin and glucose concentrations may not be associated with the time that

resumption of ovulation occurred after realimentation of nutritionally anovulatory beef heifers. Alternatively, if insulin and/or glucose provide the signal for resumption of ovulation, it may take 25-30 days for the effect to be manifested at the hypothalamo-pituitary-ovarian axis.

Insulin and glucose concentrations in the postpartum beef cows are not predictive of luteal activity (Vizcarra et al., 1996). Intracerebraventricular infusion of insulin in growth restricted ovariectomized ewes did not alter LH secretion (Hileman et al., 1993) and suppression of postmeal insulin secretion by diazoxide in previously underfed rats did not prevent secretion of LH (Williams et al., 1996). Even though phlorizin-induced hypoglycemia prevented the increase in insulin and LH concentrations that normally follow early weaning in postpartum beef cows (Rutter and Manns, 1987), glucose infusion in postpartum cows did not alter LH secretion (Rutter et al., 1989). It is likely that insulin and glucose concentrations in plasma must be maintained above a threshold for normal hypothalamo-pituitary function in cattle.

Realimentation of nutritionally anestrous heifers resulted in decreased concentrations of NEFA in plasma. Plasma concentrations of NEFA are inversely related with feed intake or energy balance in ruminants (Peters, 1986; Lucy et al., 1991). Even though realimentation of nutritionally anestrous heifers reduced concentrations of NEFA in plasma, a gradual increase in NEFA concentrations was observed preceding resumption of ovulation. During the onset of nutritionally induced anovulation, concentrations of NEFA were greater in R compared with M heifers (Chapter III). However concentrations of NEFA in R heifers during cycle -1 (the first anovulatory cycle) were less compared with cycle -2 (the last ovulatory cycle) indicative of fat depletion or a gradual reduction in

metabolic rate in chronically underfed heifers. Feed restriction reduces resting metabolic rate in heifers (Yambayamba et al., 1996) and steers (Lapierre et al., 1992). Realimentation of chronically underfed heifers resulted in recovery of resting metabolic rate to levels observed in control heifers by 36 d after initiation of refeeding (Yambayamba et al., 1996). It is likely that realimentation of nutritionally anestrous heifers results in a gradual increase in fat depots and increased metabolic rate preceding resumption of ovulation.

Implications

Availability of metabolic fuels and/or BCS are major factors influencing reproductive efficiency. Nutritionally induced anovulation occurred when heifers had a BCS of 3.8 and heifers had a BCS of 4.6 at resumption of ovulation after realimentation. Therefore, less body fat stores are needed to maintain ovarian cycles than are needed to reinitiate cycles after nutritionally induced anovulation. Rapid gain after nutritionally induced anovulation in beef heifers results in a shorter interval between anestrus and resumption of ovulation. However, resumption of cyclicity with rapid gain requires more feed because BW at resumption of cyclicity of HGAIN was greater compared with LGAIN heifers. Thus, limit fed high energy diets after a period of anestrus are probably more cost effective compared with ad libitum high energy diets.

Table 1. Composition of diets

Item	HGAIN	LGAIN	MAINTENANCE
Ingredients, as fed %			
Corn distillers grain	23.2	20.0	-
Rolled corn	38.2	32.9	37.5
Cottonseed hulls	7.7	6.7	21.7
Alfalfa pellets	3.9	3.4	32.5
Prairie hay	22.7	33.3	-
Cane molasses	3.5	3.0	3.0
SBM	-	-	5.0
Limestone 38%	.5	.5	-
Salt	.2	.2	.3
Zinc oxide	.002	.002	-
Vitamin A-30,000	.02	.02	-
Vitamin E--50%	.02	.02	-
Calculated values as fed			
Kg	10.0	6.8	4.5
DM%	89.7	86.6	88.9
Total NEm, Mcal	16.2	10.2	6.7
Total NEg, Mcal	9.7	6.1	3.6
CP %	10.4	9.6	12.2

Table 2. Influence of daily gain during realimentation of nutritionally anovulatory heifers fed at two different levels of nutrition on interval between anovulation and resumption of ovulation and BW and BCS at first ovulation or luteinization

Criteria	HGAIN	LGAIN	MSE
Days to first ovulation or luteinization after realimentation, d	57[a]	80[b]	75
BW at anovulation, kg	295[a]	300[a]	117
BW at first ovulation or luteinization, kg	387[a]	343[b]	94
Change in BW from anovulation to resumption of ovulation, %	28[a]	16[b]	10
BCS at anovulation	3.8[a]	3.8[a]	.1
BCS at first ovulation or luteinization	4.7[a]	4.4[a]	.2

[a,b] Means within a row lacking a common superscript differ (P<.05).

Table 3. Characteristics of dominant follicles during Wan, W-2, W-1 and W0 in HGAIN and LGAIN heifers

	Wave				
Criteria	Wan	W-2	W-1	W0	MSE
Growth rate, mm/d	.9[a]	1.2[b]	1.5[c]	1.6[c]	.04
Duration of growing phase, d	5.2[a]	6.4[b]	6.4[b]	7.0[b]	.7
Max diameter, mm	9.2[a]	11.7[b]	13.2[c]	15.3[d]	1.1
Duration of static phase, d	3.8[a]	4.9[b]	5.0[b]	2.5[c]	.5
Regression rate, mm/d	1.0[a]	1.2[b]	1.5[c]	-	.02
Duration of regression phase, d	5.0[a]	6.0[b]	5.9[b]	-	.4
Wave persistence, d	14.0[a]	17.2[b]	17.4[b]	-	4.8

[a, b, c, d] Means within a row lacking a common superscript differ (P<.05).

Table 4. Characteristics of first subordinate follicles during Wan, W-2, W-1 and W0 in HGAIN and LGAIN

Criteria	Wave				
	Wan	W-2	W-1	W0	MSE
Growth rate, mm/d	.8[a]	1.2[b]	1.4[c]	1.7[d]	.02
Duration of growing phase, d	4.0[a]	3.3[b]	2.9[bc]	2.4[c]	.3
Max diameter, mm	7.3[a]	8.3[b]	8.2[b]	8.2[b]	.6
Duration of static phase, d	2.9[a]	2.4[b]	2.0[c]	1.4[d]	.08
Regression rate, mm/d	.7[a]	1.1[b]	1.4[c]	1.8[d]	.03
Duration of regression phase, d	4.3[a]	3.6[b]	2.8[c]	2.2[d]	.2
Wave persistence, d	11.2[a]	9.3[b]	7.7[c]	6.0[d]	1.9

[a, b, c, d] Means within a row lacking a common superscript differ ($P<.05$).

