BODY ENERGY RESERVES AND STEROIDS REGULATE GONADOTROPINS AND REPRODUCTION IN BEEF COWS

By

MICHAEL L. LOOPER

Bachelor of Science
University of Arkansas
Fayetteville, Arkansas
1992

Master of Science
University of Arkansas
Fayetteville, Arkansas
1994

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 1999

BODY ENERGY RESERVES AND STEROIDS

REGULATE GONADOTROPINS AND

REPRODUCTION IN BEEF COWS

Thesis Approved:

Robert P. Wettemann

Thesis Adviser

Leon J. Spicer

James Mabry

Rodney D. Rhinehart

Gerald W. Horn

Wayne B. Powell

Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the people that helped make this dissertation possible.

I would like to express sincere appreciation to my adviser, Dr. Robert P. Wettemann for his assistance, constructive advisement and patience during my graduate studies. I appreciate all the opportunities Dr. Wettemann has allowed me during my stay at Oklahoma State. I wish to thank Dr. Rod Geisert for his friendship and guidance during my studies. I especially appreciate his input and expertise during the laboratory phase of my graduate program. I hope some day to instruct others with the same energy as Dr. Geisert. A special thanks is extended to Dr. Jerry Malayer for the use of his laboratory facilities. It was a pleasure having the opportunity to work with Dr. Malayer. I would like to express my sincere appreciation to Dr. Leon Spicer and Dr. Gerald Horn for their advisement during my graduate program and serving as members of my committee.

It is impossible for good research to be conducted without competent people. I gratefully appreciate the guys at the South Range for all their fun and hard work throughout my graduate studies. I thank Mark Anderson, Randy Jones and David Cox for their time and assistance in data collection. I especially want to thank Randy for the NCAA tickets and Mark for the Lazy E Cantina experience. Special thanks to Steve Welty at the Nutrition/Physiology Barn for his assistance in data collection. I would like to extend my appreciation to LaRuth Mackey for her expertise in the laboratory. I would

still be pipetting a LH assay if not for LaRuth. Thanks go out to Vivienne Woods and Connie Chamberlain for their helpful lab assistance. I would like to thank Dr. Jorge Vizcarra for all of his helpful suggestions and ultimate patience during my studies. Thanks to Paula Cinnamon for her helpful technical assistance during the completion of this dissertation.

I was especially fortunate to be surrounded my excellent graduate students while at Oklahoma State. I appreciate the friendship of all the graduate students, especially John Bossis, Bart Cardwell, Steven Cooper, Clay Lents, Tulio Prado, and Kim Vonnahme. Special thanks to Clay Lents for his camaraderie; true friends are hard to find.

As always, I wish to thank my parents for their unwavering support of all my endeavors throughout life. I now realize how fortunate I am to have two wonderful parents. I cannot forget my brother and his wife, Shawn and Lori. Thanks.

I wish to thank my wife, Joneta, for her understanding and patience during the completion of this dissertation. You have given my life new meaning. Now we can get started on our life together. I love you.

To God be the Glory, great things He hath done.

TABLE OF CONTENTS

LIST OF TABLES

Chapter III

Chapter IV

Chapter V

LIST OF FIGURES

Chapter III

Chapter IV

Chapter V

CHAPTER I

INTRODUCTION

There were 1.9 million farms in the United States as of January 1, 1998 (USDA Census of Agriculture, 1997). Forty-two percent (804,595) of these farms were involved in some aspect of beef production. Oklahoma Agricultural Statistics (1997) revealed 73,000 total farm operations in the state with 64,000 involved in beef production.

According to USDA Census of Agriculture (1997), there are 33.7 million beef cows in the United States. Oklahoma ranks third in the U.S. in both the number of beef cows (1.96 million) and number of calves (1.93 million) (Oklahoma Agricultural Statistics, 1997). Total value of all cattle and calves in Oklahoma as of January 1, 1998, was estimated at 3.1 billion dollars.

Annual calf crop in the U.S. is estimated at 70 to 75% under a variety of environmental and management conditions (USDA, 1986, 1987, 1988). A 10% increase in the annual calf crop (85%) would allow the production of the same kilograms of beef from 12% fewer cows. Oklahoma beef producers could decrease the number of cattle by 230,000 cows without influencing beef production.

Cattle are the least efficient of the domestic farm species usually producing one calf per year. Beef cows have approximately 80 d after parturition to become pregnant

and maintain a 12 month calving interval. Little can be done to manipulate the gestation period; however, if the interval from parturition to conception can be shortened, production efficiency can be increased. Cows would have two opportunities to conceive and to maintain a yearly calving interval if the anestrous period was reduced to 60 d. It is essential to understand the physiological mechanisms responsible for resumption of ovarian activity in postpartum beef cows so that the anestrous period can be minimized.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Animal reproduction involves interplay between numerous neuronal and hormonal signals that allow for successful production of viable offspring. Of utmost importance in the regulation of reproductive processes is the hypothalamic decapeptide, gonadotropin-releasing hormone (GnRH), which is synthesized primarily in a small group of loosely-oriented neurons distributed throughout the diagonal band of Broca, the medial and lateral preoptic areas and the anterior hypothalamic area (Leshin et al., 1988). The GnRH neurons in the preoptic area form an 'inverted Y' (Lehman et al., 1986) which is directed toward the median eminence. After synthesis, GnRH is released in a pulsatile manner from the median eminence into the hypophysial portal system (Antunes et al., 1978) by an unidentified GnRH pulse generator (Barraclough and Gorski, 1961; Knobil, 1980). The primary site of GnRH action is specific, high affinity binding sites on the membrane surface of gonadotrope cells in the anterior pituitary gland (Marian and Conn, 1983). The binding of GnRH to its receptors elicits the synthesis and release of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary gland into the peripheral circulation.

Gonadotropins bind to receptors in the gonads and regulate steroidogenesis. Androgens are secreted by LH-stimulated thecal cells in the ovary (Fortune and Armstrong, 1977), and aromatase activity and estradiol production is induced in granulosa cells by FSH (Armstrong and Papkoff, 1976). In cattle, LH is the major luteotrophic hormone (Hansel et al., 1973). Concentrations of steroids in the peripheral circulation regulate the synthesis and release of gonadotropins.

Synthesis and release of gonadotropins is modulated by steroids throughout the estrous cycle and during pregnancy. Steroids alter gonadotropins through positive and negative feedback effects at the hypothalamus and/or the anterior pituitary gland. Increased concentrations of estradiol and decreased progesterone are associated with behavioral estrus (Henricks et al., 1971; Wettemann et al., 1972; Chenault et al., 1975). Increased concentrations of estradiol induce a preovulatory surge of LH causing ovulation (Wettemann et al., 1972; Chenault et al., 1975). Exogenous estradiol produces a preovulatory-like LH surge in ovariectomized cows (Short et al., 1973). Kesner et al. (1981) found increased concentrations of estradiol initially reduced LH release from the anterior pituitary gland, then increased GnRH-induced LH release in steers and ovariectomized cows. During pregnancy, concentrations of estradiol are increased while concentrations of progesterone are similar to those observed during the luteal phase of the estrous cycle (Edqvist et al., 1973; Smith et al., 1973). Gonadotropin stores in the pituitary gland are diminished at parturition due to prolonged exposure to increased concentrations of steroids (Chamley et al., 1976; Moss et al., 1980).

It is well established that long-term undernutrition will decrease release of gonadotropins in rats (Campbell et al., 1977), heifers (Day et al., 1986; Bossis et al.,

1999), cows (Richards et al., 1989a) and lambs (Foster and Olster, 1985). Severe feed deprivation causes a state of anovulation in most animals and has been termed "pseudo-hypophysectomy" (Pomerantz and Mulinos, 1939). Short term feed restriction of rats (Campbell et al., 1977) and monkeys (Cameron and Nosbisch, 1991) reduces circulating concentrations of gonadotropins. Fasting for 10 d reduced serum FSH concentrations without affecting concentrations of LH in obese men. However, pulse frequency and amplitude of LH and FSH were not influenced by fasting (Klibanski et al., 1981).

Elucidation of the mechanisms influencing gonadotropin release (i.e., steroids, nutrition) and factors affecting the extension of the postpartum anestrous period of beef cows would allow increased reproductive efficiency and decrease production cost by reducing the number of cows necessary for sufficient beef production. Objectives of the present studies were: 1) to characterize effects of nutrition and body energy reserves on concentrations of LH and FSH in ovariectomized beef cows, 2) to evaluate the influence of body energy reserves on endocrine and behavioral events associated with the first ovulation in postpartum beef cows, and 3) to evaluate the effects of nutrition and steroids on serum and pituitary gland concentrations of LH and FSH, mRNA for gonadotropin subunits and GnRH receptor, and concentrations of GnRH receptor in pituitary glands of beef cows.

Bovine Brain

Anterior Pituitary Gland

The anterior pituitary gland of mature beef cows weighs from 1.6 to 2.0 grams (Beal et al.,1978; Rasby et al., 1991; Vizcarra et al., 1997) which is only .3 to .4% of the

total weight of the bovine brain (Sisson and Grossman, 1953). Due to the dual embryonic origin, the pituitary gland (hypophysis) is divided into two distinct divisions: the adenohypophysis (anterior) and neurohypophysis (posterior) (Herring, 1908a, 1908b; Atwell and Marinus, 1918). The anterior pituitary gland originates from an outgrowth of the primitive nasal cavity called Rathke's pouch and is comprised of three tissue types: pars tuberalis, pars intermedia and pars distalis (Wislocki and King, 1936).

The vascularity of the anterior pituitary gland is unique in the fact that the primary capillaries form a plexus around the median eminence transporting blood to the secondary capillaries of the pars distalis (Green, 1951). Blood passes through the posterior gland to reach the targeted anterior pituitary gland (Green, 1951).

The anterior pituitary gland synthesizes and releases eight hormones including thyroid-stimulating hormone (TSH), growth hormone (GH), prolactin, adrencorticotropin hormone (ACTH), melanocyte-stimulating hormone (MSH), β-endorphin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Fevold et al., 1931; Greep, 1935; Nikitovitch-Winder and Everett, 1958, 1959). Synthesis of LH and FSH may occur in the same gonadotrope cell or in different cells (Childs et al., 1987). These glycoproteins are composed of two dissimilar, non-covalently linked α (alpha) and β (beta) subunits (Pierce and Parsons, 1981). The α-subunit is common to both hormones as well as thyroid-stimulating hormone. The specificity of the hormone is determined by the β-subunit (Pierce and Parsons, 1981).

Hypothalamic Control of the Anterior Pituitary Gland

In delivering the Lister Memorial Lecture in the summer of 1930, Dr. Harvey Cushing shared the current theory that the pituitary gland regulated certain areas of the brain. The idea that products of the anterior lobe of the pituitary were directed toward the brain, while the posterior pituitary gland released its secretions into the third ventricle had lingered for almost thirty years (Cushing, 1930a, 1930b). Wislocki and King (1936) soon challenged these concepts. Intraperitoneal and subcutaneous injections of trypan blue stained the anterior lobe of the pituitary gland and the attached stalk of the cat, rabbit and monkey. Nerve fibers were observed passing from the hypothalamus to the posterior pituitary gland in rabbits and monkeys (Wislocki and King, 1936). Injections of indian-ink into the aorta of rats, guinea pigs, rabbits and dogs further attenuated vascular connection of the hypothalamus and the pituitary gland, as well as an intrapituitary vascular connection (Green and Harris, 1947).

Discovery of a "neurovascular link" between the hypothalamus and the pituitary gland inspired researchers to examine regulation of the pituitary gland by the brain (Green and Harris, 1947). Blood flow was observed from the median eminence to the anterior pituitary gland in rats (Green and Harris, 1949). Halasz et al. (1962) found that transplantation of the pituitary gland into different regions of the rat brain indicated a region of hypothalamic control; the hypophsiotrophic area or the mediobasal hypothalamus. Target organs of the pituitary gland (i.e., thyroid, adrenal and testis) were preserved when the hypophysiotrophic area was disconnected from the rest of the brain suggesting control of hormone secretion from the anterior pituitary gland is confined to this area of the brain (Halasz and Pupp, 1965).

The quest for the characterization of the "substances" released from the hypothalamus ignited fierce competition among endocrinologists. Two laboratories independently isolated porcine (Schally et al., 1971) and ovine (Amoss et al., 1971) gonadotropin-releasing hormone (GnRH). Both Roger Guillemin and Andrew Schally received the Nobel Prize for Medicine in 1977 for their discovery of peptide hormones of the brain. However, Schally was first to isolate and determine the structure of the important decapeptide hormone, luteinizing hormone releasing factor, or GnRH (Wade, 1981).

Evidence that increased GnRH release was associated with an increase in LH release was first obtained in the rat (Sarkar et al., 1976). Zolman et al. (1973) was the first to demonstrate that natural and synthetic porcine GnRH produced LH release in heifers and bulls in vivo and in cultures of pituitary cells from steers. In vivo studies with ewes (Clarke and Cummins, 1982) and bull calves (Rodriguez and Wise, 1989) demonstrated a temporal relationship between GnRH and LH. Recently, Gazal et al. (1998) found a strong correlation (0.95) between pulsatile release of GnRH into cerebrospinal fluid of the third ventricle and plasma concentrations of LH in mature beef cows.

Estrous Behavior of Cows

Successful reproduction in cows must include a functional hypothalamic-pituitary-ovarian axis that interacts via endocrine, paracrine and autocrine factors. A milieu of hormones (i.e., GnRH, LH, FSH, estrogens and progestins) coordinate events

associated with normal estrous cycles. The estrous cycle of dairy and beef cows is a dynamic period ranging from 18-24 d with a mode of 20 d (Asdell et al., 1949; Lasley and Bogart, 1943). Concentrations of estrogens increase during the 3-4 d follicular phase of the cycle (Henricks et al., 1971; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Chenault et al., 1975), whereas progesterone dominates the 15 d luteal phase of the estrous cycle (Stabenfeldt et al., 1969; Henricks et al., 1970).

Estrous behavior indicates the initiation of a series of physiological processes involved in estrous cycle commencement. Trimberger (1948) found the duration of estrus ranged from 2.5 to 28 h with a mean of 17.8 h in dairy cows observed thrice daily for behavioral estrus. Duration of estrus averaged 7.1 h (range 33 min to 35.8 h) in dairy cows (n = 2055 observations) continuously observed by a radiotelemetric system (HeatWatch®; Dransfield et al., 1998). Continuous observation via video recording revealed duration of estrus averaged 4.4 h (range 1 to 18 h) in beef cows confined in pens with cement floors (Hurnik and King, 1987). Stevenson et al. (1996) found duration of estrus in beef cows averaged 14.8 h (range 3 to 26 h) using a radiotelemetric system. Several environmental and managerial factors affect estrous behavior in cattle. Continuous observation of estrus by video recording indicated that most mounting activity (70%) occurred between 1900 and 0700 h in dairy cows (Hurnik et al., 1975). However, Dransfield et al. (1998) found the distribution of mounts indicative of the onset and end of estrus were similar throughout the day. Increasing the number of cows in estrus from one to four or more increased the intensity of estrus from 11 to 50 mounts, respectively (Hurnik et al., 1975). Similarly, duration of estrus was increased when the number of cows in estrus at one time was increased from one to three cows (7.5 to 10.1 h,

respectively) (Hurnik et al., 1975). Dairy cows observed for estrus on a dirt surface had greater intensity and duration of estrus compared with cows on concrete (Britt et al., 1986; Vailes and Britt, 1990). Mounting activity was greatest in dairy cows observed twice daily when ambient temperatures were less than 25°C compared with temperatures above 30°C (Gwazdauskas et al., 1983; Gwazdauskas, 1985).

Release of LH and FSH occurs at behavioral estrus in most domestic farm animals. Estrogens and progestins govern gonadotropin release through positive and negative feedback, respectively. The preovulatory surge of gonadotropins is due to the positive feedback of increased concentrations of estradiol (Beck and Convey, 1977; Kesner et al., 1981). Conversely, increased concentrations of progesterone from the corpus luteum during the luteal phase have negative effects on concentrations of LH (Rahe et al., 1980; Walters et al., 1982). Elimination of progesterone either by $PGF_{2\alpha}$ or CL removal increased concentrations of LH in heifers (Louis et al., 1974; Fogwell et al., 1978; Imakawa et al., 1986) and ewes (Gust et al., 1984). A preovulatory LH surge occurred in heifers 55 h after treatment with $PGF_{2\alpha}$ (Imakawa et al., 1986) and cows displayed a preovulatory LH surge 44 h after lutectomy (Stumpf et al., 1989).

Estrogens

Manifestation of estrous behavior is due to the actions of estrogens, primarily estradiol on the brain (Clegg et al., 1958; Blache et al., 1991). It is well established that estrogens stimulate estrous activity in cows. Administrating estradiol benzoate induces behavioral estrus in ovariectomized cows (Asdell et al., 1945; Carrick and Shelton, 1969), as well as ovariectomized heifers (Rajamahendran et al., 1979). Immunization

against estradiol prevents expression of behavioral estrus in intact beef heifers (Martin et al., 1978).

Effects of estradiol on estrous behavior occur in an "all-or-none" fashion which means once a threshold level is reached, estrous behavior is independent of increasing estradiol concentrations (Cook et al., 1986; Coe and Allrich, 1989). Carrick and Shelton (1969) indicated a consistent trend for increased dosages of estradiol to decrease the interval from estradiol treatment to the onset of estrus and to increase estrous duration. Using a wide range of physiological estradiol concentrations (300 to 4,800 μg), Cook et al. (1986) found that behavioral interactions of ovariectomized cows and heifers in estrus were not different among estradiol concentrations. Duration of estrus was similar for all estradiol dosages with the exception of 4,800 μg of estradiol, which increased the duration of estrus by approximately 8 h. Superovulated dairy heifers had three times the maximum estradiol concentrations of nonsuperovulated heifers; however, duration of estrus was only 2.3 h longer in superovulated heifers compared with nonsuperovulated heifers and estrous traits were similar between groups (Coe and Aldrich, 1989). Duration of estrus was prolonged (range 8 to 64 h) when cows were administered estradiol benzoate at 36 and 48 h after exposure to progesterone compared with cows receiving a single injection of estradiol 36 h after progesterone exposure (range 0 to 23 h) (Britt et al., 1986). Heifers exposed to supraphysiological concentrations of estradiol became refractory to subsequent estradiol treatment (Carrick and Shelton, 1969). Sensitivity to exogenous estrogen returned when heifers were pretreated with progesterone for 5 d suggesting progesterone resensitized the hypothalamus and/or anterior pituitary gland to estradiol (Carrick and Shelton, 1969).

Concentrations of estradiol are relatively constant during the estrous cycle of beef cattle and increase dramatically prior to estrus (Henricks et al., 1971; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Glencross et al., 1973; Bossis et al., 1999). Plasma concentrations of estradiol begin to increase 3-4 d before estrus and are maximal on the day prior to estrous behavior (Henricks et al., 1971; Wettemann et al., 1972). Maximum concentrations of estradiol decrease to minimal concentrations within 1-2 d after estrus (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Glencross et al., 1973). Chenault et al. (1975) found maximum concentrations of estradiol decreased within 14 h after estrus in dairy cows.

Estradiol is synthesized and released from preovulatory follicles in the ovary (Ireland and Roche, 1982a; Ireland and Roche, 1983). Cannulation of utero-ovarian veins verified that a single large antral follicle was responsible for increased concentrations of estradiol during proestrus and estrus in cows (Ireland et al., 1984). Falck (1959) suggested both theca and granulosa cells of rat ovarian follicles were involved in the production of estradiol from the ovary conceiving the idea of the "two-cell theory". Under the influence of gonadotropins, steroidogenesis occurs in two cell types: the LH-responsive theca interna and the FSH-responsive granulosa cells (Fortune and Armstrong, 1977; Fortune and Armstrong, 1978). Luteinizing hormone acts via receptors on the theca cells to increase production of cAMP which activates genes that encode for cholesterol side-chain cleavage and 17α-hydroxylase and $C_{17,20}$ lyase which are required for androgen synthesis (Erickson et al., 1985). Increased enzyme activity (17α-hydroxylase) occurs as bovine follicles mature (Rodgers et al., 1986). Androstenedione is the principal aromatizable steroid produced by bovine theca cells

predominately through the Δ^5 pathway (Lacroix et al., 1974; Fortune, 1986). Androgens produced by the theca cells diffuse across the follicular basement membrane to be utilized as substrates in estrogen biosynthesis by granulosa cells (Baird, 1977). Bovine granulosa cells supply progestin precursors to the theca cells allowing more androgens to be produced (Fortune, 1986). Androgen aromatization is regulated by FSH in granulosa cells (Dorrington et al., 1975). Aromatase activity is absent in immature, hypophysectomized rats but can be induced by FSH (Armstrong and Papkoff, 1976). Dieleman and Blankenstein (1984) found aromatization decreases approximately 14 h after the preovulatory LH surge in cows.

Frequent pulses of LH with low amplitude occur during the follicular phase of the bovine estrous cycle (Rahe et al., 1980). Pulse frequency of LH is less with greater amplitudes during the progesterone dominated luteal phase (Rahe et al., 1980). Estrogens have a positive effect on the synthesis and secretion of LH from the anterior pituitary gland at estrus. Increased serum concentrations of estradiol are parallel to increases in concentrations of luteinizing hormone (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Chenault et al., 1975; Lemon et al., 1975). Concentrations of LH begin to increase 5-6 d prior to estrus, with a preovulatory surge near the onset of estrus (± 3 h) (Henricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). Pituitary content of LH and FSH are at maximal concentrations on d 18-20 of the estrous cycle when concentrations of estradiol are increased (Hackett and Hafs, 1969). Pituitary LH and FSH decreased 89 and 73%, respectively, from d 18 to d 2 of the subsequent estrous cycle. Furthermore, the preovulatory decrease of pituitary FSH precedes that of LH by 2 d (Hackett and Hafs,

1969). Pituitary and plasma concentrations of LH are negatively correlated (-.88) in the absence of steroid feedback in ovariectomized heifers (Swanson et al., 1971).

The preovulatory surge of LH remains at maximal concentrations for 6 to 10.6 h (Henricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). Kesner et al. (1981) suggested estradiol induces the LH surge in cows by increasing the sensitivity of the pituitary to GnRH and then increasing GnRH. Estradiol initially reduces the ability of the pituitary to release LH either by decreasing responsiveness of the pituitary to GnRH or reducing GnRH release in cows. Concentrations of LH are reduced several hours (10-15 h) prior to the LH surge, although the pituitary is capable of responding to exogenous GnRH, suggesting that estradiol reduces GnRH release below the threshold concentration that normally induces LH release. Secretion of GnRH resumes around 12 h after estradiol stimulation and induces a LH surge (Kesner et al., 1981). Maximum LH concentrations occurred when the diameter of the dominant follicle was greatest in dairy heifers prior to first estrus (Swanson et al., 1972). Exogenous estradiol treatment of ovariectomized cows and heifers (Short et al., 1973; Beck and Convey, 1977; Imakawa et al., 1986), and ovariectomized ewes (Moss et al., 1981; Kasa-Vubu et al., 1992) induced LH release similar to endogenous preovulatory surges. Exogenous prostaglandin-induced luteal regression in beef heifers increased concentrations of estradiol and LH in serum simultaneously (Imakawa et al., 1986).

Release of gonadotropins after treatment with GnRH is dependent upon the stage of the estrous cycle, indicative of steroidal regulation of gonadotropins. In beef heifers, quantity of LH and FSH release was increased when GnRH was administered at or near estrus compared with GnRH treatment during the luteal phase (Kaltenbach et al., 1974;

Zolman et al., 1974). Serum concentrations of LH were greater in heifers treated with GnRH after estradiol administration compared with GnRH treatment before estradiol (Beck and Convey, 1977).

Serum concentrations of FSH increased with maximum concentrations of estradiol in intact cattle (Akbar et al., 1974; Dobson, 1978). Insertion of estradiol capsules after removal of progesterone implants elicited a surge of FSH similar to LH in ovariectomized ewes (Kasa-Vubu et al., 1992). Prostaglandin-induced corpus luteum regression increased pulse frequency of both LH and FSH in cows (Schallenberger et al., 1984). Ovariectomized cattle treated with estradiol failed to induce a preovulatory surge of FSH; however, pituitary content of FSH was increased 20 h after estradiol treatment (Schoenemann et al., 1985). Only in vitro treatment with GnRH and not estradiol increased FSH release in bovine pituitary cells (Padmanabhan and Convey, 1981). Additional regulation of FSH by ovarian peptides may explain these differences. Pituitary concentrations of FSH in Holstein heifers increased continuously from 3 to 30 d after ovariectomy (Swanson et al., 1971). Two glycoproteins that play a role in FSH regulation have been isolated from bovine follicular fluid: activin which stimulates and inhibin which inhibits FSH production (Robertson et al., 1985, 1988). Ireland et al. (1983) found administration of charcoal-treated follicular fluid depressed FSH release without influencing release of LH in ovariectomized heifers. Removal of the regulatory effects of the ovary dramatically increases concentrations of FSH.

The precise location in which estrogens have their effect on gonadotropin release has been subject to intense controversy. Estrogens can act either indirectly at the

hypothalamus, directly at the anterior pituitary gland or at both sites to regulate gonadotropin release.

Effects of estrogens on the hypothalamus. The frequency and amplitude of GnRH pulses from the hypothalamus vary depending upon the steroidal environment. The preovulatory LH surge coincides with an obvious sustained GnRH surge in several species including rats (Sarkar and Fink, 1979), ewes (Clarke and Cummins, 1985), monkeys (Pau et al., 1993), mares (Irvine and Alexander, 1994) and cows (Gazal et al., 1998). In ovariectomized rats, exposure to estradiol at concentrations similar to those during proestrus and estrus increased concentrations of GnRH in pituitary stalk blood (Sarkar and Fink, 1979). Estradiol induced a preovulatory-like surge of GnRH in cerebrospinal fluid of ovariectomized cows that coincided with a LH surge (Gazal et al., 1998). Exogenous estradiol has a biphasic effect on GnRH release in ewes (Caraty et al., 1989). Initially, estradiol decreases pulse frequency and amplitude of GnRH followed by an abrupt surge of GnRH. This preovulatory GnRH surge is followed by the LH surge (Caraty et al., 1989; Moenter et al., 1990). Estradiol may further influence GnRH secretion by modifying its episodic pattern of release. Evans et al. (1995) found estradiol treatment altered the pulsatile pattern of GnRH release. Distinct pulses of GnRH in ovariectomized ewes were observed in the early phase (1 to 5 h after increased estradiol) of the preovulatory LH surge. This was followed by an increase in concentrations of GnRH lacking definite episodic release (Evans et al., 1995).

Increasing concentrations of estradiol modulate GnRH release from the hypothalamus. However, estrogen receptors are absent on GnRH neurons in a number of

species including rats (Shivers et al., 1983), monkeys (Herbison et al., 1995) and sheep (Lehman and Karsch, 1993). The question remains, how does estradiol effect GnRH release in the hypothalamus? Estradiol implants placed in confined areas of the ventromedial nucleus of the hypothalamus of ovariectomized ewes caused estrous behavior and increased plasma LH concentrations (Blache et al., 1991). Estrogen implants placed in other areas of the hypothalamus (preoptic area, anterior hypothalamus, lateral hypothalamus and posterior hypothalamus) failed to induce a preovulatory LH surge; however, two ewes implanted in the lateral hypothalamus did exhibit estrous behavior (Blache et al., 1991). Increasing evidence in the ewe indicates that neurons responsible for the release of the inhibitory amino acid, gamma amino butyric acid (GABA), have estrogen receptors. Herbison et al. (1993) used immunocytochemistry techniques and antibodies specific for the estrogen receptor, the enzyme glutamic acid decarboxylase (the synthetic enzyme for gamma amino butyric acid) and GnRH to examine cells containing estrogen receptors in the preoptic and anterior hypothalamic areas of ewes. Estrogen receptors were absent on GnRH neurons; however, GABA neurons adjacent to GnRH neurons possessed estrogen receptors and may mediate the estrogen influence on GnRH neurons. Proopiomelanocortin (POMC) neurons in the rat have estrogen receptors (Jirikowski et al., 1986). Leshin et al. (1988) found POMC neurons are in close proximity to GnRH neurons in cows suggesting a pathway in which estradiol may regulate GnRH synthesis and release.

Effects of estrogens on the anterior pituitary gland. Estradiol increases the sensitivity of the anterior pituitary gland to GnRH treatment in a number of species including cows

(Kaltenbach et al., 1974; Kesner et al., 1981). The number of GnRH receptors (GnRH-R) increase prior to or during estrus in rats (Clayton et al., 1980; Savoy-Moore et al., 1980), hamsters (Adams and Spies, 1981), ewes (Turzillo et al., 1994) and cows (Schoenemann et al., 1985) suggesting estradiol upregulation. Exogenous estrogen treatment increases the number of GnRH-R on the anterior pituitary gland of ovariectomized ewes (Moss et al., 1981), rats (Kaiser et al., 1993) and cows (Schoenemann et al., 1985). Estradiol has a biphasic effect on LH release in ovariectomized cattle. Schoenemann et al. (1985) found serum concentrations of LH decreased 3 h after estradiol treatment followed by a preovulatory-like LH surge. Pituitary gland concentrations of LH and FSH were maximal at 20 h after estradiol. In vitro treatment of rat (Drouin et al., 1976) and cow (Padmanabhan et al., 1978; Baratta et al., 1994) pituitary cells with estradiol increased LH release and synthesis. A single injection of estradiol increases the number of GnRH-R 2.5 fold in the pituitary gland of ewes where the hypothalamus and pituitary gland are disconnected (Gregg and Nett, 1989). Goodman and Karsch (1980) proposed estradiol effects occur primarily at the pituitary gland since estradiol treatment of ovariectomized ewes for 10-11 d decreased LH pulse amplitude but not pulse frequency.

Progesterone

The generally accepted role of progesterone on behavioral estrus and regulation of gonadotropins is one of inhibition (Gomes and Erb, 1965). Progesterone given at dosages to mimic concentrations found on d 4-10 of the cycle, d 11-16 of the cycle or supraphysiological concentrations, linearly decreased the incidence of behavioral estrus in dairy cows (Davidge et al., 1987). Progesterone has been proposed to act as a "brake"

on LH secretion in the cow (Walters et al., 1982) and as an "organizer" of the estrous cycle in the ewe (Hauger et al., 1977). Pulsatile release of LH is altered during the estrous cycle of the cow depending upon the dominant steroid (Rahe et al., 1980). Withdrawal of progesterone is necessary for increased concentrations of estradiol and subsequent estrus in cattle (Fogwell et al., 1978; Walters et al., 1982) and sheep (Goodman et al., 1981). Removal of progesterone-releasing intravaginal devices (PRIDs) increased concentrations and pulse amplitude of LH and FSH in heifers (Ireland and Roche, 1982b). Pulse frequency of LH was increased after PRID removal while FSH pulse frequency was unchanged. Schallenberger et al. (1984) found pulse frequency of LH and FSH increased within 3 h after prostaglandin-induced luteal regression in heifers. Basal concentrations and pulse amplitudes of LH were increased while basal concentrations and amplitudes of FSH were decreased. Implants supplying concentrations of progesterone similar to the luteal phase blocked LH and FSH surges in ovariectomized ewes (Kasa-Vubu et al., 1992). Carrick and Shelton (1969) suggested progesterone treatment resensitized the hypothalamus and/or the anterior pituitary gland of ovariectomized cows exposed to supraphysiological concentrations of estradiol.

The action of progesterone on LH suppression seems to be an indirect effect at the hypothalamus. Progesterone treatment decreased GnRH in pituitary stalk blood of ovariectomized rats (Sarkar and Fink, 1979) and sheep (Karsch et al., 1987). Recognizing the close association between pulsatile release of GnRH and LH, Goodman and Karsch (1980) proposed that GnRH secretion was directly altered by progesterone since pulse frequency of LH was diminished after progesterone treatment in ovariectomized ewes. Pulsatile infusion of GnRH reinitiated normal menstrual cycles in female monkeys with

hypothalamic lesions that abolished endogenous GnRH release (Wildt et al., 1981b). Progesterone implants failed to block estradiol-induced LH release in lesioned monkeys suggesting progesterone acts at the hypothalamus (Wildt et al., 1981b). Similarly, progesterone implants did not alter the estradiol-induced LH surge in GnRH-pulsed, hypothalamic-pituitary disconnected sheep (Clarke and Cummins, 1984). Progesterone administered to ovariectomized ewes suppressed serum concentrations of LH; however, the number of GnRH-R and pituitary gland content of LH were not altered (Moss et al., 1981). In vitro treatment of bovine pituitary cells with progesterone did not affect basal or GnRH-induced LH release (Padmanabhan and Convey, 1981). Addition of progesterone to pituitary cell culture blocked estradiol-induced increases in LH release. Baratta et al. (1994) found that progesterone treatment of midluteal phase, bovine pituitary cells did not affect tonic LH release but suppressed GnRH-induced LH release. Secretion of FSH in bovine pituitary cell culture was unchanged in the presence of progesterone (Padmanabhan and Convey, 1981). However, progesterone decreased FSH secretion in ovine pituitary cell cultures within 12 h of treatment in the presence of insulin (Batra and Miller, 1985).