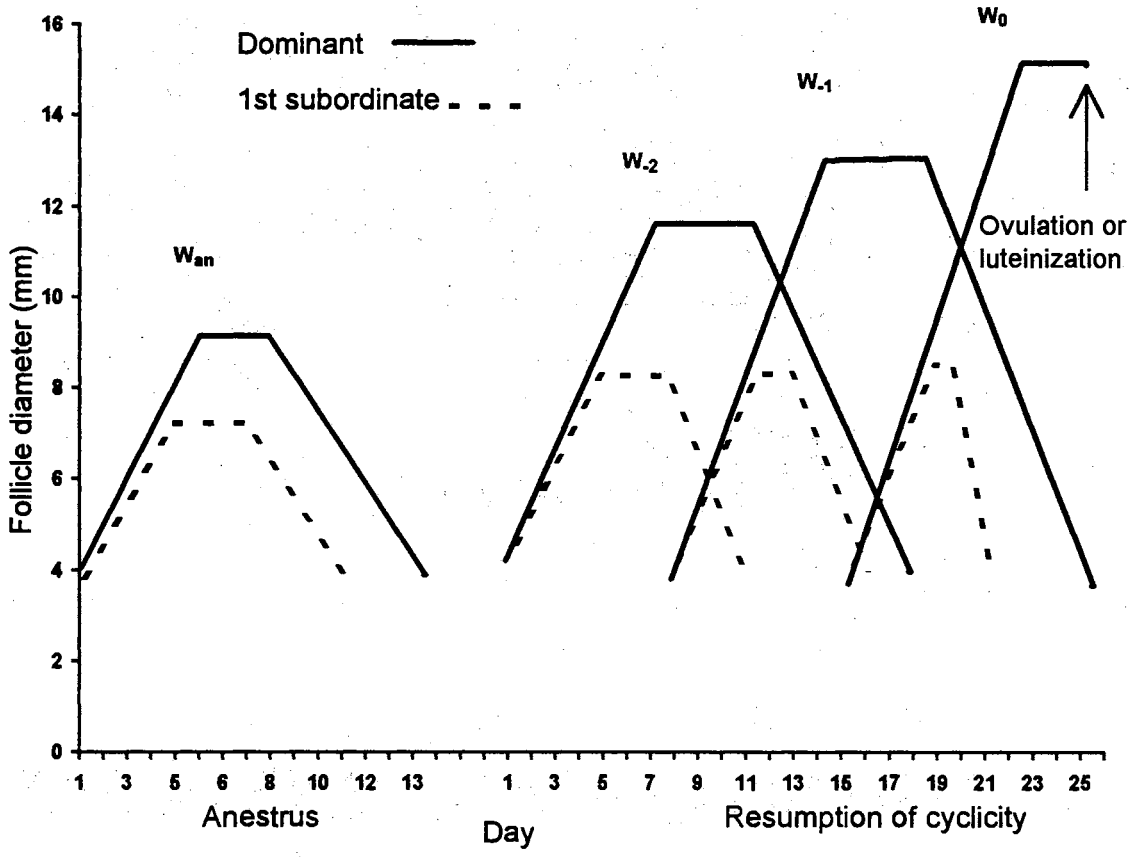

Figure 1. Diameters of dominant and first subordinate follicles during anovulation and resumption of ovulation after realimentation.

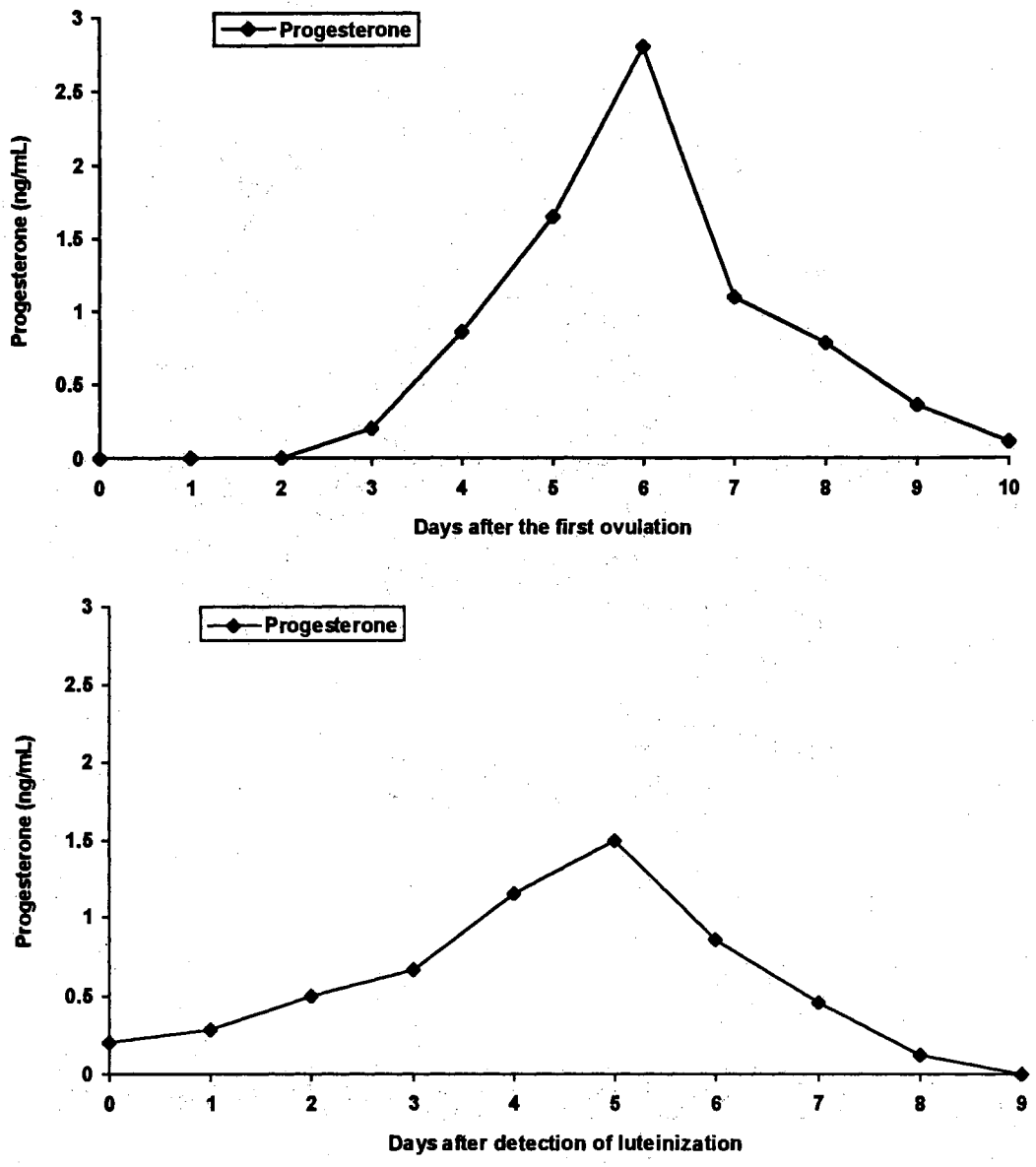

Figure 2. Concentrations of progesterone after first ovulation (a, n = 9) or luteinization (b, n = 2).