Steroids alter gonadotropin release throughout the estrous cycle. Dynamic negative (both progesterone and estradiol) and positive (estradiol) feedback control the pulsatile pattern of gonadotropin release. Behavioral estrus is induced by increased concentrations of estradiol and decreased progesterone, and estrous activity is influenced by endogenous concentrations of estradiol. Progesterone acts at the hypothalamus to alter GnRH release while estradiol influences gonadotropin release directly at the anterior pituitary gland. The hypothalamus and/or anterior pituitary gland of cows exposed to

maximal concentrations of estradiol during gestation may be resensitized by increasing concentrations of progesterone.

Ovulation in Cows

Ovulation is initiated by the preovulatory LH surge and resembles an inflammatory-like process resulting in the rupture of the follicle and release of the ovum from the ovary (Espey, 1994). Ovulation usually occurs approximately 28-32 h after the onset of estrus in both dairy and beef cattle (Trimberger, 1948; Walker et al., 1996; Looper et al., 1998).

A protrusion termed the "stigma" develops at the apex of the preovulatory follicle at the time of ovulation (Espey, 1967). The apical wall of the preovulatory follicle in rabbits diminishes to less than 20% of its original thickness (Espey, 1967). This distension is not due to change in the intrafollicular pressure. Espey and Lipner (1963) found a constant pressure of 15-20 mm Hg present during ovulation in the preovulatory follicle of the rat. Contraction of smooth muscle cells surrounding the follicle is thought to play a role in the ovulatory process (Pendergrass and Talbot, 1979; Self et al., 1989). However, Kobayashi et al. (1984) found ovulation in the rabbit occurs without contractions of the follicular smooth muscle.

The current hypothesis proposes ovulation to be similar to an inflammatory response (Espey, 1994). Increases in blood volume in the rat ovary increased from .6 μL at the initiation of ovulation induced by hCG to 1.37 μL 4 h after hCG administration (Tanaka et al., 1989). This increase in blood flow is presumably due to vasodilatation

produced from increasing kinins present in the ovary (Espey, 1994) and decreasing concentrations of histamine in mast cells lining blood vessels of the ovary (Krishna et al., 1989).

Prostaglandins produced via the arachidonate pathway are thought to be involved in activation of collagenolytic activity necessary for degradation of the follicular wall (Reich et al., 1985; Murdoch et al., 1986). Concentrations of prostaglandins are increased in sheep follicles 12 h after the preovulatory surge of LH (Murdoch et al., 1981). The rate limiting enzyme for prostaglandin synthesis, prostaglandin G/H synthase-2 (PGHS-2), is induced by hCG in rats, cows and mares (Liu et al., 1997; Sirois and Dore, 1997). Time during ovulation when prostaglandin G/H synthase-2 was expressed varied among species; however, ovulation of all species occurred within 10 h after PGHS-2 induction (Liu et al., 1997; Sirois and Dore, 1997).

First Postpartum Ovulation

Resumption of ovarian function in postpartum beef cows resemble luteal events associated with puberty in ruminants. Gonzalez-Padilla et al. (1975) found two distinct increases in concentrations of progesterone in heifers prior to first ovulation. Similarly, in postpartum beef cows, first ovulation after parturition is usually followed by a transient increase in concentrations of progesterone for 4 to 5 d (Williams and Ray, 1980; Humphrey et al., 1983; Perry et al., 1991). A normal luteal phase (17-21 d) preceded by estrus often follows this transient increase in progesterone (Pratt et al., 1982; Perry et al., 1991).

Short luteal phases can be one of two types: corpora lutea having a short lifespan with a transient increase in progesterone (Odde et al., 1980) or normal corpora lutea with reduced concentrations of progesterone (Pratt et al., 1982). Short-lived corpora lutea may be induced by GnRH administration (Kesler et al., 1980) or short-term calf removal (Odde et al., 1980).

First ovulation in postpartum beef cows may or may not be accompanied by behavioral estrus. Forty-two percent of nonsuckled cows observed twice daily for behavioral estrus ovulated without exhibiting estrus, while 70% of suckled cows did not have behavioral estrus associated with the first postpartum ovulation (Graves et al., 1968). Similarly, Rawlings et al. (1980) found concentrations of progesterone were increased in 4 of 5 cows before detected estrus determined by Kamar heat mount detectors and a bull. Using ultrasonography to monitor corpus luteum formation and visual observation thrice daily for estrous behavior, Perry et al. (1991) found that 10 suckled beef cows did not exhibit behavioral estrus prior to first ovulation after parturition, while one cow exhibited estrus and two cows had increased estrous activity.

Progesterone concentrations are reduced during transient luteal phases compared with normal luteal phases. Concentrations of progesterone before the first postpartum estrus were 15-20% of concentrations of progesterone during the subsequent normal luteal phases of beef cows (Arije et al., 1974). In primiparous heifers exhibiting short luteal phases, progesterone concentrations averaged less than 1 ng/mL in 7-8 d cycles (Corah et al., 1974; Williams and Ray, 1980). Plasma progesterone increased in postpartum Hereford cows for 4 d, declined for the next 5 d, and then increased to concentrations indicative of normal luteal activity (Rawlings et al., 1980). Werth et al.

(1996) found concentrations of progesterone averaged 2.3 ng/mL 6.6 d prior to the first estrus after parturition which was followed by normal luteal function.

Source of the first increase in plasma progesterone at puberty and after calving is considered to be of ovarian origin. Increases in progesterone in pubertal heifers are associated with luteal tissue found below the surface of the ovary (Berardinelli et al., 1979). Luteal tissues could not be visualized nor palpated after ovariectomy; however, histological examination of ovaries revealed condensed luteal tissue. In pubertal ewes, the luteal structure associated with the transitory increase in progesterone is more pronounced, averaging 7.0 ± 1.7 mm (Berardinelli et al., 1980). Daily ultrasonography of beef cows from 25 d postpartum until normal luteal activity revealed a short-lived CL associated with the transient increase in progesterone (Perry et al., 1991).

Abnormal follicle size is not responsible for short-lived corpora lutea. Maximum diameter of the ovulatory follicle is similar between the first (short luteal phase) and second ovulation (normal luteal phase) in suckled beef cows (13.5 vs 13.3 mm for first and second ovulation, respectively; Perry et al., 1991). Ovulation after weaning of calves is associated with short luteal phases (Odde et al., 1980). Follicle diameters of cows with weaned calves expected to have short-lived CL were similar to control cows (17.6 mm vs 16.8 mm, respectively; Braden et al., 1989). Corah et al. (1974) suggested luteinized follicles also might contribute to this transient increase in progesterone. Rectal palpation of ovaries in postpartum beef cows revealed increased numbers of medium and large follicles occurred at the time of transient increases in progesterone (Rawlings et al., 1980). This indicates luteinized follicles may contribute to increased concentrations of progesterone.

Several mechanisms have been postulated to describe the events associated with short luteal phases including 1) subnormal follicular development prior to ovulation, 2) inadequate concentrations of LH, and/or 3) a premature increase in concentrations of prostaglandins (Hunter, 1991; Garverick et al., 1992).

First ovulation in postpartum beef cows is usually associated with a short-lived CL (Perry et al., 1991) and concentrations of progesterone are usually less than during a normal luteal phase (Corah et al., 1974; Williams and Ray, 1980). Estrus and a normal luteal phase follows the short-lived CL. First ovulation after parturition may or may not be accompanied by behavioral estrus and duration and intensity of estrus may be reduced.

Follicle Development Associated with Subnormal Luteal Function. Follicles forming short-lived corpora lutea are usually less developed and lack adequate gonadotropin receptors. There are less LH and FSH receptors in follicles of postpartum cows expected to have short-lived CL than normal cycling cows (Braden et al., 1989). Progesterone treatment reduces the incidence of short cycles in postpartum cows with weaned calves (Ramirez-Godinez et al., 1981). Number of LH receptors in the ovary was greater in anestrous, suckled, postpartum beef cows treated with progesterone than control cows; however, FSH receptors for treated and control cows did not differ (Inskeep et al., 1988).

Binding of LH to thecal cells induces estradiol production necessary for normal development of the corpus luteum in anestrous ewes (Hunter et al., 1986). Progesterone treatment for 2 to 14 d (McLeod et al., 1982; McLeod and Haresign, 1984) delayed the preovulatory LH surge in seasonally anestrous ewes administered GnRH at 2 h intervals compared with nontreated control ewes. Normal luteal function occurred in all

progesterone pretreated ewes, while only 14 to 25% of anestrous ewes not treated with progesterone had normal luteal function. McLeod and Haresign (1984) proposed that progesterone may modify the response of the ovulatory follicle to gonadotropin release.

Concentrations of estradiol in follicular fluid may be less in follicles expected to form short-lived CL. Progesterone treatment of anestrous, suckled beef cows for 9 d increased concentrations of estradiol in follicular fluid of the largest follicle at 10 and 20 h after norgestomet treatment compared with control cows (Garcia-Winder et al., 1987). Follicles from anestrous, suckled cows treated with progesterone for 9 d were heavier due to increased amounts of follicular fluid which contained more estradiol than nontreated cows (Inskeep et al., 1988). Similarly, estradiol concentrations were four-fold greater in follicles from normal cycling cows compared with follicles from cows with weaned calves expected to form short-lived corpora lutea (Braden et al., 1989).

Concentrations of Serum Gonadotropins Associated with Short-lived CL. Short-lived CL may be produced by GnRH treatment of anestrous ewes (O'Shea et al., 1984) and postpartum beef cows (Kesler et al., 1980). Abnormal CL produced following the induction of ovulation by GnRH treatment is reduced by pretreatment with progesterone (Ramirez-Godinez et al., 1981). Normal and short-lived corpora lutea do not differ during early development. Corpora lutea weight, progesterone content and hCG binding are similar on d 3 and 4 of the estrous cycle for anestrous ewes treated with injections of GnRH (every 2 h) with or without pretreatment with progesterone (Hunter et al., 1988). However, all three variables were decreased in CL from ewes without progesterone pretreatment which were expected to be short-lived (Hunter et al., 1988). Maximal

concentrations of gonadotropins are necessary for support of newly formed CL (Hansel and Convey, 1983). Early weaned cows had reduced serum concentrations of FSH prior to the first estrous cycle compared with the second estrous cycle (Ramirez-Godinez et al., 1982; Garcia-Winder et al., 1986). However, administration of FSH to GnRH-treated postpartum beef cows did not alter plasma estradiol concentrations or reduce the formation of abnormal corpora lutea (Lishman et al., 1979).

Concentrations of LH, as well as frequency, amplitude and duration of pulses of LH did not differ between cows with normal cycles compared with weaned cows expected to have short estrous cycles (Ramirez-Godinez et al., 1982; Garverick et al., 1988). Binding of hCG to CL of GnRH-treated anestrous ewes was similar 4 d after GnRH treatment in ewes pretreated with progesterone compared with non-progesterone treated ewes (Hunter et al., 1988). However, binding of hCG was reduced 5 d after GnRH treatment in anestrous ewes not pretreated with progesterone. Amounts of mRNA encoding for LH receptor was reduced (48%) in short-lived corpora lutea of postpartum beef cows at day 8 of the estrous cycle compared with normal CL (Smith et al., 1996).

Uterine Control of Luteal Activity. Premature release of prostaglandins by the uterus may be a contributor to short-lived corpora lutea. Intrauterine infusions of the prostaglandin synthesis inhibitor, indomethacin, increased the lifespan of corpora lutea in GnRH-treated cows (Troxel and Kesler, 1984). Copelin et al. (1989) demonstrated that immunization against prostaglandin $F_{2\alpha}$ would extend the lifespan of the first postpartum corpus luteum that is expected to be short-lived. Hysterectomy of early-weaned cows maintained CL until $PGF_{2\alpha}$ administration while control cows subjected to a sham

surgery had 8 to 10 d estrous cycles (Copelin et al., 1987). Short-lived CL are often caused by premature induction of luteolysis.

Normal luteolysis in ruminants relies on the interaction between prostaglandins and oxytocin (McCracken et al., 1984; Hansel and Dowd, 1986). Changes in $PGF_{2\alpha}$ are usually determined by quantification of the stable $PGF_{2\alpha}$ metabolite, 15-keto-13,14 dihydro $PGF_{2\alpha}$ (PGFM). Peripheral concentrations of PGFM accurately reflect uterine $PGF_{2\alpha}$ secretion (Guilbault et al., 1984). Dairy cows that had short-lived CL had greater serum concentrations of oxytocin associated with an increase in PGFM compared with cows that had normal CL (Peter et al., 1989). In vitro production of prostaglandins by luteal cells was greater in cows induced to ovulate by administration of hCG without prior progesterone treatment, compared with cows pretreated with progesterone implants (Hu et al., 1990). Postpartum cows (Zollers et al., 1989) and ovariectomized ewes (Vallet et al., 1990) pretreated with progesterone had reduced systemic concentrations of PGFM after injection with oxytocin compared with animals without prior progesterone treatment.

Progesterone treatment for 5 d reduced concentrations of oxytocin receptor in the endometrium of ovariectomized ewes (Vallet et al., 1990). Ninety-two percent of progesterone pretreated, anestrous ewes administered multiple injections of GnRH ovulated compared with 54% of ewes given multiple injections of GnRH without prior exposure to progesterone (Hunter et al., 1989). Ewes induced to ovulate with GnRH (injections every 2 h) without pretreatment with progesterone had increased plasma concentrations of oxytocin and PGFM 3 to 5 d after the end of GnRH treatment. On d 5 after GnRH treatment, oxytocin binding sites in the endometrium were found only in

ewes not pretreated with progesterone (Hunter et al., 1989). Similarly, early weaned beef cows without progesterone treatment had increased concentrations of oxytocin receptors and decreased number of progesterone receptors in the endometrium compared with cows expected to have normal-lived corpora lutea (Zollers et al., 1993).

Nutritional Effects on Secretion of Gonadotropins

Undernutrition hinders the reproductive process in rats (Campbell et al., 1977), heifers (Day et al., 1986; Yelich et al., 1995; Bossis et al., 1999), cows (Richards et al., 1989a) and lambs (Foster and Olster, 1985). Pomerantz and Mulinos (1939) proposed malnutrition acted as "pseudohypophysectomy" in rats that lost greater than 10% of their body weight when consuming inadequate diets. The severity of nutritional deprivation determines if reproductive processes will be compromised.

Chronic Nutritional Deprivation

Long-term feed restriction diminishes pulsatile release of the gonadotropins. Pulse frequency of LH is decreased after severe undernutrition in rats (McClure and Saunders, 1985; Sisk and Bronson, 1986), monkeys (Dubey et al., 1986), humans (Boyar et al., 1974), ewes (Foster and Olster, 1985), heifers (Day et al., 1986; Yelich et al., 1995; Bossis et al., 1999) and cows (Richards et al., 1989a).

The site at which nutrition influences gonadotropin release presumably is the hypothalamus since the pituitary gland is uncompromised in underfed animals. Exogenous GnRH treatment of feed restricted monkeys (Dubey et al., 1986), ewes (Foster et al., 1989; Kile et al., 1991) and cattle (Beal et al., 1978; Rasby et al., 1991;

Vizcarra et al., 1997) caused LH release, usually to concentrations greater than ad libitum fed animals. In contrast, gonadotropin content of the pituitary glands of humans may be depleted after nutritional deprivation. Serum concentrations of FSH were decreased in men on d 8 of a 10 d fast and did not increase after GnRH infusion (.2 μg/min) for 4 h (Klibanski et al.,1981).

Severe feed restriction (4 g of food/day) for 39 d in male rats decreased pulsatile LH 57% of control rats while concentrations of FSH in feed restricted rats were decreased only 14% of controls (Sisk and Bronson, 1986). Serum concentrations of LH and FSH were reduced 77 and 63%, respectively, in ovariectomized ewes fed a diet of 60% maintenance for 127 d (Kile et al., 1991). Pituitary concentrations of LH and FSH were reduced 80 and 56%, respectively, in feed restricted ewes. Reduction of pulsatile GnRH release may favor synthesis and release of FSH over that of LH. In monkeys, greater pulse frequency of GnRH increased pulsatile release of LH compared with FSH, whereas decreased GnRH pulse frequency increased FSH secretion (Wildt et al., 1981a). Concentrations of FSH were increased in feed restricted heifers prior to nutritional anestrus compared with FSH concentrations before the initiation of feed restriction (Rhodes et al., 1996; Bossis et al., 1999). Nutritional deprivation decreases concentrations of estradiol in heifers (Rhodes et al., 1996; Bossis et al., 1999) which may allow for increased FSH concentrations. Vizcarra et al. (1997) found that GnRH treatment (1 pulse/4 h) reduced pituitary concentration of FSH, but not LH in nutritionally anestrous cows. Presumably, a slower pulse frequency of GnRH is needed for FSH secretion compared with that required for LH release.

Acute Nutritional Deprivation

Influence of acute or short-term feed restriction on gonadotropins depends primarily on the extent of the restriction and the species involved. After a 4 d fast, pituitary content, as well as serum concentrations of both LH and FSH were decreased in adult male rats (Bergendahl et al., 1989). Orchidectomized rats fed 50% ad libitum diets for 7 d had decreased LH pulse frequency compared with ad libitum fed rats (Dong et al., 1993). Campbell et al. (1977) demonstrated complete starvation of rats for 7 d decreased serum concentrations of LH and FSH 75 and 32%, respectively, compared with concentrations in control rats. The decrease in LH secretion associated with nutritional deprivation may be due to increased sensitivity of the pituitary to the negative effects of estradiol. Ovariectomized rats treated with estradiol and fasted for 48 h had reduced concentrations, pulses and amplitudes of LH compared with unfasted, estradiol treated rats (Cagampang et al., 1991). Wethers fed to lose body weight and treated with estradiol for 72 h had reduced LH pulse frequency compared with estradiol-treated wethers fed to gain or maintain body weight (Beckett et al., 1997b). However, concentrations of serum LH were reduced in ovariectomized heifers fed energy-restricted diets in the absence of estradiol (Imakawa et al., 1987). McShane and Keisler (1991) found that sensitivity of the pituitary to estradiol was not increased in feed restricted ewes. Estradiol may regulate gonadotropin release at different regions of the brain in fasted animals compared with fed animals. Ovariectomized rats implanted with estradiol into the A1 region and paraventricular nucleus of the brain had reduced concentrations of LH after a 48 h fast compared with fasted rats implanted with estradiol in other regions of the brain (Nagatani et al., 1994). Estradiol implants placed in restricted regions of the ventromedial

hypothalamus of ovariectomized ewes resulted in estrous behavior and increased LH secretion (Blache et al., 1991).

Short-term feed restriction in nonhuman primates influences gonadotropin release similar to the effect in rats. One day of fasting adult male rhesus monkeys decreased LH pulse frequency within the first 4-6 h after fasting was initiated (Cameron and Nosbisch, 1991). Refeeding at normal nutritional intakes the day after fasting restored LH pulse frequency to a frequency greater than observed on a day of normal feeding. Influences of fasting on LH pulse frequency in the monkey are independent of the psychological stress associated with food restriction. After a 2 d fast, male rhesus monkeys were administrated liquid nutrients via an indwelling gastric cannulae without reducing the psychological stress of fasting (Schreihofer et al., 1993b). Monkeys infused with liquid nutrients displayed agitation similar to continuously fasted monkeys; however, pulsatile LH release was reestablished. Overfeeding monkeys ($\geq$ 300% maintenance diet) the day prior to a 24 h fast prevented decreased LH secretion usually observed with fasting (Schreihofer et al., 1993a).

Concentrations of LH and FSH in men were not affected after a 24 h fast (Loucks et al., 1994); however, plasma concentrations of LH and FSH, and pulse frequency of LH in men were decreased after a 48 h fast (Cameron et al., 1991). Concentrations of LH and FSH in serum were reduced in men ($\pm$ 25% normal body weight) completely fasted for 5 d (water only) (Veldhuis et al., 1993). Pulse amplitude of LH was reduced while LH pulse frequency was not influenced by a 5 d fast.

The effects of acute feed restriction on gonadotropin release have not been directly investigated in ruminants. Three of four Holstein heifers fasted (2 kg of

straw/day) for 8 d during the estrous cycle (d 8 to 16 of the cycle) had reduced LH concentrations on d 7 of fasting (McCann and Hansel, 1986). In contrast, cows fed restricted diets had reduced concentrations of LH compared with maintenance fed cows only after severe nutrient restriction (26 wk; Richards et al., 1989a). Bossis et al. (1999) found that concentrations of LH were reduced in heifers fed restricted diets (32 wk) compared to maintenance-fed heifers during the estrous cycle prior to nutritional anovulation but not during the last ovulatory cycle.

Influence of Steroid Hormones on Genes Encoding for Gonadotropins

Although much is known about the regulation of LH and FSH secretion by steroid hormones, recent advancement of molecular techniques has allowed insight into pretranslational regulation of gonadotropins by GnRH, steroids and ovarian peptides. Secretion of GnRH controls synthesis and secretion of gonadotropins via receptors on the gonadotrophs; consequently, regulation of GnRH receptors is a critical step in controlling secretion of gonadotropins.

Gonadotropin-releasing Hormone Receptor

The gonadotropin-releasing hormone receptor (GnRH-R) is composed of seven hydrophobic regions that span the plasma membrane of gonadotroph cells of the anterior pituitary gland (Duello and Nett, 1980; Marian and Conn, 1983). Gonadotropin-releasing hormone binds to bihormonal gonadotrophs (producing both LH and FSH), as well as to monohormonal cells (producing only LH or FSH) (Childs et al., 1987; Liu et al., 1988).

Pockets formed from loops of the seven transmembrane domains of the receptor allow GnRH to bind and activate the receptor (Stojilkovic et al., 1994). The carboxy-terminal cytoplasmic tail, which is thought to be coupled with G proteins in other receptors of this type, is absent in the GnRH-R; therefore, the GnRH-R depends upon other regions to activate G proteins (Dohlman et al., 1991). The binding of GnRH to its receptor causes a conformational change that leads to a dissociation of heterotrimeric G proteins activating the α-subunit of the G protein (Stojilkovic et al., 1994). The α-subunit of the G protein activates phosphoinositide-specific phospholipase C (PLC) which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) forming 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG), which are major second messengers (Conn, 1986). The increased concentrations of $InsP_3$ induce oscillatory increases in Ca^{2+} release from intracellular stores via $InsP_3$ binding to receptors on intracellular vesicles (Stojilkovic et al., 1992). Increased intracellular concentrations of Ca^{2+} and DAG activate protein kinase C (PKC). Both Ca^{2+} and PKC serve as signal transducers to control several cellular responses, including the control of primary and secondary gene responses (Stojilkovic et al., 1994).

With the development of the mouse gonadotropin cell line, αT3-1 (Windle et al., 1990), the complementary DNA (cDNA) encoding GnRH-R was cloned and characterized in the mouse (Reinhart et al., 1992; Tsutsumi et al., 1992), rat (Kaiser et al., 1992), human (Chi et al., 1993), sheep (Brooks et al., 1993; Illing et al., 1993) and cow (Kakar et al., 1993). The GnRH-R of the human, sheep and cow contains 328 amino acids, while the mouse and rat receptor have 327 amino acids (Kakar et al., 1993; Stojilkovic et al., 1994). Homology of the mouse receptor is 97, 89, 87 and 86% with the rat, human, sheep and cow receptors, respectively (Kakar et al., 1993; Stojilkovic et al.,

1994). Northern blot analyses indicate four hybridizing species in sheep of 5.4-6, 3.6-4, 2.3 and 1.3 kilobases (Brooks et al., 1993; Illing et al., 1993). The 2.3 kb mRNA does not encode a full length receptor (Illing et al., 1993). In the cow, the predominant mRNA is 5.0 kb in size, followed by less abundant transcripts at 3.5, 2.5 and 1.5 kb (Kakar et al., 1993).

Number of GnRH-R changes during the estrous cycle of most species due to varying concentrations of steroids, as well as GnRH. Regulation of GnRH-R numbers reflects changes in GnRH-R gene expression (Stojilkovic et al., 1994). Pulsatile release of GnRH increases the amount of GnRH-R mRNA while continuous delivery of GnRH tends to decrease receptor gene expression (Belchetz et al., 1978). Intermittent infusion of GnRH to seasonally anestrous ewes (Turzillo et al., 1995b) and rats (Yasin et al., 1995) increased the amount of GnRH-R mRNA in the pituitary gland. In vitro treatment of rat pituitary cells with GnRH at a pulse every 30 min or every hour for 24 h increased amounts of mRNA for GnRH-R (Kaiser et al., 1997). Similarly, in vitro perifusion of pituitary gland fragments from metestrous and proestrous rats with pulsatile GnRH increased mRNA for GnRH-R (Bauer-Dantoin et al., 1995). Continuous infusion of GnRH decreased mRNA for GnRH-R in nutritionally anestrous cows (Vizcarra et al., 1997) and wethers (Wu et al., 1994). Chronic treatment (7 d) of sheep pituitary cells with a GnRH agonist decreased amounts of GnRH-R mRNA (Wu et al., 1994). Immunization against GnRH decreased steady state concentrations of GnRH-R mRNA after 2-4 d in orchidectomized sheep (Adams et al., 1997; Sakurai et al., 1997). Blockade of neurosecretion from the hypothalamus in rats by pentobarbital decreased GnRH-R mRNA expression (Bauer-Dantoin et al., 1995). Daily injections of the GnRH

antagonist, Lupron, decreased amounts of GnRH mRNA by 75 to 80% in male rats compared with control rats (Pinski et al., 1996). Turzillo et al. (1995a) found disconnection of the hypothalamus from the pituitary did not reduce concentrations of GnRH-R mRNA in ewes. Female rats tend to have greater sensitivity to changes in amplitude and pulse frequency of GnRH than male rats (Yasin et al., 1995). Longer intervals between pulses and less amplitudes of GnRH are needed to increase mRNA for GnRH-R in females compared to males.

Changes in the amount of GnRH-R mRNA that occur during the estrous cycle are indicative of changes in concentrations of steroid hormones. Removal of the gonads increased GnRH-R mRNA in pituitaries of rats (Kaiser et al., 1993) and sheep (Illing et al., 1993) indicating chronic exposure to steroids down-regulates gene expression of GnRH-R. Concentrations of GnRH-R mRNA are increased during the preovulatory period in sheep (Brooks et al., 1993; Turzillo et al., 1994) and rats (Bauer-Dantoin et al., 1993). Concentrations of GnRH-R mRNA are increased 3-fold on the afternoon of proestrus in rats, preceding the gonadotropin surge by 6 h (Bauer-Dantoin et al., 1993).

Acute treatment of ovariectomized ewes (Hamernik et al., 1995; Turzillo et al., 1995a), wethers (Adams et al., 1996; Sakurai et al., 1997) and ovariectomized rats (Kaiser et al., 1993; Bauer-Dantoin et al., 1995; Yasin et al., 1995) with estradiol increases GnRH-R mRNA. However, chronic treatment with estradiol for 7 d decreased GnRH mRNA levels in ovariectomized rats (Kaiser et al., 1993). Concentrations of GnRH-R mRNA rapidly decreased after removal of exogenous estradiol in wethers (Adams et al., 1996). Estradiol treatment of hypothalamic-pituitary disconnected ovariectomized ewes increased pituitary concentrations of GnRH-R mRNA indicating

estradiol acts directly at the pituitary gland (Turzillo et al., 1995a). Estradiol treatment induced increases in mRNA for GnRH-R in pituitaries of wethers immunized against GnRH but increases were not as dramatic as in the presence of GnRH (Adams et al., 1997). In vitro treatment of ovine pituitary cells for 48 h with estradiol dramatically increased GnRH-R mRNA (Sealfon et al., 1990; Wu et al., 1994).

Effects of progesterone on concentrations of GnRH-R mRNA are less clear than those of estradiol and may depend on previous exposure to estradiol. Chronic exposure (7-8 d) to exogenous progesterone reduced concentrations of GnRH-R mRNA in wethers compared with controls (Sakurai et al., 1997). However, wethers implanted with progesterone and infused with estradiol had concentrations of GnRH-R mRNA similar to wethers treated only with estradiol (Sakurai et al., 1997). Progesterone treatment of estrogen primed rats did not decrease GnRH-R mRNA expression during the gonadotropin surge; however, receptor gene expression was reduced following the LH surge in rats (Bauer-Dantoin et al., 1995). Progesterone treatment of sheep pituitary cells significantly decreased GnRH-R mRNA compared with gene expression in controls (Wu et al., 1994). The estradiol-induced increase in GnRH-R mRNA was partially inhibited when progesterone was added to sheep pituitary cell cultures (Sealfon et al., 1990; Wu et al., 1994). Turzillo et al. (1994) suggested that reduced concentrations of progesterone are necessary for GnRH-R mRNA up-regulation. In intact ewes, pituitary content of mRNA for GnRH-R increased 12 h after $PGF_{2\alpha}$ induced luteolysis. This increase in mRNA GnRH-R occurred at a time when concentrations of progesterone were decreased and prior to significant increases in concentrations of estradiol. This indicates that progesterone and not estradiol may mediate the pretranslation of GnRH-R. Amounts of

GnRH-R mRNA in intact ewes on d 14 of the estrous cycle were similar to those in ovariectomized ewes (Hamernik et al., 1995). Minimal concentrations of estradiol and progesterone in circulation may be necessary for up-regulation of GnRH mRNA.

Structure of Gonadotropins

Luteinizing hormone and FSH are glycoproteins consisting of two, noncovalently linked subunits designated α and β. Within a species, the α-subunit is common among LH and FSH, as well as with thyroid-stimulating hormone and chorionic gonadotropin (human and equine). The β-subunit is unique to each hormone and confers biological specificity (Pierce and Parsons, 1981).

α-subunit gene and protein. Since the human α-subunit gene sequence was reported (Fiddes and Goodman, 1979), the gene encoding the α-subunit has been isolated and sequenced in the bovine (Erwin et al., 1983; Nilson et al., 1983), mouse (Chin et al., 1981) and rat (Godine et al., 1982). A single gene containing 4 exons and 3 introns encode for the α-subunit in humans, cattle, mice and rats (for review see Gharib et al., 1990). In all species, the α mRNA ranges in size from 730 to 800 nucleotides translating into a 24 amino acid precursor followed by the mature 96 amino acid peptide (92 aa in the human) (Gharib et al., 1990). The protein sequence (96 aa) for the α-subunit is identical for all four glycoproteins and contains two oligosaccharides N-linked to asparagines (Pierce and Parsons, 1981). The tertiary structure of the α-subunit is attained by five internal disulfide bonds (Pierce and Parsons, 1981). Intracellular concentrations

of the common α-subunit are in excess of the LHβ-subunit mRNA in cattle (Fetherston and Boime, 1982) and sheep (Landefeld et al., 1984).

LHβ-subunit gene and protein. A single gene encodes for the LHβ-subunit in humans (Talmadge et al., 1984), rats (Tepper and Roberts, 1984) and cattle (Maurer, 1985); however, the related human CGβ-subunit is encoded by seven genes (Talmadge et al., 1984). The LHβ-subunit gene in the bovine contains 3 exons and 2 introns with a length of 1.1 kilobases (Virgin et al., 1985). Bovine LHβ-subunit mRNA is a 550 nucleotide translating into a 120 amino acid protein (Virgin et al., 1985). Six disulfide bonds assist in the three dimensional structure of both LHβ- and FSHβ-subunits (Pierce and Parsons, 1981). Additionally, the LHβ-subunit has one N-linked carbohydrate chain attached to an asparagine residue (Pierce and Parsons, 1981; Gharib et al., 1990).

FSHβ-subunit gene and protein. Similar to the LHβ gene, a single gene encodes the FSHβ-subunit in humans (Jameson et al., 1988), rats (Gharib et al., 1989) and cattle (Kim et al., 1988) and consist of 3 exons and 2 introns. In the bovine, the FSHβ gene is 4 kilobases in length (Kim et al., 1988). A long 3′-UT region (1.2 kilobases), uncommon to the LHβ-subunit, is in the bovine FSHβ gene (Gharib et al., 1990). The mature FSHβ protein is slightly smaller than the LHβ protein with 118 amino acids (Gharib et al., 1990). Two N-linked oligosaccharides are bound to asparagine residues in the FSHβ-subunit (Pierce and Parsons, 1981; Gharib et al., 1990).

Regulation of Gonadotropin Subunit mRNAs by GnRH

Release of GnRH from the hypothalamus is required for pulsatile secretion of gonadotropins in monkeys (Knobil, 1980), sheep (Clarke and Cummins, 1982) and cattle (Anderson et al., 1981). Pulsatile GnRH treatment increases gonadotropin gene expression in mice (Saade et al., 1989), rats (Dalkin et al., 1989; Haisenleder et al., 1990), sheep (Mercer et al., 1988) and cattle (Vizcarra et al., 1997). Amounts of LHβ and FSHβ mRNA were decreased 60 and 90%, respectively, in male rats treated with a GnRH antagonist for 10 d (Perheentupa and Huhtaniemi, 1990). The α-subunit mRNA was not affected in male rats treated with the GnRH antagonist. Pulses of GnRH every 30 min increased FSHβ mRNA after 6 h and α-subunit mRNA after 12 h in female rats (Haisenleder et al., 1990). After 12 h of 30 min pulses of GnRH, LHβ mRNA increased in rats, then decreased to amounts similar to control rats. Removal of hypothalamic input by disconnecting the hypothalamus from the pituitary decreased amounts of α- and LHβ-subunit mRNA in ovariectomized ewes (Hamernik et al., 1986). Immunization of ovariectomized cows against GnRH decreased amounts of mRNA for α- and LHβ-subunits (Stumpf et al., 1992).