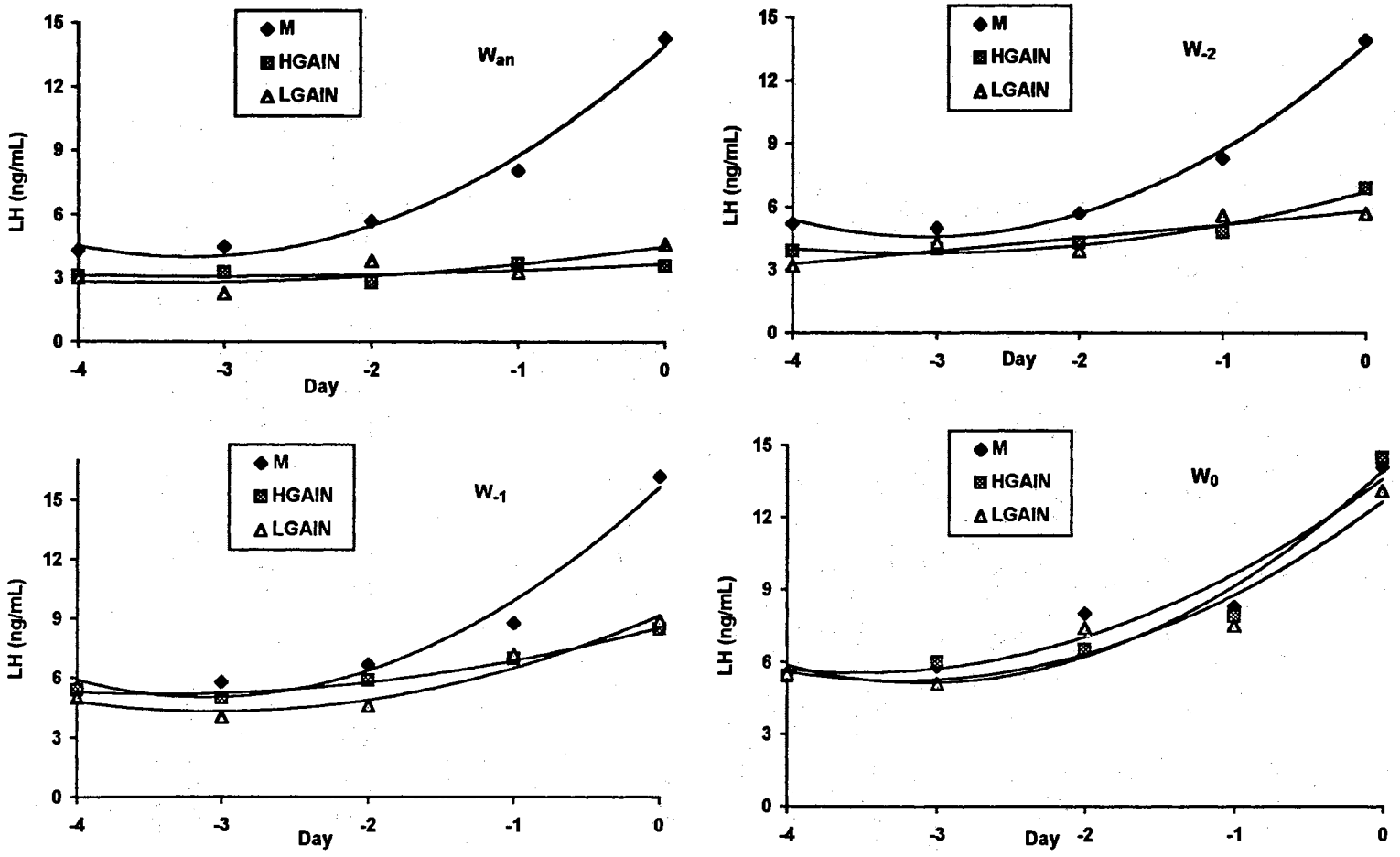

Figure 3. Least square means (symbols) and least square regressions (lines) for concentrations of LH in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves when heifers were anovulatory and before initiation of refeeding (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Treatment x wave x day effect ($P<.01$; MSE=5.46), where d 0 is the day that the dominant follicle had a maximum diameter in anovulatory heifers and one day before ovulation in cyclic heifers.

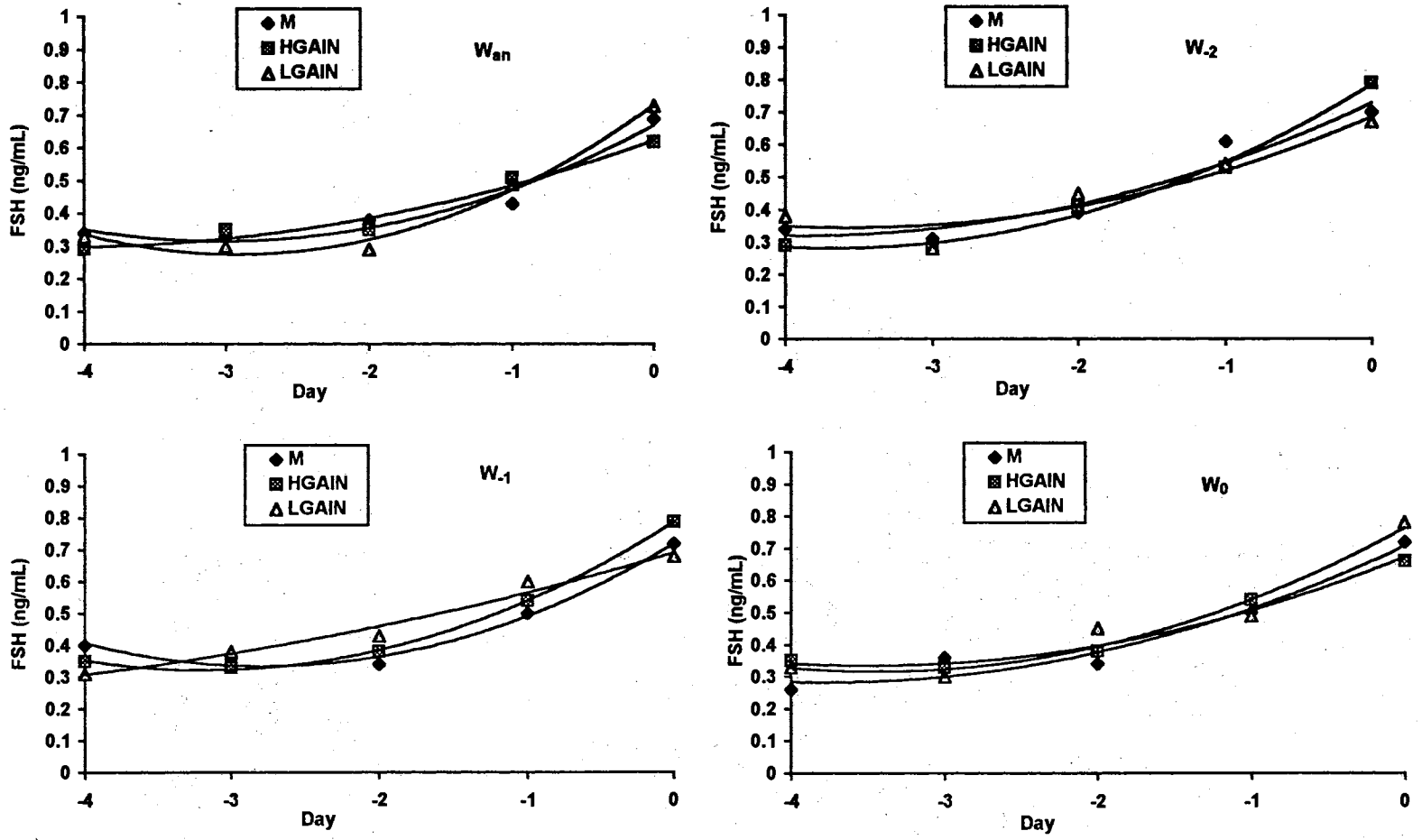

Figure 4. Least square means (symbols) and least square regressions (lines) for concentrations of FSH in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves where heifers were anovulatory and before initiation of refeeding (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Day effect (P<.01; MSE=5.46), where d 0 is the day that the dominant follicle had a maximum diameter in anovulatory heifers and one day before ovulation in cyclic heifers