Biosynthesis of gonadotropin subunits is differentially regulated by the release pattern (amplitude and frequency) of GnRH from the hypothalamus. In castrated male rats, GnRH treatment at frequent intervals (8 and 30 min) increased steady state amounts of α-subunit and LHβ mRNA (Dalkin et al., 1989). Maximal amounts of FSHβ mRNA were associated with slower GnRH pulse intervals of 120 min (Dalkin et al., 1989; Haisenleder et al., 1991). Similarly, in vitro treatment of rat pituitary cells with GnRH

stimulated maximal amounts of α-subunit and LHβ mRNA at a pulse frequency of 30 min; maximal amounts of FSHβ were associated with GnRH pulses every 120 min (Kaiser et al., 1997). Changes in GnRH-R may be responsible for differential regulation of mRNA for gonadotropin subunits. Kaiser et al. (1995) transfected GH_3 cells (rat pituitary cells generated from a rat adenoma) with a GnRH-R cDNA vector containing a heterologous promoter, and regulatory regions (transcriptional start site) of the human α-, rat LHβ- and rat FSHβ-subunits fused to luciferase reporter genes. Pituitary cells cotransfected with LHβ-subunit fused to luciferase reporter genes and increasing amounts of GnRH-R cDNA had increased luciferase activity for α and LHβ after GnRH treatment. Stimulation of luciferase activity of FSHβ by GnRH was reduced as transfection of GnRH-R cDNA increased (Kaiser et al., 1995).

Regulation of Gonadotropin Subunit mRNAs by Steroid Hormones

It is well established that removal of gonadal steroids by gonadectomy increases concentrations of gonadotropins in the circulation. Only in the last 15 years has the regulatory role of steroids on the synthesis of gonadotropins been elucidated (for review see Nett, 1990).

Gonadectomy

In early studies, mRNA was translated in cell-free systems, and the translated product was characterized by polyacrylamide electrophoresis allowing for an indirect measurement of gonadotropin subunits. Using this system, castration increased amounts

of all three gonadotropin subunits in the pituitary gland of both male and female rats compared with intact animals (Godine et al., 1980). Counis et al. (1983) found that ovariectomy of rats increased concentrations of mRNA encoding for common α-subunit and LHβ-subunit compared with intact animals. Pituitary glands of steers had 5-fold greater synthesis of α- and LHβ-subunits compared with pituitary glands of intact cows (Fetherston and Boime, 1982). Wethers had increased amounts of FSHβ mRNA (Alexander and Miller, 1982) and ovariectomized lambs had increased α-subunit mRNA (Landefeld et al., 1984) compared with intact animals.

A more direct method of quantifying amounts of mRNA for subunits became available with the isolation of gonadotropin subunit cDNAs. Utilizing RNA-cDNA hybridization assays, Abbot et al. (1985) found that both α- and LHβ-subunits were increased 4 d after castration in male rats. Amounts of mRNA for α- and LHβ-subunits were increased within 24 h after castration in male rats (Papavasiliou et al., 1986). However, amounts of mRNA for the subunits increased more slowly in ovariectomized female rats. Significant increases occurred on d 4 and 7 after ovariectomy for α- and LHβ-subunits, respectively, in female rats (Papavasiliou et al., 1986). Ovariectomy gradually increased amounts of mRNA for all three gonadotropin-specific subunits in female rats with LHβ mRNA increasing more dramatically than α- and FSHβ-subunits (Gharib et al., 1987). Amounts of gonadotropin subunit mRNAs increase in male rats after castration. Amounts of FSHβ mRNA increase 4-fold 7 d post castration and decline to amounts observed in intact animals by 28 d after castration (Gharib et al., 1987). Ovariectomized pubertal heifers had greater amounts of steady state α-subunit and LHβ mRNAs compared with intact animals (Roberson et al., 1992). More LH-containing

gonadotropes were in pituitary gland cells one month after gonadectomy in rats compared with intact animals (Ibrahim et al., 1986). Using a nuclear run-off assay, where nuclei of pituitary cells are incubated with radiolabeled nucleotides, gonadotropin subunit mRNA synthesis was greatest in pituitary glands from ovariectomized rats compared with pituitary glands from intact and estrogen-treated rats (Shupnik et al., 1988).

Estrogen

Stimulatory effects of gonadal removal on amount of gonadotropin mRNAs in rats were inhibited with a single injection of estradiol (Counis et al., 1983). Chronic administration of estradiol to rats for 7 d after gonadectomy reduced the α-, LHβ- and FSHβ-subunits to amounts observed in intact animals (Gharib et al., 1987). Daily injections of estradiol in sheep reduced mRNA for FSHβ by 75 and 90% after 3 and 6 days, respectively (Alexander and Miller, 1982). Chronic exposure to estradiol has a triphasic effect on amount of mRNA for gonadotropins in ovariectomized ewes (Herring et al., 1991). Acute exposure to estradiol (12 h) decreased the amounts of mRNA for all three gonadotropin subunits in ovariectomized ewes followed by increases in α-subunit and LHβ mRNA after 24 h with no affect on FSHβ mRNA. Further exposure to estradiol decreased amounts of mRNA for α and LHβ after 4 d, and amounts of FSHβ mRNA were decreased after 8 d (Herring et al., 1991). Estradiol treatment of ovariectomized, hypothalamic-pituitary disconnected ewes for 24 h reduced amounts of LHβ mRNA by 38% compared with untreated ewes, and amounts of mRNA for α and FSHβ were not influenced (Di Gregorio and Nett, 1995). This indicates that estradiol may act directly at the pituitary gland to regulate steady state amounts of mRNA.

Concentrations of estradiol increase simultaneously with concentrations of gonadotropins at estrus. With increased concentrations of LH and FSH it would seem likely that amounts of mRNA for the gonadotropins would increase similarly; however, there are conflicting reports. Estradiol treatment of ovariectomized rats induced a preovulatory surge of LH without increasing amounts of mRNA for LHβ; however, the α-subunit was increased 2-fold during the LH surge (Haisenleder et al., 1988). A previous report from the same laboratory indicated that LHβ mRNA was increased during the proestrus LH surge while the amount of α-subunit mRNA was unchanged (Zmeili et al., 1986). Acute exposure (16 h) to estradiol increased translatable α-subunit 3-fold in intact ewes (Landefeld et al., 1984). Amounts of mRNA for the α-subunit were unchanged whereas FSHβ mRNA was decreased during an estradiol-induced surge of LH in ovariectomized ewes (Hamernik and Nett, 1988). If concentrations of gonadotropins were increasing, LHβ mRNA was similar to untreated ovariectomized ewes. However, when gonadotropins were decreasing, LHβ mRNA was reduced (Hamernik and Nett, 1988). Concentrations of estradiol similar to concentrations that occur during the follicular phase of the estrous cycle did not stimulate gene expression of α- and LHβ-subunits in ovariectomized cows (Cupp et al., 1995).

Progesterone

Effects of progesterone on gonadotropin subunit mRNAs are less pronounced and not as definite as those associated with estradiol. During gestation (d 50 to 140), amounts of mRNA for LHβ declined in ewes (Wise et al., 1985). Concentrations of progesterone and estradiol are increased during this time of gestation in ewes (Moss et al., 1980).

Ovariectomized ewes treated with estradiol and progesterone for 69 d to mimic concentrations of steroids during late gestation had reduced amounts of mRNA for α- and LHβ-subunits compared with ovariectomized ewes (Nett et al., 1990). Exogenous pulsatile GnRH for an additional 42 d after exposure to steroids did not increase amounts of α- and LHβ-subunits compared with ovariectomized ewes with or without steroid treatment. Exposure of ovariectomized, hypothalamic-pituitary disconnected ewes to progesterone during estradiol treatment decreased α-subunit and FSHβ mRNA without altering mRNA for LHβ compared with ewes treated with estradiol alone (Di Gregorio and Nett, 1995). Exposure of sheep pituitary cell cultures to progesterone decreased amounts of FSHβ mRNA by 68% and α mRNA by 30% within 24 h (Phillips et al., 1988). However, administration of the progesterone antagonist RU 486 did not restore LHβ-subunit amounts in lactating rats (Lee et al., 1989). Progesterone treatment did not alter translatable α- or LHβ-subunits in ovariectomized rats compared with controls (Counis et al., 1983). Progesterone treatment via implants for 3 wk did not affect amounts of mRNA for α-, LHβ- or FSHβ-subunit in ovariectomized sheep (Hamernik et al., 1987). The influence of progesterone on gonadotropin-specific subunits is unclear. Progesterone affects synthesis and release of gonadotropins at the hypothalamus by altering GnRH secretion. Progesterone alone may have little or no affect on the synthesis of gonadotropins; however, progesterone, in conjunction with estradiol, may act synergistically to suppress LHβ mRNAs.

Disagreement among studies utilizing steady state amounts of mRNA for gonadotropins as a measurement for gene expression can be explained by differences in animal models (intact vs ovariectomized), as well as the physiological state of the animal

at the time of pituitary gland harvest. Steady state amounts of mRNA for the gonadotropins may or may not reflect plasma concentrations of gonadotropins. Winters (1996) found a significant correlation ($r = .60$) between plasma concentrations of FSH and mRNA for FSHβ-subunit in intact rats. However, there was no correlation between plasma FSH and mRNA for FSHβ in castrated rats. Plasma concentrations of α- and LHβ-subunits did not reflect amounts of mRNA for these gonadotropins in the pituitary gland of either castrated or intact rats (Winters, 1996).

Discrepancies when comparing amounts of mRNA between studies may be due to slight differences in time at which pituitary glands were collected. Alexander and Miller (1982) proposed that the half-life of common α-subunit was 51 h while the β-subunit is less stable with a half-life of 12-16 h (Hall and Miller, 1986; Hamernik and Nett, 1988; Di Gregorio and Nett, 1995). Accurate determination of treatment differences in mRNA for gonadotropin subunits would require collection of pituitary glands at more frequent intervals. Use of techniques that directly measure gonadotropin gene transcription (i.e., nuclear run-off assay) also may reduce variable results between studies (Hamernik, 1995).

Nutritional Effects on Gonadotropin Subunits

Malnutrition alters secretion of gonadotropins; therefore, amounts of the mRNA encoding for the glycoproteins should be affected by nutrition. Amounts of α-subunit mRNA in intact male rats were decreased 42% after a 4 d fast compared to control rats, while LHβ- and FSHβ-subunit mRNAs were not affected (Bergendahl et al., 1989). Six days of fasting decreased both α- and FSHβ-subunits, while the LHβ-subunit increased in

fasted rats compared with normal fed rats. Deprivation of food for 5 d did not alter amounts of mRNA for α- and FSHβ-subunits for intact and orchidectomized rats (Bergendahl and Huhtaniemi, 1994). Feed restriction for 5 d increased LHβ-subunit mRNA in acute, as well as chronically castrated male rats compared with intact animals (Bergendahl and Huhtaniemi, 1994). The effects of undernutrition on the synthesis of gonadotropins in rats are not clear. Presence of gonads may influence how nutrition effects gonadotropin synthesis in rodents.

Ovariectomized ewes receiving minimal concentrations of estradiol (~ 5 pg/mL) and fed restricted diets (60% of NRC requirements) for 127 d had reduced amounts of mRNA for α-, LHβ- and FSHβ-subunits compared with normal fed ewes (Kile et al., 1991). Mature ovariectomized ewes fed a restricted diet (60% of control animals) for 6 wk followed by a more severe feed restriction (40% of control diet) for 14 wk had similar amounts of α-, LHβ- and FSHβ-subunits at 20 wk compared with normal fed ewes (Thomas et al., 1990). Landefeld et al. (1989) found ad libitum feeding of ovariectomized, nutritionally restricted, growth-retarded lambs for 14 d increased amounts of mRNA for all gonadotropin subunits compared with lambs remaining on a low plane of nutrition. Orchidectomized sheep fed to maintain or lose body weight for 7 wk had increased amounts of FSHβ mRNA while mRNA for LHβ was unchanged compared with orchidectomized sheep fed ad libitum diets (Beckett et al., 1997a). Nutrient restriction tends to be detrimental to the synthesis of mRNAs encoding for gonadotropin subunits. Conflicting reports on nutritional effects of gonadotropin synthesis is probably due to the severity of the restriction and/or the differences in age, maturity and body condition of the animals at initiation of feed restriction.

Chronic nutritional deprivation reduces reproductive performance primarily by decreasing LH secretion. Short-term fasting decreases gonadotropin secretion in rodents, humans and nonhuman primates; however, the effects of acute nutritional deprivation on gonadotropin release in cattle have not been elucidated. Resumption of luteal activity after parturition is necessary for efficient reproductive processes. The first postpartum ovulation in cattle is associated with a transient increase in progesterone. How body energy reserves at parturition influence endocrine and behavioral events associated with first ovulation in postpartum beef cows has not been clarified. Steroid hormones regulate release of gonadotropins during gestation and throughout the estrous cycle. Pituitary and serum concentrations of gonadotropins are diminished at parturition and during the early post partum period due to prolonged exposure to estrogens during gestation. Inadequate body energy stores at parturition further attenuates reduced reproductive performance of cattle during the postpartum anestrous period. Estrogens and progestins coordinate, through positive and negative feedback systems, events associated with normal estrous cycles. Mechanisms by which steroids influence serum and pituitary concentrations of LH and FSH, mRNA for gonadotropin subunits and GnRH receptor, and concentrations of GnRH receptor in pituitary glands of nutritionally induced, anovulatory cows is not established.

CHAPTER III

INFLUENCE OF BODY ENERGY RESERVES AND NUTRITION ON LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE IN OVARIECTOMIZED BEEF COWS

Abstract: Ovariectomized Hereford and Angus x Hereford cows were used to determine the effect of body energy stores and nutrient intake on concentration, pulse frequency and amplitude of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in serum. In Experiment 1, nutritionally induced anovulatory cows were fed to either maintain (M; n = 4) or gain body weight (G; n = 4) for 4 mo to determine the effects of chronic nutritional deprivation on gonadotropin secretion. Blood samples were collected at 10 min intervals for 8 h. In Experiment 2, ovariectomized cows in moderate body condition were randomly assigned to receive either a restricted (R; n = 4) or maintenance diet (M; n = 4) for 6 d to determine effects of acute nutritional deprivation on gonadotropin secretion and blood metabolites. Concentrations of LH and FSH in serum of cows were quantified the day before treatment (d 0), and on d 3 and 6. Concentrations of glucose, nonesterified fatty acids (NEFA), insulin-like growth factor-I (IGF-I) and leptin were determined in daily plasma samples. In Experiment 1, thin cows that gained weight and body condition score (BCS) had greater ($P < .05$) concentrations, pulse frequencies and amplitudes of LH and FSH than did thin cows that maintained BCS. Acute feed

restriction for 6 d (Experiment 2) did not influence concentration, pulse frequency or amplitude of LH and FSH in ovariectomized, moderate condition cows. Concentrations of glucose and leptin were not altered during 6 d of feed restriction; however, concentrations of NEFA were increased and IGF-I were decreased in R cows. We conclude that long term feed deprivation of ovariectomized beef cows decreases LH and FSH concentration, pulse frequency and amplitude, whereas feed restriction for 6 d alters energy metabolism without influencing secretion of gonadotropins.

Key Words: Beef Cows, Nutritional Deprivation, Gonadotropins

Introduction

Inadequate nutrition is associated with suppression of gonadal activity in many species. Long-term feed restriction diminishes pulsatile release of LH in ewes (Foster and Olster, 1985; Foster et al., 1989), heifers (Day et al., 1986; Bossis et al., 1999) and cows (Richards et al., 1989a). Pulsatile GnRH treatment of nutritionally induced anovulatory cows initiates LH secretion and ovarian function (Bishop and Wettemann, 1993; Vizcarra et al., 1997) indicating that the anterior pituitary gland is functional.

Influence of acute feed restriction on gonadotropins depends primarily on the extent of the restriction and species involved. Feed deprivation for 4 to 7 d decreased serum (Campbell et al., 1977; Bergendahl et al., 1989) and pituitary (Bergendahl et al., 1989) concentrations of LH and FSH in intact male rats. Amplitude of LH pulses in serum was reduced in ovariectomized rats fasted for 5 d compared with nonfasted rats (Dyer et al., 1985). The preovulatory surge of LH was blocked in proestrous rats when food was removed for 24 h (McClure and Saunders, 1985). Fasting for 1 to 2 d reduces

plasma concentration and pulse frequency of LH in male monkeys (Cameron and Nosbisch, 1991; Schreihofer et al., 1993a, 1993b). Infusion of nutrients to fasted monkeys via gastric cannulae (Schreihofer et al., 1993b) as well as overfeeding prior to fasting (Schreihofer et al., 1993a) prevented the decrease in LH secretion in fasted animals. Concentrations of LH and FSH, as well as LH pulse frequency, were decreased in serum of men fasted for 48 h (Cameron et al., 1991).

Nutritional status of animals is probably mediated to the brain by a number of metabolic signals. Concentrations of NEFA increase and glucose decrease in plasma of feed restricted cattle (Richards et al., 1989b; Yelich et al., 1995; Bossis et al., 1999). Concentrations of GH increase and IGF-I decrease in plasma of cattle during chronic feed restriction (Breier et al., 1986; Bossis et al., 1999).

Effects of acute feed restriction on gonadotropin release and blood metabolites have not been elucidated in ruminants. Objectives of these experiments were: 1) to determine the effect of chronic nutritional deprivation on concentration, pulse frequency and amplitude of LH and FSH in ovariectomized beef cows and 2) to examine the effects of acute nutritional deprivation on secretion of LH and FSH, and concentrations of glucose, NEFA, IGF-I and leptin in plasma of ovariectomized beef cows in moderate body condition.

Materials and Methods

Experiment 1. Hereford and Angus x Hereford cows in good to moderate body condition (BCS = 5; 1 = emaciated, 9 = obese; Wagner et al., 1988) were fed to lose 1% of initial body weight per wk (approximately 26 wk) until they became anovulatory (BCS = 4).

Cows were ovariectomized and randomly assigned to either maintain (M) or gain (G) diets for 4 mo. When M cows had a body weight of 288 ± 4 kg and a BCS = 4.2 ± .1, and G cows had a body weight of 493 ± 68 kg and a BCS = 5.2 ± .4, they were confined in individual stalls in a barn at 21 ± 4°C and 14 h of light. During the sampling period, G cows were fed 7 kg of prairie hay (CP = 5.8%), 1.4 kg of alfalfa pellets (CP = 17%) and 35 g of a mineral mix (46.1% salt, 50.0% dicalcium phosphate, .4% copper sulfate, .5% zinc oxide and 3.0% mineral oil) each day, and M cows were fed 2.25 kg of prairie hay and 35 g of a mineral mix. Cows were fitted with a polyvinyl jugular cannula (id, 1.68 mm; od, 2.39 mm; BB 317 v11, Bolab, Lake Havasu City, AZ) two days prior to treatment to allow frequent collection of blood samples.

Blood was collected at 10 min intervals for 8 h from G (n = 4) and M (n = 4) cows. Samples were allowed to clot for 24 h at 4°C and centrifuged at 2,500 x g for 20 min. Serum was decanted and stored at - 20°C until concentrations of LH and FSH were quantified.

Experiment 2. Eight Hereford and Angus x Hereford cows in moderate body condition (BW = 483 ± 17 kg; BCS = 5.1 ± .1) were ovariectomized 4 mo prior to the initiation of treatment. Prior to treatment, cows were fed 7 kg of prairie hay, 1.4 kg of alfalfa pellets and 35 g of a mineral mix daily. Cows were confined in stalls and fitted with cannulae as described for Experiment 1. Cows were randomly assigned to receive either a restricted diet [R; n = 4; 2.25 kg of prairie hay (CP = 5.8%) and 35 g of a mineral mix] or maintenance diet [M; n = 4; 7 kg of prairie hay (CP = 5.8%), 1.4 kg of alfalfa pellets (CP

= 17%) and 35 g of a mineral mix] daily for 6 days in two replications (n = 4; summer of 1995 and n = 4; summer of 1996). The day before treatment (d 0), and on d 3 and 6, blood samples were collected at 10 min intervals for 8 h and serum was collected to quantify concentrations of LH and FSH. Blood samples were collected daily (d 0 to 6) via tail venipuncture in 10 mL tubes containing EDTA (.1 mL of a 15% solution), cooled to 4°C, centrifuged (2,500 x g for 15 min) within 2 h, and plasma was decanted and stored at - 20°C until concentrations of glucose, NEFA, IGF-I and leptin were quantified.

Hormone and metabolite assays. Concentrations of LH were quantified in serum (250 μL) samples by RIA (Bishop and Wettemann, 1993) using NIH LH-B9 as the standard. Intra- and interassay coefficients of variation were 8 and 19%, respectively (n = 5 assays). Concentrations of FSH were determined in duplicate by RIA with USDA-bFSH-I-2 as the standard (Vizcarra et al., 1997). Intra- and interassay coefficients of variation were 5 and 11% (n = 5 assays).

Concentrations of IGF-I in daily plasma samples were determined in a single assay by RIA (Echternkamp et al., 1990) after an acid ethanol extraction. Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used as the standard and intraassay coefficient of variation was 2%. Leptin concentrations were quantified by RIA (Linco Multi-Species Leptin RIA Kit, Linco, Inc., St. Charles, MO). Sensitivity of the assay was 1.0 ng/mL. Addition of 2 and 5 ng of human leptin to 1 mL plasma resulted in recovery of 84 and 111%, respectively. When 60 and 80 μL of plasma were assayed,

concentrations were parallel to the standard curve. Intraassay coefficient of variation was 8%.

Concentrations of glucose in plasma were quantified by an enzymatic colorimetric procedure (Sigma, No. 510, Sigma Chemical Co., St. Louis, MO). Intra- and interassay coefficients of variations were 4 and 14%, respectively (n = 2 assays). Concentrations of NEFA in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C, Wako Chemicals Inc., Dallas TX) with modifications (McCutcheon and Bauman, 1986) and expressed as microequivalents of palmitate per liter. Intra- and interassay coefficients of variation were 6 and 11%, respectively (n = 2 assays).

Pulse analyses. Pulse frequency and amplitude of LH and FSH were determined using the pulsar program (Merriam and Wachter, 1982). The G values for both LH and FSH were G1=99, G2=4.5, G3=4, G4=3.5 and G5=99. To determine if variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variations in concentrations, appropriate G values are chosen for each experiment. The value for G1 is usually set at 99 to prevent identifying a one sample increase in concentration as a pulse, which by our definition of a pulse can not occur due to the frequency at which samples were collected. The G5 value also was set at 99 to avoid false positive determination of a small increase, followed by a return to baseline concentration, as a pulse.

Statistical analyses. Data were analyzed by analyses of variance using the MIXED procedure of SAS (1996) to determine the effects of chronic nutritional deprivation on

hormone concentrations. Analyses of variance were performed using the GLM procedure of SAS (1996) to determine the effect of treatment on pulse amplitude and frequency. Effects of acute nutritional deprivation (Exp. 2) on concentration, pulse frequency and amplitude of LH and FSH were determined by the MIXED procedure of SAS (1996). Concentrations of blood metabolites from daily plasma samples (d 1 to d 6) were analyzed as repeated measures using MIXED procedure of SAS (1996). Concentrations of IGF-I on d 0 were used as covariates. Treatment means were compared using the PDIFF statement of SAS (1996) when protected by a significant ($P < .05$) treatment effect.

Results

Experiment 1. Nutritionally induced anovulatory cows fed to gain body weight (G) had greater ($P < .05$) LH concentrations than did cows that maintained body weight (M) (6.9 vs 3.6 ng/mL; Table 1). Similarly, FSH concentrations in serum were greater ($P < .06$) in G cows (2.1 ng/mL) than M cows (1.6 ng/mL; Table 2).

Number and amplitude of LH pulses were greater ($P < .05$) in cows gaining weight than in cows maintaining weight (Table 1). Number and amplitude of FSH pulses were greater ($P < .05$) in G cows than M cows (Table 2).

Experiment 2. Feeding 30% of maintenance energy requirements (NRC, 1984) for 6 d did not influence ($P > .1$) concentrations of LH and FSH in ovariectomized beef cows in moderate condition (Table 3 and 4). Concentrations of LH were 4.5 ± .1 ng/mL in M

cows and 5.1 ± .1 ng/mL in R cows. Concentrations of FSH were 2.5 ± .1 ng/mL and 2.7 ± .1 ng/mL in M and R cows, respectively.

Acute feed deprivation for 6 d did not influence ($P > .1$) the number of LH and FSH pulses in ovariectomized beef cows (Table 3 and 4). Pulse frequencies (pulses/8 h) of LH were 8.4 ± .2 and 6.9 ± .7 for M and R cows, respectively. Numbers of FSH pulses per 8 h were 6.3 ± .6 for M and 4.8 ± .6 for R cows.

Amplitudes of LH and FSH pulses were not altered ($P > .1$) by nutritional restriction for 6 d (Table 3 and 4). Amplitudes of LH pulses were 3.1 ± .2 and 3.4 ± .2 ng/mL for M and R cows, respectively, and amplitudes of FSH pulses were .8 ± .1 and 1.0 ± .1 ng/mL for M and R cows, respectively.

Concentrations of glucose in plasma were not influenced ($P > .1$) by acute feed deprivation. During d 0 through 6, concentrations were 71.8 ± .8 and 69.5 ± .8 mg/dL for M and R cows, respectively (Figure 1).

There was a treatment x day effect ($P < .05$) for NEFA in plasma. Concentrations of NEFA were greater ($P < .05$) in R cows than in M cows from d 2 through d 6 of treatment (Figure 2).

There was a treatment x day effect ($P < .05$) on plasma IGF-I concentrations. Concentrations of IGF-I were greater ($P < .05$) in M cows than in R cows on d 5 and 6 of feed restriction (Figure 3).

Acute feed deprivation did not influence ($P > .1$) concentrations of leptin in plasma. Concentrations of leptin averaged 2.3 ± .1 ng/mL for both M and R cows (Figure 4).

Discussion

Feeding nutritionally induced anovulatory beef cows to gain body weight increased concentrations, pulse frequencies and amplitudes of LH and FSH. Long term feed restriction causes diminished pulsatile release of LH in rats (McClure and Saunders, 1985; Sisk and Bronson, 1986), monkeys (Dubey et al., 1986), humans (Boyar et al., 1974), ewes (Foster and Olster, 1985), heifers (Day et al., 1986; Bossis et al., 1999) and cows (Richards et al., 1989a). The major effect of undernutrition on gonadotropin secretion is probably at the hypothalamus since the anterior pituitary gland responds to GnRH in feed restricted animals. Pulsatile infusion of GnRH to underfed monkeys (Dubey et al., 1986), ewes (Kile et al., 1991) and cows (Bishop and Wettemann, 1993; Vizcarra et al., 1997) causes pulsatile LH secretion.

Concentrations of LH in anovulatory cows fed maintenance diets were 52% of the concentrations in anovulatory cows fed to gain weight, whereas FSH concentrations in maintenance fed, anovulatory cows were 76% of the concentrations in anovulatory cows fed to gain weight. Nutritional deprivation may have less influence on FSH than LH in ovariectomized cows. Removal of ovarian regulatory peptides (i.e., inhibin) by ovariectomy increases concentrations of FSH in serum of cattle (Ireland et al., 1983). Although GnRH stimulates the release of both LH and FSH from the anterior pituitary gland, nutritional restriction may differentially regulate the release of gonadotropins by altering GnRH release. Increased pulse frequency of GnRH increases pulsatile release of LH, whereas decreased GnRH pulse frequency increased FSH secretion in monkeys (Wildt et al., 1981a). Vizcarra et al. (1997) found that GnRH treatment (1 pulse/4 h) reduced pituitary content of FSH, but not LH in nutritionally anestrous cows.

Concentrations of FSH were increased in restricted heifers prior to nutritional anestrus (Rhodes et al., 1996; Bossis et al., 1999). Nutritional restriction reduces release of GnRH (Rasby et al., 1991) which may allow FSH secretion.

Nutritional restriction of ovariectomized beef cows in moderate condition for 6 d did not alter secretion of LH and FSH. Removal of 85% of the ruminal contents (d 0) and feed restriction for 3 d did not influence concentration, pulse frequency and amplitude of LH in steers (Ojeda et al., 1996). However, fasting Holstein heifers (1 kg straw) for 8 d reduced LH concentrations (McCann and Hansel, 1986). Differences in results between studies may be attributed to the animal models used and presence or absence of the gonads. Ovariectomy reduces plasma estradiol (Beck and Convey, 1977) and removes peptides that control gonadotropin release (Ireland et al., 1983; Robertson et al., 1985, 1988). Removal of the regulatory effects of the ovary dramatically increase concentrations of LH in cattle (Schallenberger and Peterson, 1982; Anderson et al., 1985) possibly obscuring the effects of nutritional deprivation.

Influence of acute feed restriction on secretion of LH and FSH depends primarily on the extent of the restriction and the species involved. Pituitary content, as well as serum concentrations of both LH and FSH were decreased in adult male rats after a 4-d fast (Bergendahl et al., 1989). However, serum concentrations of LH were not affected, but LH pulse frequency was decreased in orchidectomized rats fed 50% ad libitum diets for 7 d (Dong et al., 1993). A 24-h fast of adult male rhesus monkeys decreased LH pulse frequency (Cameron and Nosbisch, 1991), and plasma concentrations of LH and FSH were decreased, and LH pulse frequency reduced after a 48-h fast in men (Cameron et al., 1991). One day of fasting did not alter serum concentrations of LH and FSH in

women, but LH pulse frequency was decreased and pulse amplitude was increased (Loucks et al., 1994).

Nutritional status of an animal is perceived by the hypothalamo-pituitary axis through a series of metabolic signals. The exact signal(s) responsible for the regulation of gonadotropin release is unknown. Blood metabolites, such as glucose and amino acids, have been proposed to stimulate release of GnRH (Rutter et al., 1983; Cameron et al., 1985; Richards et al., 1989b). Infusion of glucose increased LH concentration and pulse frequency in lactating, anestrous beef cows treated with GnRH (Garmendia, 1986). However, glucose infusion (iv) did not alter LH secretion in cows with adequate body condition (McCaughey et al., 1988). Abomasal infusion of amino acids increased pulsatile LH secretion in ovariectomized, feed restricted lambs (Hall et al., 1992). Male monkeys fasted for 2 d had reduced LH concentrations and pulse frequency in serum; however, infusion of glucose and amino acids restored pulsatile release of LH (Schreihofer et al., 1993b).

Chronic nutritional deprivation of beef cows (20 wk of feed restriction) results in reduced body energy reserves and decreased concentrations of glucose (Richards et al., 1989b; Grimard et al., 1995). Primiparous beef cows in moderate BCS at parturition (BCS = 6) had greater concentrations of glucose during the breeding season than cows with decreased body condition (Vizcarra et al., 1998). Feed deprivation for 6 d did not influence glucose concentrations in ovariectomized cows in moderate condition in the present study.

Plasma concentrations of NEFA are increased during negative energy balance, such as during the postpartum period in dairy cows (Lucy et al., 1991), and during

chronic feed restriction in beef cows (Richards et al, 1989b). Concentrations of NEFA are positively associated with body condition in cows fed on a high plane of nutrition (Vizcarra et al., 1998), whereas, a negative relationship exists between NEFA and body condition in cows fed restricted diets (Richards et al., 1989b). Cows restricted for 6 d in the present study had greater concentrations of NEFA than maintenance cows, and gonadotropin secretion was not altered. Concentrations of NEFA were increased in cows fed restricted diets prior to the onset of anestrus (Richards et al., 1989b) and in heifers on a low plane of nutrition (Yelich et al., 1995). Realimentation of nutritionally anestrous heifers increased NEFA concentrations prior to resumption of ovulation (Bossis, 1999). Although NEFA concentrations in plasma may be an indicator of metabolic status, luteal activity can not be accurately predicted using concentrations of NEFA in postpartum cows (Vizcarra et al., 1998).

Restricted nutrient intake in ruminants uncouples the positive relationship of the GH/IGF-I axis, and concentrations of GH in serum are increased while IGF-I concentrations are reduced (Breier et al., 1986; Bossis et al., 1999). Concentrations of IGF-I were reduced after 6 d of feed restriction. Similarly, short-term fasting (48 h) of heifers decreased plasma concentrations of IGF-I (Spicer et al., 1992). Armstrong et al. (1993) found that increased concentrations of NEFA were associated with increased concentrations of GH in serum of feed restricted heifers. Some aspects of nutrition on reproduction are probably mediated through the IGF-I system (Schillo, 1992). Increased concentrations of IGF-I in plasma are associated with reduced postpartum intervals in beef cows (Rutter et al., 1989) and greater luteal progesterone secretion in dairy cows (Spicer et al., 1990). The effect of IGF-I on reproduction may be at the anterior pituitary

gland and (or) ovary. Treatment of GH-deficient mice with exogenous GH resulted in increased concentrations of IGF-I and subsequent increases in LH release (Chandrashekar and Bartke, 1993). Steroidogenesis by granulosa and thecal cells in the bovine ovary is stimulated by IGF-I (Spicer and Echternkamp, 1995).