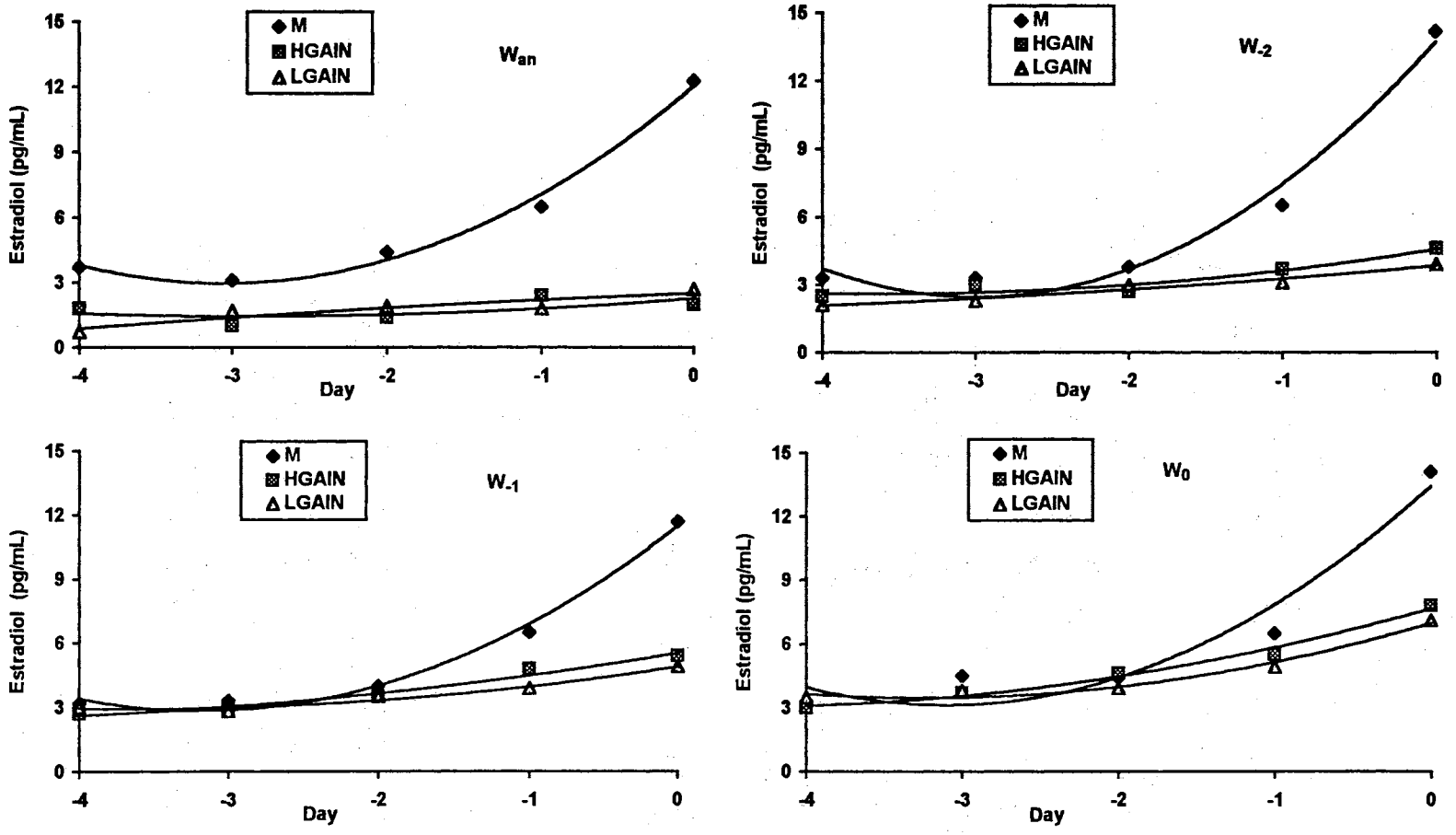

Figure 5. Least square means (symbols) and least square regressions (lines) for concentrations of estradiol in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves where heifers were anovulatory and before initiation of refeeding (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Treatment x wave x day effect ($P<.05$; MSE = 3.57), where d 0 is the day that the dominant follicle had a maximum diameter in anovulatory heifers and one day before ovulation in cyclic heifers.

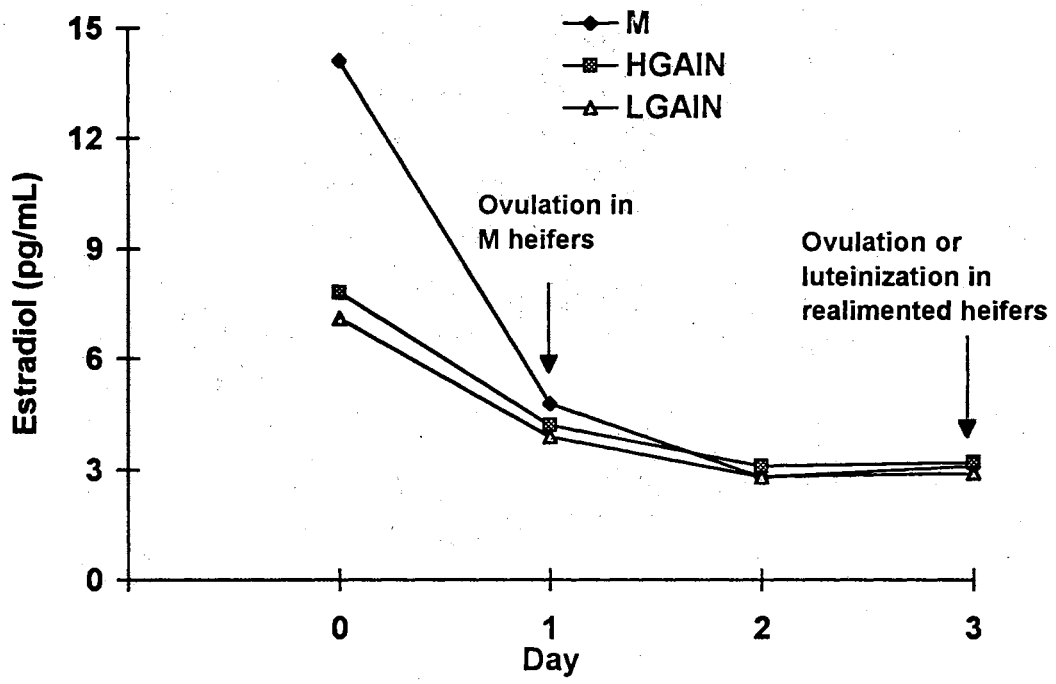

Figure 6. Estradiol concentrations in M, HGAIN and LGAIN during the first ovulation or luteinization, after realimentation, where d 0 is the day that the dominant follicle had a maximum diameter in anovulatory heifers and in cyclic heifers.