The protein product of the obese (*ob*) gene, leptin, plays a role in regulation of energy balance in some species (Barash et al., 1996; Houseknecht et al., 1998). However, acute feed restriction did not influence concentrations of leptin in ovariectomized cows in moderate condition. Exogenous leptin decreases body weight and fat deposits in *ob/ob* mice while increasing energy expenditure (Pelleymounter et al., 1995). Synthesis and release of neuropeptide Y (NPY), the neurotransmitter responsible for feed intake, is inhibited by leptin (Wang et al., 1997). Leptin has been implicated as a potential signal to the brain for communication between nutritional status of an animal and the reproductive system. The *ob/ob* mice lack the ability to produce leptin and are sterile (Swerdloff et al., 1978). Exogenous leptin increased uterine and ovarian weight, testis weight and concentrations of gonadotropins in *ob/ob* mice, indicating that leptin may be a metabolic signal (Barash et al., 1996). Yu et al. (1997) proposed that leptin acts to inhibit NPY and increases LH secretion. However, in vitro studies indicate that leptin inhibits steroidogenesis in bovine thecal and granulosa cells (Spicer and Francisco, 1997, 1998). Although leptin may be a mediator of reproduction in rodents, its function in nutritional regulation of reproduction in ruminants has not been determined.

Implications

Chronic feed deprivation reduced concentration, pulse frequency and amplitude of LH and FSH in serum of beef cows. Acute feed restriction of cows in moderate condition does not influence the pulsatile release of LH and FSH. Acute nutritional deprivation suppresses gonadotropin secretion in rodents and primates; however, long-term nutritional deprivation is necessary to alter gonadotropins or influence reproduction in beef cows.

Table 1. Least squares means for concentration, pulse frequency and pulse amplitude of LH in ovariectomized, anovulatory cows fed to gain or maintain body weight (Exp. 1)

Criteria	Body weight		MSE
	Gain	Maintain	
Concentration, ng/mL	6.9[a]	3.6[b]	4.8
Pulse frequency, pulse/8 h	7.5[a]	5.3[b]	2.3
Pulse amplitude, ng/mL	5.9[a]	2.1[b]	3.8

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

Table 2. Least squares means for concentration, pulse frequency and pulse amplitude of FSH in ovariectomized, anovulatory cows fed to gain or maintain body weight (Exp. 1)

Criteria	Body weight		MSE
	Gain	Maintain	
Concentration, ng/mL	2.1[a]	1.6[b]	.1
Pulse frequency, pulse/8 h	6.5[a]	2.8[b]	4.0
Pulse amplitude, ng/mL	.6[a]	.3[b]	.1

[ab]Means within a row without a common superscript differ ($P < .06$).

Table 3. Least squares means for concentration, pulse frequency and pulse amplitude of LH on d 3 and d 6 in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2)

	Diets				
	d 3		d 6		
Criteria	M	R	M	R	MSE
Concentration, ng/mL	4.9	5.8	4.1	4.4	3.2
Pulse frequency, pulses/8 h	9.0	7.3	7.8	6.5	4.1
Pulse amplitude, ng/mL	3.3	3.9	2.9	2.9	2.7

Table 4. Least squares means for concentration, pulse frequency and pulse amplitude of FSH on d 3 and d 6 in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2)

	Diets				
	d 3		d 6		
Criteria	M	R	M	R	MSE
Concentration, ng/mL	2.1	2.2	2.8	3.2	.2
Pulse frequency, pulses/8 h	6.3	4.0	6.3	5.5	4.0
Pulse amplitude, ng/mL	.5	.7	1.1	1.3	.4

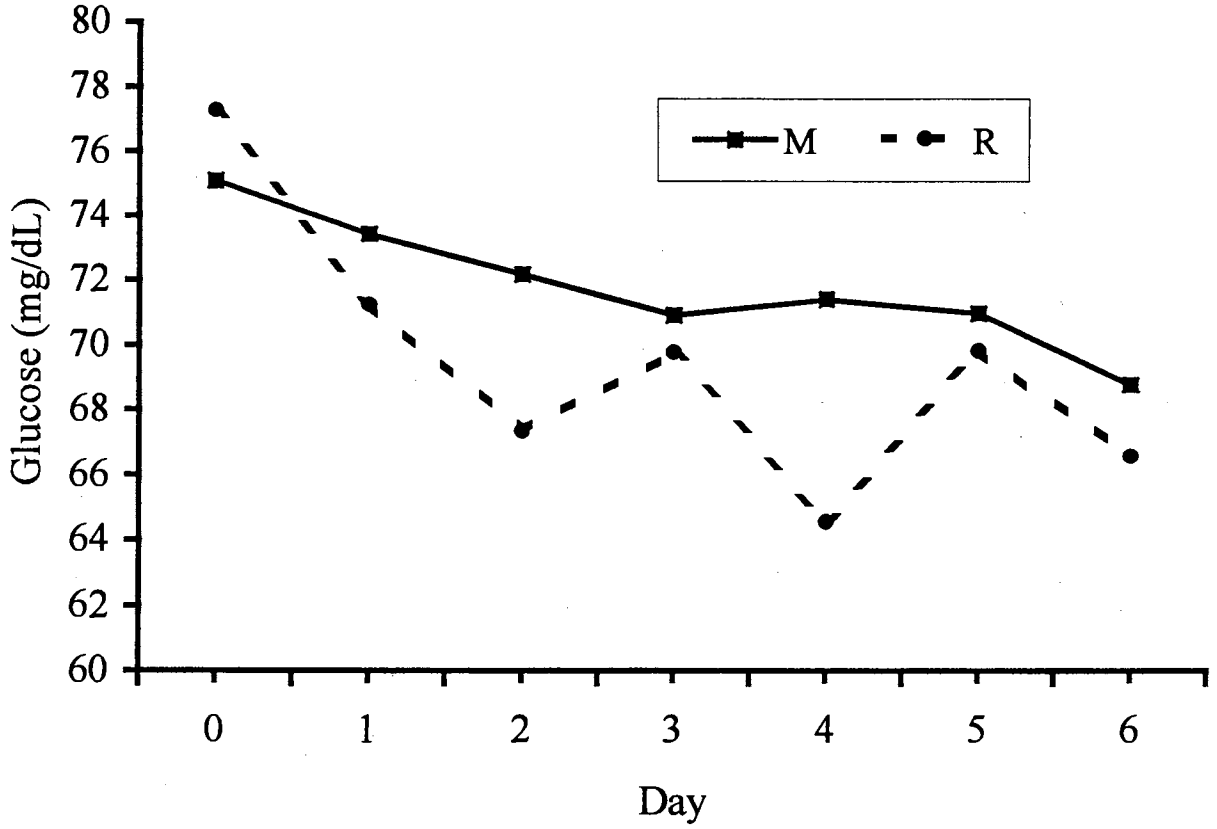

Figure 1. Least squares means for concentrations of glucose in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2) (MSE = 15.5).

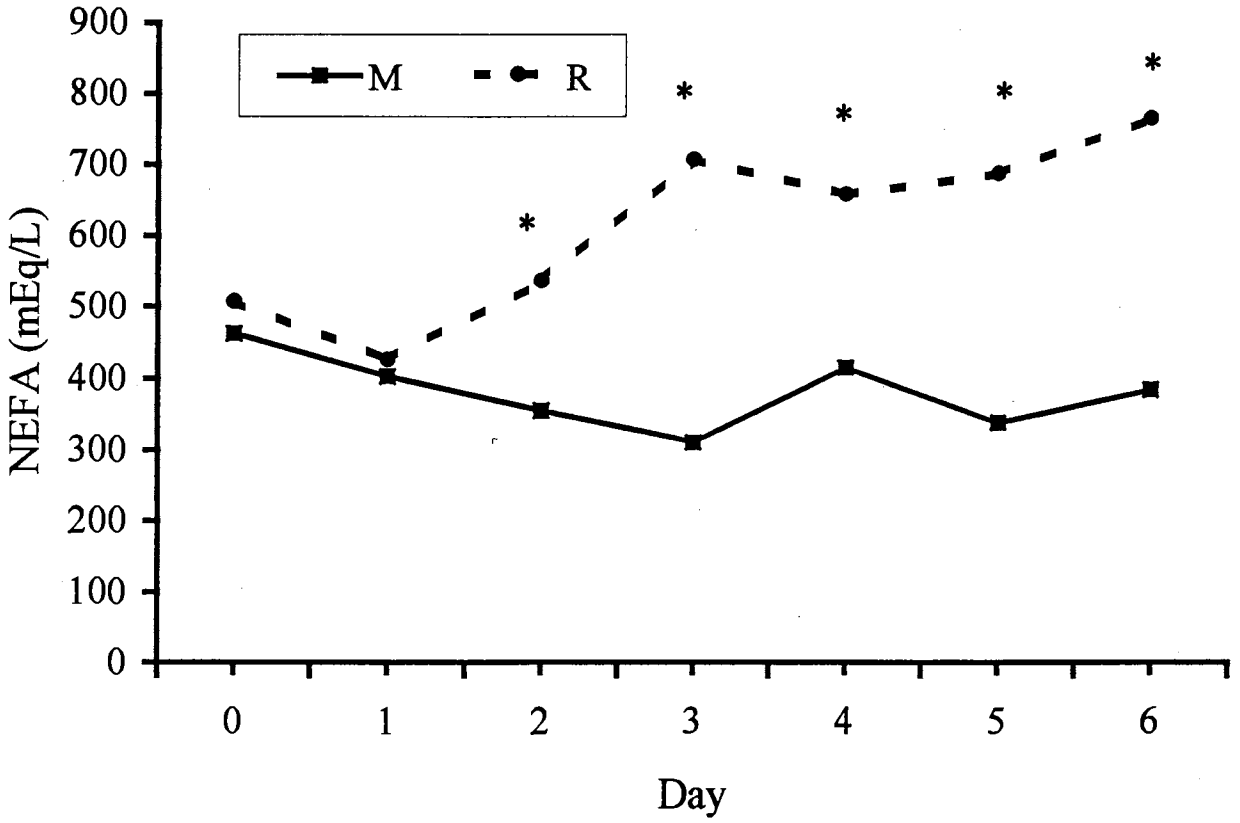

Figure 2. Least squares means for concentrations of NEFA in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2). *Treatment x day ($P < .05$; MSE = 16,764).

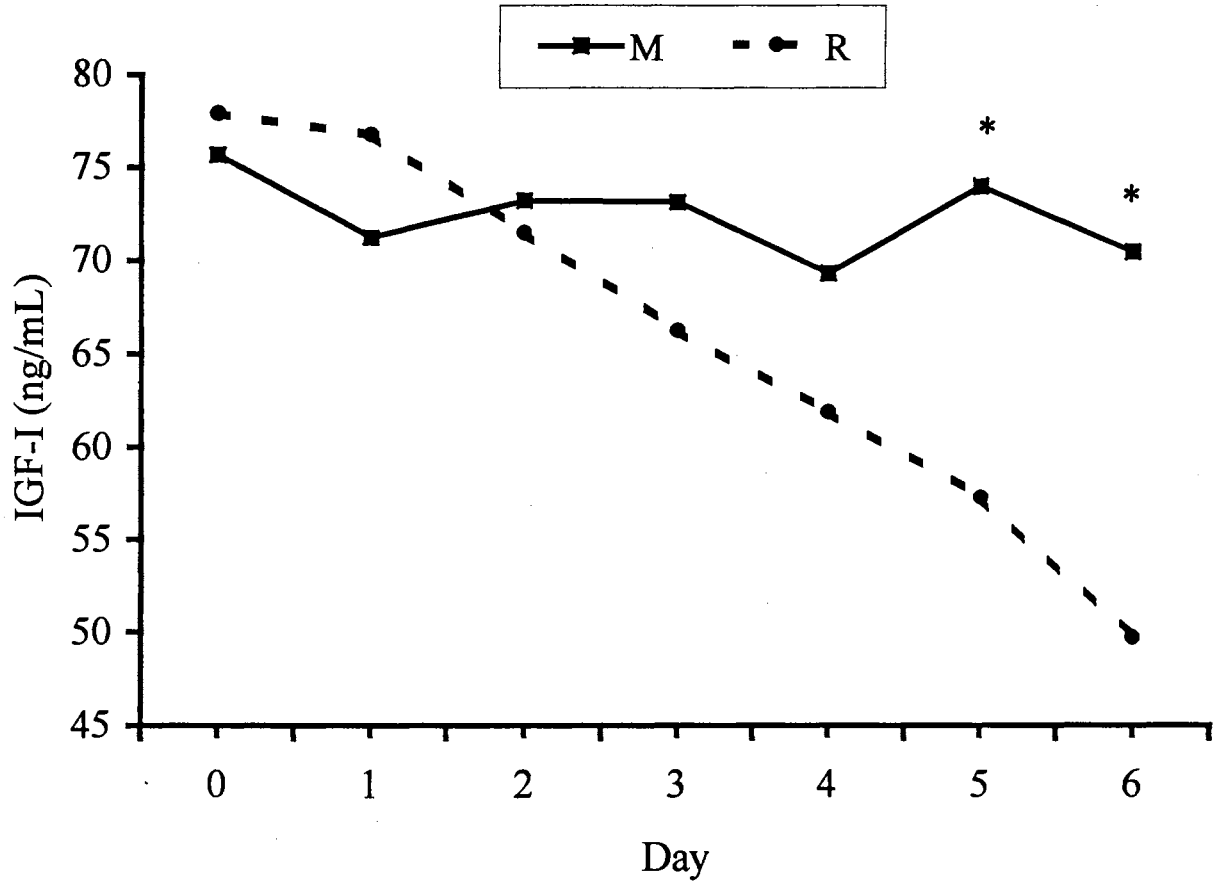

Figure 3. Least squares means for concentrations of IGF-I in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2). Treatment x day ($P < .05$; MSE = 32.8) (*$P < .05$).

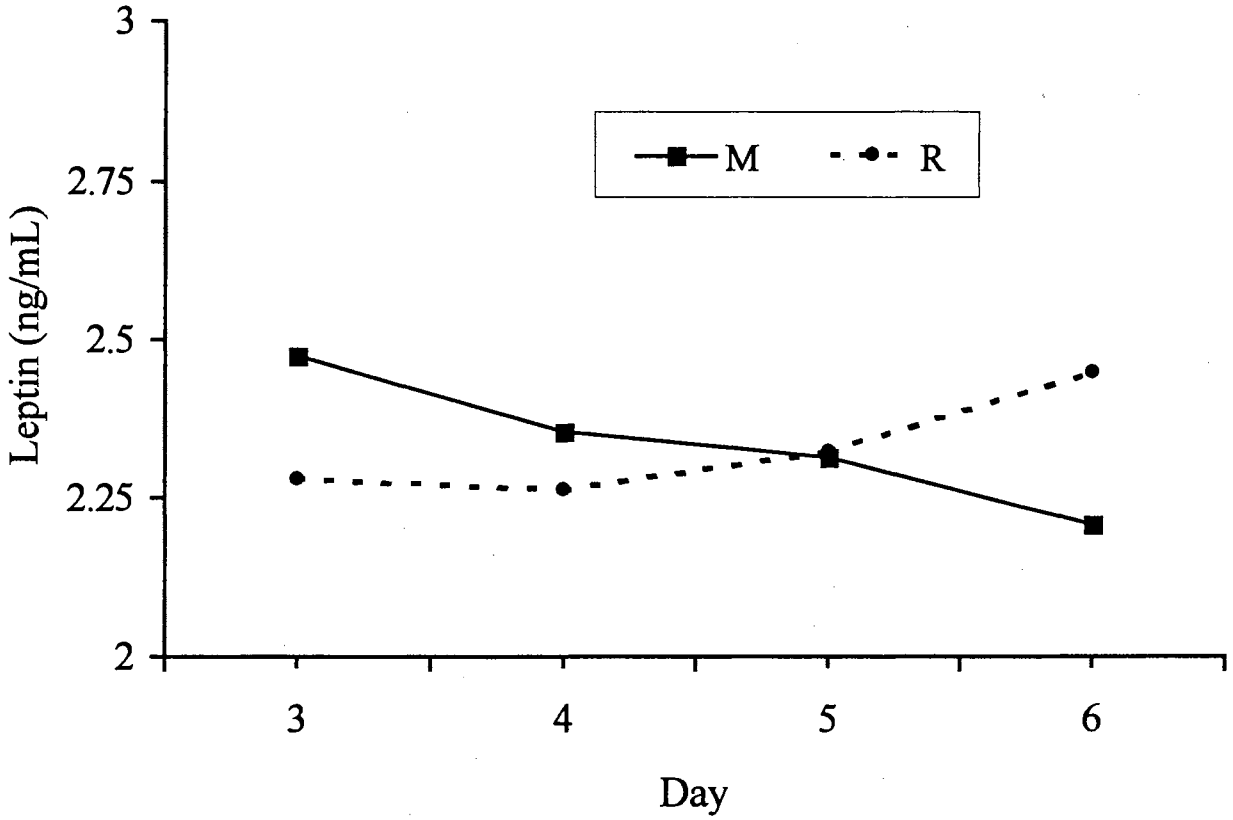

Figure 4. Least squares means for concentrations of leptin in ovariectomized cows in moderate condition fed maintenance (M) or restricted (R) diets for 6 d (Exp. 2) (MSE = .1).