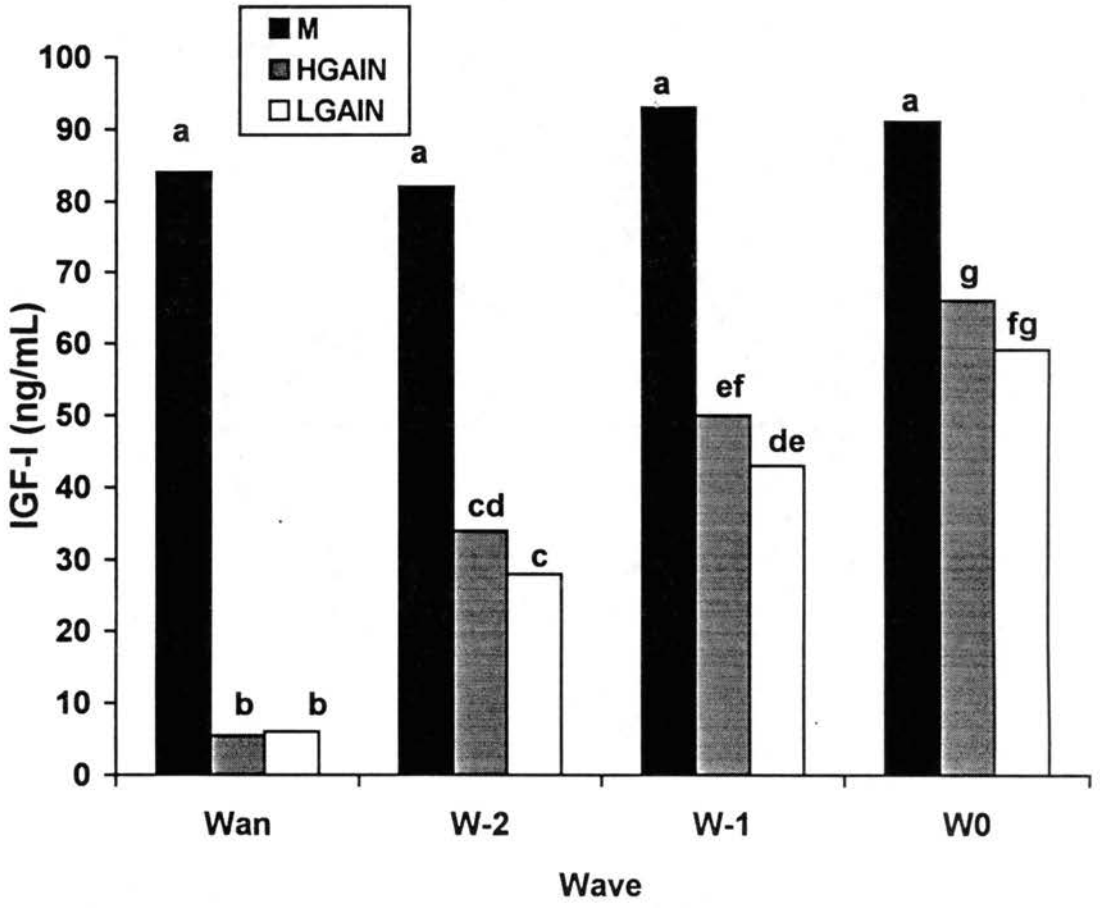

Figure 7. Least square means for concentrations of IGF-I in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Concentrations for days (-4, -3, -2, -1,and 0) are averaged over treatment and wave. Treatment x wave ($P<.0001$; MSE = 153).

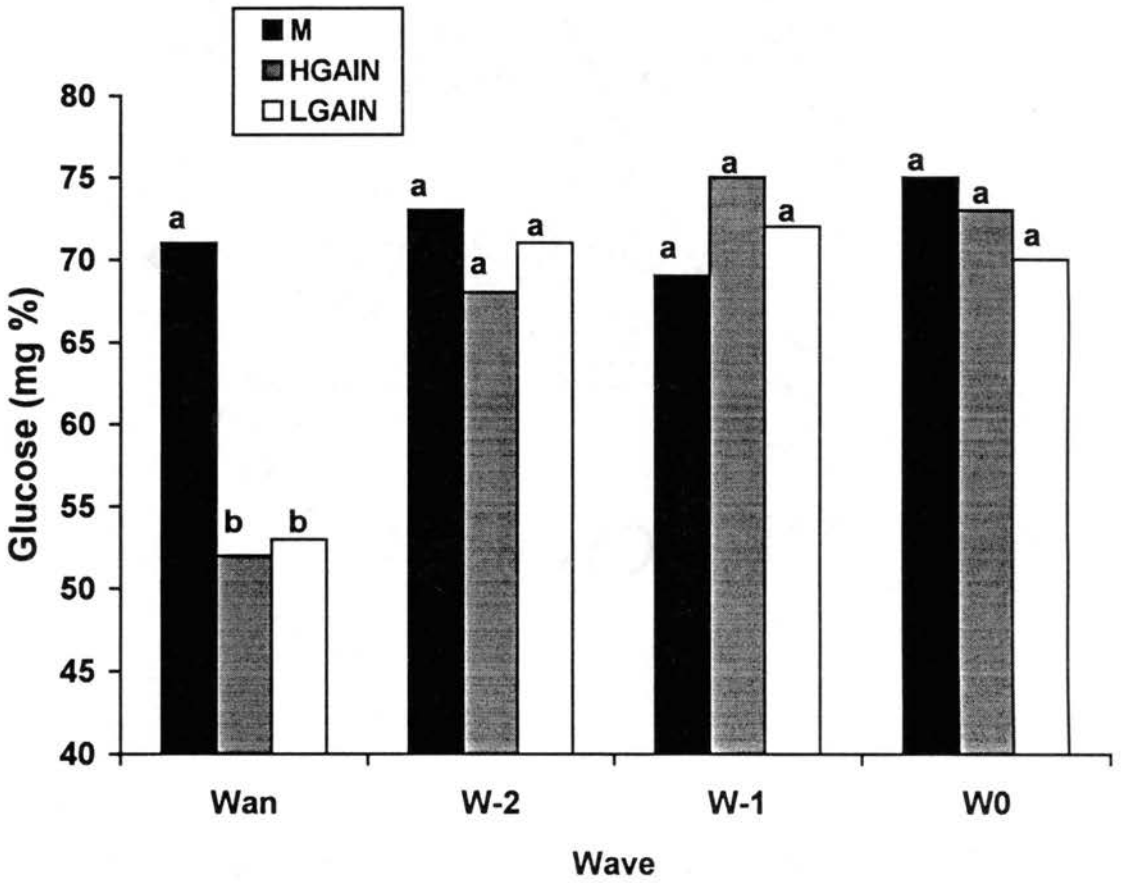

Figure 8. Least square means for concentrations of glucose in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Concentrations for days (-4, -3, -2, -1,and 0) are averaged over treatment and wave. Treatment x wave ($P<.01$; MSE = 81).

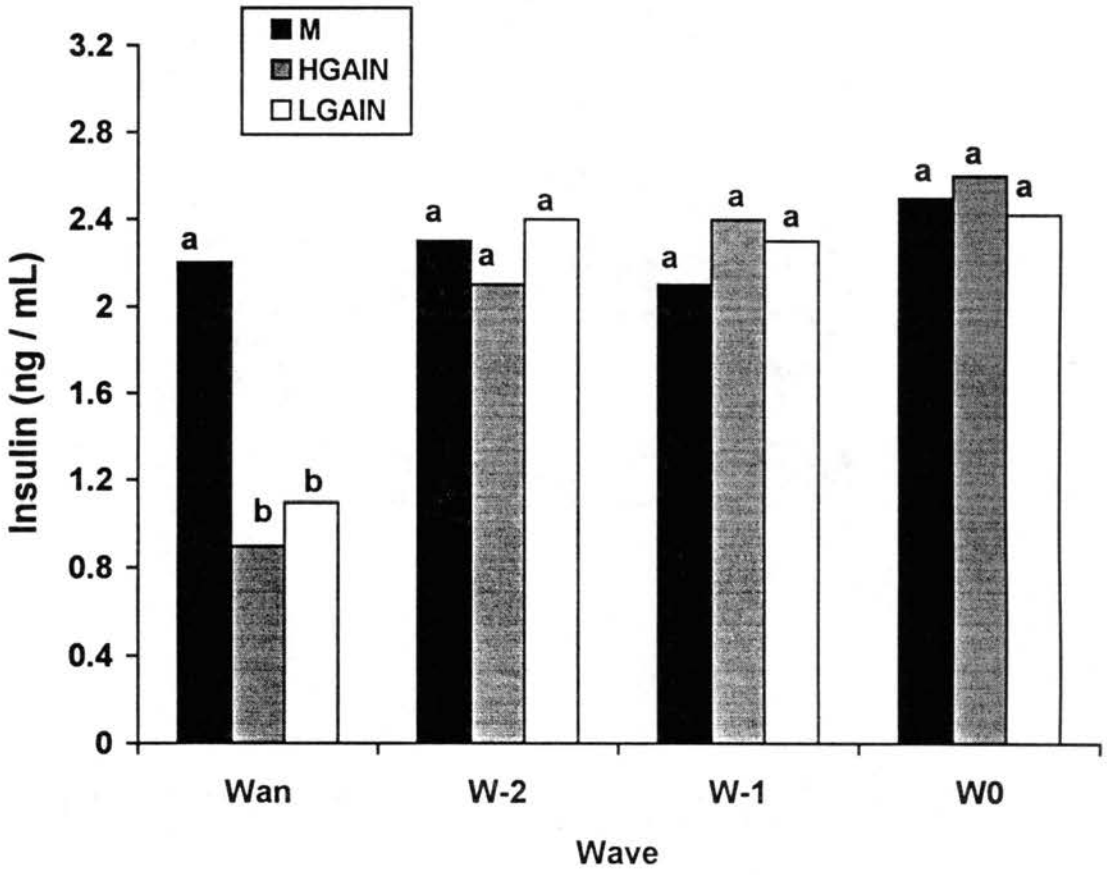

Figure 9. Least square means for concentrations of insulin in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Concentrations for days (-4, -3, -2, -1,and 0) are averaged over treatment and wave. Treatment x wave ($P<.01$; MSE = .34).

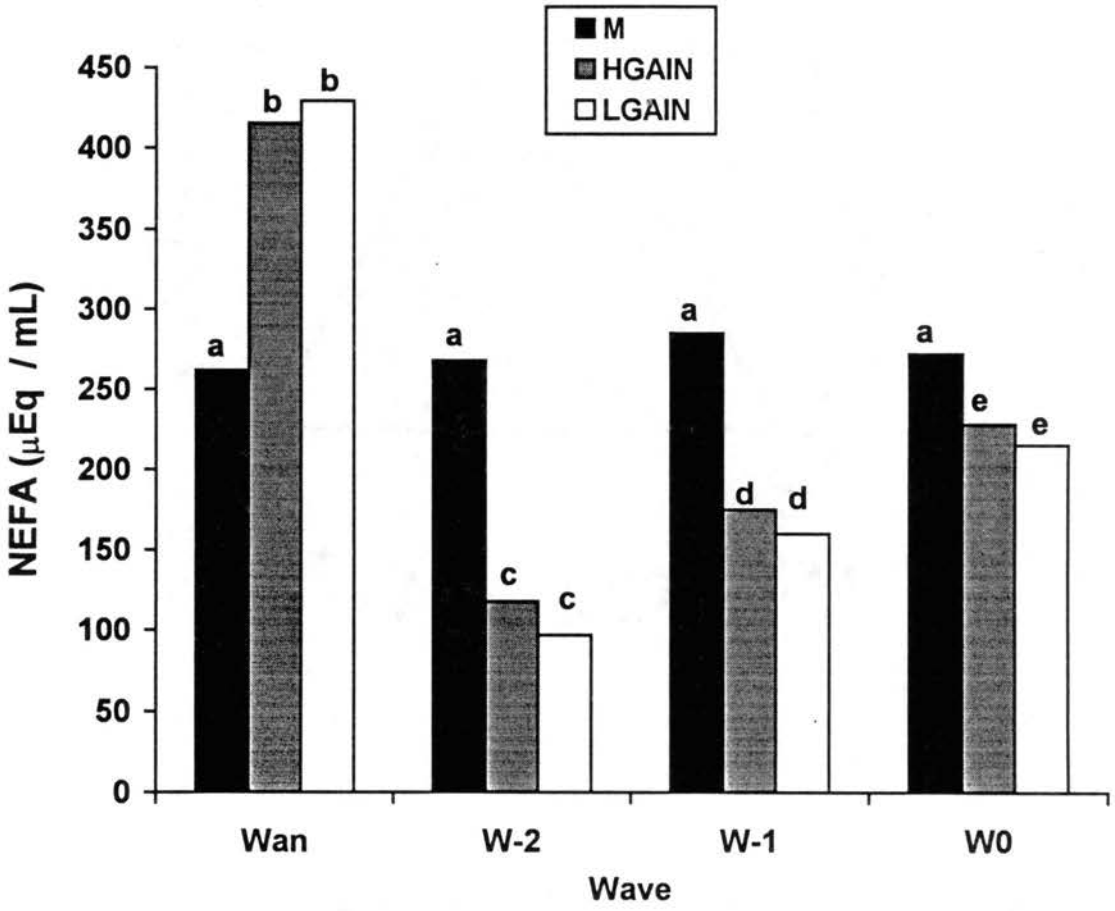

Figure 10. Least square means for concentrations of NEFA in cyclic heifers (M), or heifers gaining .6 (LGAIN) or 1.5 kg/d (HGAIN) during follicular waves when heifers were anovulatory and before initiation of realimentation (Wan), two (W-2) or one (W-1) waves before ovulation or luteinization (W0) and W0. Concentrations for days (-4, -3, -2, -1,and 0) are averaged over treatment and wave. Treatment x wave ($P<.0001$; MSE = 864).

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine mechanisms by which nutrition and body energy reserves influence ovarian function, gonadotropin secretion and concentrations of metabolic hormones and metabolites in beef heifers. The specific objectives of this research were: 1) to evaluate follicular growth and determine concentrations of LH, FSH and GH in serum and progesterone, estradiol, IGF-I, insulin, glucose and NEFA in plasma during the last two cycles before the onset of nutritionally induced anovulation in beef heifers, and 2) to monitor sequential changes in the pattern of follicular growth and concentration of LH, FSH, estradiol, IGF-I, insulin, glucose and NEFA during the transition from nutritionally induced anovulation to resumption of ovulation after realimentation at two different rates of gain.

During experiment 1, eighteen cyclic Angus x Hereford heifers 14-18 months of age with a BCS of 5.5 $\pm$ 0.2 and BW of 378 $\pm$ 15 kg were used to determine endocrine and ovarian changes preceding nutritionally induced cessation of ovarian cycles. In two replication, a total of six heifers were fed to maintain BCS (M group) and growth, and twelve heifers were fed a restricted diet (R group) to lose approximately 1 % of their BW/wk. At the initiation of the study, estrous cycles of all heifers were synchronized with two injections of $PGF_2\alpha$ (Lutalyse) at an 11 d interval. Starting on d 13 of the induced

cycle, heifers were given $PGF_{2\alpha}$ every 16 d thereafter to synchronize and maintain 16 d estrous cycles. Transrectal ultrasonography was performed daily every second cycle to monitor the ovaries from d 8 until ovulation (d 1 of the subsequent cycle). When heifers had lost 12 % of their initial body weight, ultrasonography was performed every cycle until R heifers became anovulatory. Size and growth rate of the ovulatory follicle and maximum corpus luteum size were determined during the last ovulatory cycle (cycle -2) and the subsequent anovulatory cycle (cycle -1). Concentrations of LH, FSH and GH were quantified in blood samples collected every 10 min for 8 h on d 2 and d 15 during cycles -2 and -1. Concentrations of estradiol, glucose, insulin, IGF-I and NEFA were quantified in daily plasma samples collected from d 8 until d 0 and progesterone in daily samples from d 8 until d 15 during cycles -2 and -1.

Reducing feed intake of R heifers resulted in loss of BW and BCS and failure of ovulation an average of 25 wk after initiation of feed restriction. At the time of anovulation, R heifers had lost 22% of their initial BW and 30% of their initial BCS. During the same period, M heifers gained 19% of their initial BW and maintained their BCS.

Restriction of intake to R heifers reduced growth rate and maximum size of the ovulatory follicle during the last two cycles before anovulation. Growth rate and maximum size of the dominant follicle after induced luteolysis in restricted heifers were not different between cycle -2 and cycle -1. This indicates that reduction in size doesn't compromise the ability of a dominant follicle to ovulate.

Even though there was a substantial decrease in the size of the ovulatory follicle in R heifers compared with M heifers during cycle -2, concentration of estradiol-17β in

plasma of M and R heifers were similar. Maximum size of dominant follicles was similar during cycles -2 and -1 in R heifers, concentrations of estradiol were less during cycle -1 vs -2 and a preovulatory increase in estradiol did not occur during the anovulatory cycle. These observations indicate that a reduction in the maximum size of dominant ovulatory follicles in feed restricted cycling heifers is not always associated with reduced concentrations of estradiol in plasma.

Concentration and pulse amplitude of LH were reduced in R heifers during cycle -1 but not during cycle -2, while pulse frequency of LH in R heifers was reduced in both cycles -2 and -1 during the late follicular phase (d 15) compared with M heifers. We speculate that the reduction in LH pulse frequency on d 15 results in decreased maximum size of the dominant follicle, while the reduction in LH concentration on d 2 and d 15 results in decreased estradiol concentrations and failure of ovulation.

Despite the hypothesized reduction in GnRH secretion before the onset of nutritionally induced anestrus, concentration, pulse frequency and pulse amplitude FSH increased in R heifers during the late follicular phase of the anovulatory cycle. This increase in FSH secretion was associated with reduced estradiol concentrations and arrest of growth of the dominant follicle. Normally, emergence of a follicular wave is preceded by a surge of FSH which is coincident with the cessation in growth of the dominant follicle of the previous wave or an ovulation. The increased FSH concentrations preceding emergence of a follicular wave have been attributed to declining amounts of inhibitory substances (estradiol, inhibin or other proteins) originating from the dominant follicle of the previous wave. During the first anovulatory cycle, arrest of growth of the dominant

follicle due to insufficient LH secretion in R heifers might have stimulated increased FSH concentrations that are normally observed before the emergence of a new wave.

Feed restriction resulted in reduced maximum size of the CL during the last two cycles before the onset of anovulation, and this was associated with reduced concentrations of progesterone in plasma.

Concentrations of NEFA in plasma were greater in R compared with M heifers before the onset of nutritionally induced anestrous. Concentrations of NEFA in R heifers during cycle -1 were less compared with cycle -2, indicative of fat depletion or reduced fat mobilization in chronically underfed heifers. Concentrations of NEFA in plasma are a good indicator of metabolic status if body fat exists. However, it is unlikely that NEFA can directly affect the hypothalamo-pituitary axis and secretion of gonadotropins.

Increased concentrations of NEFA in the plasma of R heifers were associated with increased concentrations of GH in serum. Growth hormone induces a variety of metabolic effects including fat mobilization by increasing lipolysis and decreasing lipogenesis. Concentrations and pulse amplitude of GH in serum were greater in R heifers compared with M heifers but pulse frequency was not affected by chronic restricted intake. Despite increased GH secretion, concentrations of IGF-I in plasma were markedly reduced by restriction of feed intake, suggesting that the tissues are insensitive to GH in undernourished animals. In contrast with GH, the effects of IGF-I include enhanced glycogenesis and lipogenesis. It has been suggested that increased GH and decreased IGF-I and insulin in feed restricted animals represents a mechanism for preferential utilization of mobilized substrates to maintain homeostasis and provide metabolic fuels for reproductive function (Brier et al., 1988; Hileman et al., 1991). Reduction in GH binding

to hepatic membranes and reduced liver mass might be associated with "uncoupling" of the GH-IGF-I axis during feed restriction. The effects of GH on reproductive tissues are probably mediated through IGF-I. The substantial reduction in concentrations of IGF-I in plasma during cycle -2 were not associated with reduced concentrations of estradiol in plasma but were associated with reduced maximum diameter of the ovulatory follicles, maximum CL size and reduced LH pulse frequency.

Feed restriction resulted in a 50 % reduction in concentrations of insulin in plasma. The 50% reduction in peripheral insulin concentrations during cycle -2 was not associated with reduced peripheral estradiol concentrations but was associated with decreased ovulatory follicle diameter. Feed restriction and loss in BW and/or BCS were associated with decreased glucose concentrations in plasma during the last two cycles before the onset of nutritionally induced anovulation.

In the second study, twelve nutritionally anestrous beef heifers with a BW of 298 $\pm$ 3 kg and BCS of 3.8 $\pm$ .1 and six cyclic heifers with a BW of 453 $\pm$ 10 kg and BCS of 5.2 $\pm$ .2 (the same heifers used in experiment 1) were used in two replications to determine follicular growth and concentration of hormones and metabolites during anovulation and resumption of ovulation after realimentation at two different daily gains. Nutritionally anestrous heifers were separated into two groups (n=6) and refed to gain .6 (LGAIN) or 1.5 kg/d (HGAIN). Cyclic heifers were fed a maintenance diet (M) and estrous cycles were synchronized, with injections of $PGF_2\alpha$ (25 mg of Lutalyse; Upjohn), to a length of 16 d. Body weight at anovulation was similar for LGAIN and HGAIN heifers (295 $\pm$ 5 and 296 $\pm$ 4 kg for the HGAIN and LGAIN, respectively). Ultrasonography was

performed and blood samples were collected daily during anovulation and after realimentation until resumption of ovulation. Follicles ≥4 mm in diameter were sequentially identified and measured in one follicular wave before realimentation (Wan) and two waves (W-2, W-1) before the first wave resulting in ovulation or luteinization (W0)and during W0. Hormone and metabolite concentrations were determined in daily serum or plasma samples obtained during the last 5 d of the growing phase of dominant or ovulatory follicles in anovulatory or cyclic heifers, respectively, during Wan, W-2, W-1 and W0.

Greater daily gain in HGAIN than LGAIN heifers resulted in a shorter interval until resumption of ovulation during realimentation, but HGAIN heifers had greater BW compared with LGAIN heifers. At the time of first ovulation or luteinization, BCS was not different between HGAIN and LGAIN heifers. These results indicate that an increase in body fat is required for initiation of ovulatory cycles after nutritionally induced anovulation.

Diet (HGAIN or LGAIN) did not influence follicular characteristics during realimentation. Maximum size, growth rate, regression rate and persistence of dominant follicles increased gradually during realimentation. In contrast with dominant follicles, persistence of the largest subordinate follicles was substantial decreased during realimentation.

The gradual increase in follicular growth after realimentation was probably caused by increased LH and/or IGF-I secretion. Failure of ovulation during periods of nutritionally induced anovulation results from inadequate LH secretion during the late part

of the growing phase of dominant follicles. The importance of LH support for the final maturation of ovulatory follicles in cattle has been well documented.

Increases in LH and/or IGF-I concentrations after realimentation before resumption of ovulation was associated with increased concentrations of estradiol in plasma. Persistence of a dominant follicle and its ability to produce sufficient amounts of estradiol to induce a preovulatory surge of LH depends on the secretion of LH. Changes in responsiveness to estradiol negative feedback can not account for the gradual increase in LH secretion and resumption of ovulation after realimentation of nutritionally anestrous beef heifers. Rather the mechanisms that control the GnRH pulse generator return to normal function during realimentation of nutritionally anovulatory heifers and this allows a gradual increase in LH secretion which in turn increases ovarian output of estradiol and eventually resumption of ovulation.

Even though the magnitude of the preovulatory increase of LH and the size of the ovulatory or luteinized follicle during W0 were similar among M and HGAIN and LGAIN heifers, the magnitude of the preovulatory increase of estradiol was less in HGAIN and LGAIN compared with M heifers. This indicates that the size of a dominant follicle is not always associated with its ability to produce estradiol. The same conclusion was made when comparing the size of ovulatory follicles and concentrations of estradiol in plasma during the last two cycles before the onset of nutritionally induced anovulation. Reduced LH receptors in dominant follicles may account for the reduced magnitude of the preovulatory increase of estradiol in realimented vs cycling heifers fed maintenance diets.

The first ovulation in realimented heifers was followed by a short cycle in 9 out of 11 heifers. The other two heifers developed a luteinized follicle. The interval between

detection of luteinization and subsequent ovulation was similar with the length of the short cycle observed in the 9 heifers. Reduced concentrations of estradiol before the first ovulation after a period of anovulation results in decreased synthesis of uterine progesterone receptors allowing for a premature synthesis of uterine oxytocin receptors and earlier release of $PGF_2\alpha$. This mechanism and the delayed ovulation after the preovulatory increase of estradiol observed in the present study might explain the incidence of short cycles during resumption of ovulation after realimentation of nutritionally anestrous heifers.

Concentrations of FSH in serum of nutritionally anestrous heifers and preceding resumption of ovulation after realimentation were similar when compared with heifers fed maintenance diets that continue to cycle. It is likely that FSH is not a limiting factor for ovulation in anestrous heifers. Our results indicate that changes in FSH concentrations do not account for the recovery of the mechanisms of dominance after realimentation. Follicular fluid factor(s) other than steroids and inhibin may suppress follicular development without affecting peripheral FSH concentrations.

Realimentation of nutritionally anestrous heifers resulted in a gradual increase in concentrations of IGF-I in plasma. Even though HGAIN heifers resumed ovulation earlier than LGAIN heifers, concentrations of IGF-I in plasma in the two groups increased similarly preceding resumption of ovulation. This observation provides evidence that IGF-I could mediate some of the effects of nutrition on reproductive tissues. In the first experiment, the substantial reduction in concentrations of IGF-I in plasma during the last ovulatory cycle before the onset of nutritionally induced anovulation was not associated with reduced concentrations of estradiol in plasma but was associated with reduced

maximum diameter of the ovulatory follicles. In the second experiment, the gradual increase in IGF-I concentrations after realimentation of nutritionally anovulatory heifers was associated with a gradual increase in size of dominant follicles. These results indicate that concentrations of IGF-I in plasma are not associated with the steroidogenic capabilities of dominant follicles, but may be involved in the mechanisms controlling size of dominant follicles. It is likely that IGF-I regulates proliferation of follicular granulosa cells. Feed restriction reduces intrafollicular concentrations of IGF-I in large dominant follicles but not in follicles less than 7 mm. Reduced concentrations of IGF-I in feed restricted heifers may reduce the number of granulosa cells in large dominant follicles. The majority of luteal cells originate from granulosa cells of ovulatory follicles. Reduced CL size during feed restriction further support that reduced size of dominant follicles results from decreased granulosa cell number. This may explain why feed restriction substantially reduces the size of dominant follicles, but does not have a profound effect on size of subordinate follicles. The effects of reduced IGF-I concentrations on the hypothalamo-pituitary axis during nutritionally induced anovulation need further study since IGF-I and IGFBPs have been detected in the hypothalamus and anterior pituitary gland of beef cattle.

Realimentation of nutritionally anestrous heifers resulted in increased concentrations of insulin and glucose. Two follicular waves before the emergence of the ovulatory wave (25-30 d before the first ovulation or luteinization) concentrations of insulin and glucose in plasma of the HGAIN and LGAIN heifers were similar with those observed in maintenance heifers. This observation indicates that concentrations of insulin and glucose may not associated with the time of resumption of ovulation after

realimentation of nutritionally anestrous beef heifers. Alternatively, if insulin and/or glucose provide the signal for resumption of ovulation, it may take 25-30 days for the effect to be manifested at the hypothalamo-pituitary-ovarian axis. It is likely that insulin and glucose concentrations in plasma must be maintained above a threshold for normal hypothalamo-pituitary function in cattle.

Physiological concentrations of insulin are probably required for normal follicular steroidogenesis. There are receptors for insulin in granulosa cells of cattle, and insulin is a more potent stimulator of FSH-induced estradiol production by bovine granulosa cells compared with IGF-I. However the role of insulin on follicular steroidogenesis during realimentation of nutritionally anovulatory heifers is probably not a major one. The gradual increase in estradiol concentrations preceding resumption of ovulation was mainly associated with increased concentrations of LH in serum.

Realimentation of nutritionally anestrous heifers resulted in decreased concentrations of NEFA in plasma. Concentrations of NEFA in plasma are inversely related to feed intake or energy balance in ruminants, however, it is unlikely that NEFA concentrations can directly affect the hypothalamo-pituitary axis and secretion of gonadotropins. Even though realimentation of nutritionally anestrous heifers reduced concentrations of NEFA in plasma, a gradual increase in NEFA concentrations occurred preceding resumption of ovulation, indicative of increased fat depots and/or increased metabolic rate. Reduced fat depots and/or metabolic rate were associated with the onset of nutritionally induced anovulation. Reduction in metabolic rate during feed restriction is an energy conservation mechanism. Thus, an animal can maintain basic physiologic functions and survive during extensive periods of undernutrition. Metabolic rate is

inversely related to hypothalamic concentrations of NPY and reduced LH secretion. Thus, reduced metabolic rate during feed restriction may result in increased NPY at the hypothalamic level and reduced GnRH secretion. This supports the hypothesis that body energy reserves and basal metabolic rate may regulate cyclicity in cattle.

Secretion of LH is the major factor mediating the effects of nutrition on gonadal function. Body condition, as influenced by nutrient intake, regulates LH secretion. Reduced concentrations of LH in serum after chronic feed restriction results in anovulation, and recovery of LH secretion after realimentation of nutritionally anestrous heifers results in resumption of ovulation. Failure of dominant follicles to mature and ovulate rather than lack of follicular development, is responsible for anovulation after chronic feed restriction. The lack of sufficient estradiol production due to reduced LH secretion during the late part of the growing phase of dominant follicles is the apparent block to ovulation. In addition, sufficient intrafollicular concentrations of estradiol and IGF-I are probably required for normal maturation of dominant follicle in order to become responsive to gonadotropin support, ovulate and produce a functional CL. Future research on the induction of ovulation during periods of anovulation should aim on increasing the estrogenic activity and identifying the optimal intrafollicular environment of dominant follicles.

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