CHAPTER IV

LUTEAL ACTIVITY AND ESTRUS IN POSTPARTUM BEEF COWS

Abstract: Seventy-seven multiparous beef cows (Hereford and Angus x Hereford) calving with thin to moderate body condition scores (BCS) were utilized to evaluate estrous behavior and luteal activity. Blood samples were collected twice weekly after parturition to determine the occurrence of the first postpartum luteal activity (LA, progesterone ≥ .5 ng/mL). Weight changes and BCS were determined at 2 wk intervals and cows were exposed to bulls and observed twice daily for behavioral estrus. Luteal activity was classified as normal if four consecutive samples had ≥ .5 ng/mL of progesterone or short if ≤ 3 consecutive samples had ≥ .5 ng/mL. Postpartum interval to first normal LA was shorter ($P < .0001$) for moderate condition (BCS ≥ 4.5) cows than for thin (BCS ≤ 4) cows (58.3 ± 3.2 vs 93.3 ± 5.1 d, respectively). Interval to first estrus also was shorter ($P < .0001$) for moderate cows than for thin cows (53.3 ± 3.7 vs 89.3 ± 5.6 d, respectively). Postpartum weight change and BCS at calving did not influence the incidence of estrus associated with first normal LA. After the first estrus, 72% of cows had normal LA, 16% had short LA, and 12% lacked LA. Postpartum weight change and BCS did not influence the length of LA associated with the first estrus. Cows with four consecutive samples of progesterone ≥ .5 ng/mL had increased ($P < .05$) maximum concentrations of progesterone compared with cows with only 1 or 2 sample[s] ≥ .5

ng/mL. Seventy-eight percent of cows had a transient increase in progesterone preceding the first normal LA. Subnormal or the absence of luteal activity at the first postpartum estrus may reduce reproductive efficiency of beef cows.

Key Words: Postpartum Cow, Estrus, Luteal Activity

Introduction

Resumption of luteal activity in postpartum beef cows resembles the luteal events associated with puberty in ruminants. Transient increases in progesterone in plasma occur in heifers (Gonzalez-Padilla et al., 1975; Berardinelli et al., 1979) and ewes (Berardinelli et al., 1980) prior to first estrus. Similarly, first ovulations after parturition in beef cows are usually followed by a transient increase in concentrations of progesterone for 4 to 5 d (Williams and Ray, 1980; Humphrey et al., 1983; Perry et al., 1991). A normal luteal phase (17-21 d) preceded by estrus often follows this transient increase in progesterone (Pratt et al., 1982; Perry et al., 1991).

Increased progesterone in plasma after the first spontaneous ovulation in postpartum beef cows is similar to concentrations of progesterone produced by short-lived corpora lutea formed in cows induced to ovulate by calf separation (Odde et al., 1980; Ramirez-Godinez et al., 1982) or GnRH administration (Kesler et al., 1980; Wettemann et al., 1982). The source of progesterone is probably the CL (Perry et al., 1991); however, luteinized follicles also may produce progesterone (Corah et al., 1974; Rawlings et al., 1980).

First ovulation in postpartum beef cows may or may not be accompanied by behavioral estrus (Graves et al., 1968; Wettemann et al., 1978). Abnormal follicle size is

not responsible for short-lived CL in cows with weaned calves (Braden et al., 1989) or postpartum beef cows (Perry et al., 1991). Intensity of estrus associated with early transient increases in progesterone during the postpartum period may be minimal and estrus may not be observed (Perry et al., 1991; Shipka et al., 1998).

Short-term exposure to progesterone may be necessary for initiation of normal estrous cycles (Corah et al., 1974). Treatment of postpartum cows with progestogens before or at weaning reduced the incidence of shortened or abnormal luteal phases (Ramirez-Godinez et al., 1981). Conception rate was less in postpartum primiparous cows that did not have a transient increase in progesterone before estrus than in cows that had increased progesterone (Werth et al., 1996).

Reduced nutrient intake during the prepartum period increases the interval from parturition to first estrus in beef cows (Dunn et al., 1969; Bellows and Short, 1978; Richards et al., 1986). Inadequate body energy reserves at parturition increases the days to conception (Richards et al., 1986; Selk et al., 1988). Nutritional effects on the incidence of short luteal phases in mature beef cows has not been elucidated. The purpose of this study was to evaluate the effect of body condition at calving on duration of postpartum luteal activity and occurrence of estrus in beef cows.

Materials and Methods

Seventy-seven Hereford and Angus x Hereford cows with moderate to good body condition (BCS = 5.2 ± .04; 1 = emaciated, 9 = obese, Wagner et al., 1989) were utilized. Commencing 3.5 mo prior to the initiation of the calving season (mean calving date = April 1; range = February 26 to May 17, 1996), cows were randomly assigned to one of

two prepartum diets to either maintain (M) or lose (L) BCS. All cows grazed native grass range and were fed either .68 kg/d (L diet) or 1.36 kg/d (M diet) of a 40% crude protein supplement until parturition. After parturition, all cows grazed native grass range and were fed 1.8 kg/d of a 40% crude protein supplement. Body weight changes and BCS were determined at 2 wk intervals. Postpartum weight changes were calculated by subtracting weight after parturition (within 2 wk) from postpartum weight at 40 to 60 d. Cows were assigned to 1 of 3 categories: 1) gain (+11.4 to 45.5 kg), 2) maintain (-9.1 to +9.1 kg) and 3) lose (-11.4 to 40.9 kg). Cows were exposed to bulls and observed twice daily (30 min intervals) for behavioral estrus after parturition. Bulls were fitted with chin-ball markers and All-Weather PaintStik (LA-CO Industries, Inc., Elk Grove Village, IL) was applied to the tail head of cows weekly. Cows were considered to be in estrus when they stood to be mounted by bulls or other cows, or tail paint was rubbed off between observation periods.

Commencing at 35 d post partum, blood plasma samples were collected at 3 or 4 d intervals (Monday and Thursday) by venipuncture of the tail into 15 mL tubes containing EDTA (.1 mL of 15% solution), placed on ice, and centrifuged within 2 h (2,500 x g for 15 min). Samples were stored at -20°C until progesterone concentrations were quantified to determine the occurrence of first postpartum luteal activity (LA; progesterone ≥ .5 ng/mL). Concentrations of progesterone in plasma were quantified by 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 = 22 assays) were 7 and 4%, respectively. Sensitivity of the assay was .1 ng/mL. Luteal activity was classified as normal if 4 consecutive samples had ≥ .5 ng/mL of progesterone

(LA for at least 11 d) or short if 3 or less consecutive samples had ≥ .5 ng/mL of progesterone.

The effects of BCS at parturition and postpartum weight change on occurrence and length of luteal activity and behavioral estrus were analyzed by analyses of variance. Effect of duration of luteal activity (number of samples ≥ .5 ng/mL) on maximum concentrations of progesterone was determined by analysis of variance. Treatment means were compared using the PDIFF statement of SAS (1996) when protected by a significant ($P < .05$) treatment effect. Chi-square analyses were used to determine the effect of LA prior to first postpartum estrus on the duration of subsequent luteal activity.

Results

Body condition score of cows fed to lose weight was 3.6 ± .1 at parturition, and cows fed to maintain condition had a BCS of 4.7 ± .1 ($P < .05$; Table 1). During the first 60 d post partum, 28 cows gained an average of 23.6 ± 1.6 kg, 20 cows lost 18.6 ± 1.9 kg, and 29 cows maintained body weight (gained 1.0 ± 1.5 kg; $P < .0001$; Table 2). Interval from parturition to normal LA and to first estrus was shorter ($P < .0001$) for moderate condition cows than for thin cows (Table 1). The interval from parturition to conception was 24 d less for moderate condition cows than for thin cows ($P < .05$; Table 1). Interval from first normal LA to conception was similar ($P > .1$) for moderate condition cows (13.3 d) and thin cows (10.3 d).

Body condition at parturition and postpartum weight change did not influence the duration of luteal activity associated with first estrus. Based on our bleeding schedule (Monday and Thursday), cows could have concentrations of progesterone ≥ .5 ng/mL for

the following days: one sample = 1 to 6 d, two samples = 5 to 9 d, three samples = 8 to 13 d and four samples = 11 to 17 d. Seventy-two percent of cows had a transient (progesterone ≥ .5 ng/mL in one sample) increase in progesterone preceding the first normal luteal activity. Six percent of cows had ≥ .5 ng/mL of progesterone for two samples (Table 3). A typical transient increase in progesterone is depicted in Figure 1.

Seventy-four percent (57/77) of cows exhibited estrus during the experiment and 26% did not express or were not detected in estrus. Body condition score at parturition and postpartum weight change did not influence the incidence of estrus associated with first normal luteal activity. After the first estrus, 72% (41/57) of cows had normal luteal activity (concentrations of progesterone ≥ .5 ng/mL in 4 consecutive samples), 16% (9/57) had short luteal activity (1 to 3 samples ≥ .5 ng/mL of progesterone) and 12% lacked luteal activity (Table 3).

Duration of luteal activity influenced ($P < .05$) maximum concentrations of progesterone. Maximum concentrations of progesterone were 6.2 ± .3, 5.2 ± 1.4, 2.3 ± 1.2 and 1.2 ± 1.0 ng/mL for cows with 4, 3, 2 and 1 consecutive sample[s] with progesterone ≥ .5 ng/mL, respectively (Figure 2).

Increased concentrations of progesterone before first estrus influenced ($P < .05$) subsequent luteal function. Eighty-one percent of cows with a transient increase in progesterone ≥ .5 ng/mL in 1 or 2 samples prior to first behavioral estrus exhibited normal luteal function after estrus (Table 4). Thirty-six percent of cows lacking a transient increase in progesterone prior to first estrus had subsequent normal luteal function (Table 4).

Discussion

Body condition of cows at parturition influences the interval from parturition to estrus and ovulation. Cows in moderate body condition (BCS ≥ 4.5) had a shorter interval from parturition to first estrus and luteal activity than cows in thin body condition (BCS ≤ 4). Cows with BCS of ≤ 4 at parturition had increased postpartum intervals to first estrus and conception than did cows calving with BCS ≥ 5 (Richards et al., 1986). A greater percentage of primiparous beef cows calving with BCS ≥ 5 exhibited estrus by the end of a 60 d breeding season than did cows calving with BCS of 4 (Spitzer et al., 1995).

Interval from parturition to conception was decreased by 24 d in cows calving in moderate condition compared with thin cows. Selk et al. (1988) found that BCS at parturition was the single most important factor affecting subsequent reproductive performance in beef cows. More cows in moderate-good body condition at parturition became pregnant compared with cows in thin condition (Selk et al., 1988). Pregnancy rates for primiparous beef cows were 56, 80 and 96% for cows calving with BCS of 4, 5 and 6, respectively (Spitzer et al., 1995).

Body condition at parturition and postpartum weight change had no affect on the duration of the first postpartum luteal activity or the incidence of normal luteal activity after the first estrus. Luteal phases of reduced length occur in heifers prior to puberty (Berardinelli et al., 1979) and during resumption of luteal activity in postpartum cows (Humphrey et al., 1983; Perry et al., 1991). Seventy-eight percent of cows had short luteal activity prior to normal luteal function. Perry et al. (1991) found that all beef cows studied (n = 13) had short luteal activity at the first postpartum ovulation, and only 17% of cows exhibited behavioral estrus associated with the first postpartum ovulation.

Concentrations of progesterone were increased in 69% of crossbred primiparous heifers prior to first estrus (Werth et al., 1996).

Source of the first increase in progesterone in plasma at puberty or postpartum is probably of ovarian origin. Increased concentrations of progesterone in heifers prior to puberty is associated with luteal tissue found below the surface of the ovary (Berardinelli et al., 1979). Daily ultrasonography of postpartum cows indicated formation of a short-lived CL associated with a transient increase in progesterone (Perry et al., 1991). Lutenization of follicles may account for increases in progesterone since ovarian palpation did not reveal CL formation (Corah et al., 1974; Rawlings et al., 1980).

First normal luteal activity was preceded by estrus in 72% of the cows. Forty-two percent of nonsuckled (weaned) beef cows observed twice daily for estrus ovulated without exhibiting estrus, while 70% of suckled beef cows did not exhibit behavioral estrus associated with the first postpartum ovulation (Graves et al., 1968). Ultrasonography and estrous detection thrice daily revealed 10 of 12 suckled beef cows ovulated without exhibiting behavioral estrus after parturition (Perry et al., 1991). However, twice daily observation revealed behavioral estrus was not expressed in 8% of postpartum beef cows that had increased concentrations of progesterone (> 1 ng/mL) in serum for 1 wk followed by a decrease in progesterone (Werth et al., 1996). Concentrations of estradiol in serum prior to ovulation and maximum diameter of the ovulatory follicle was similar at the first (short luteal phase) and second ovulation (normal luteal phase) in suckled beef cows (Perry et al., 1991). Weaning of calves increases the incidence of short-lived CL (Odde et al., 1980). Follicle size was not different between beef cows with weaned calves and in normally cycling cows (Braden et

al., 1989). Cows with weaned calves had reduced concentrations of LH and FSH receptors in follicles than did normally cycling cows.

Behavioral estrus was not detected in 26% of postpartum cows observed twice daily. Intensity of estrous behavior may be reduced in postpartum beef cows. Perry et al. (1991) found that estrous activity was less intense or was absent in postpartum beef cows observed thrice daily. Ninety-five percent of dairy cows ovulated without behavioral estrus when visually observed twice daily; however, continuous estrus detection with a radiotelemetry system determined that only 42% of the cows lack estrous activity before the first postpartum ovulation (Shipka et al., 1998). Number of mounts associated with the first postpartum estrus in dairy cows was reduced compared with the subsequent estrus (1.1 vs 4 mounts, respectively; Shipka et al., 1998). Similarly, the number of mounts at the first postpartum estrus in primiparous beef cows was reduced compared with the second estrus (R. P. Wettemann, unpublished data).

Duration of luteal activity influenced maximum concentrations of progesterone in plasma of postpartum beef cows. Maximum concentrations of progesterone in cows with short luteal activity were 30% of maximum concentrations in cows with normal luteal activity. Similarly, concentrations of progesterone associated with a transient increase in progesterone were 20 to 40% of concentrations of progesterone during normal luteal activity in postpartum, suckled beef cows (Rawlings et al., 1980). Perry et al. (1991) found maximum concentrations of progesterone averaged less than 1 ng/mL after the first ovulation in postpartum cows. Transient increases in progesterone averaged 2.3 ng/mL prior to first estrus in primiparous beef cows (Werth et al., 1996). Decreased maximum concentrations of progesterone associated with short luteal activity may be due to reduced

CL size. Postpartum cows induced to ovulate with GnRH had smaller CL compared with GnRH-treated cows pretreated with progesterone (Rutter et al., 1985). Diameters of the first (short luteal phase) and second (normal luteal phase) CL in postpartum beef cows were similar through d 4 after ovulation (Perry et al., 1991). However, diameters of CL were larger from d 5 to 8 after the second ovulation.

Eighty-one percent of cows with a transient increase in progesterone before first estrus had subsequent normal luteal function, and only 36% of cows lacking a transient increase in progesterone had normal luteal function after estrus. Transient increases in progesterone in postpartum cows may be necessary for resumption of normal luteal activity. More primiparous cows with increased concentrations of progesterone prior to first estrus conceived compared with heifers lacking increased progesterone (Corah et al., 1974). Conception rate was greater in primiparous beef cows with a transient increase in progesterone than in cows lacking a transient increase in progesterone (76 vs 41%, respectively; Werth et al., 1996). Induction of ovulation in postpartum cows with GnRH (Kesler et al., 1980; Pratt et al., 1982) or calf separation (Odde et al., 1980; Ramirez-Godinez et al., 1982) causes abnormal luteal function which may be eliminated by progesterone treatment prior to GnRH or calf separation (Ramirez-Godinez et al., 1981).

Increased progesterone in plasma before normal luteal activity in postpartum beef cows may stimulate the hypophyseal-hypothalamo-ovarian axis. Gonadotropin stores in the pituitary gland are diminished at parturition due to prolonged exposure to increased concentrations of steroids during gestation (Chamley et al., 1976; Moss et al., 1980). Heifers exposed to supraphysiological doses of estradiol become refractory to subsequent estradiol treatment and fail to exhibit behavioral estrus (Carrick and Shelton, 1969).

More ovariectomized heifers treated with estradiol exhibited estrus when pretreated with progesterone for 5 d than nontreated heifers, indicating progesterone may resensitize the brain (Carrick and Shelton, 1969). Increased concentrations of progesterone may allow pituitary content of LH to increase. Increased secretion of LH occurs after progesterone treatment of anestrous cows (Garcia-Winder et al., 1987) and heifers (Anderson et al., 1996). Progesterone treatment also increases the number of progesterone receptors in the uterus (Zollers et al., 1993). Increased progesterone receptors in the uterus may control the timing of $PGF_{2\alpha}$ release and allow normal luteal activity in the subsequent estrous cycle (Cooper et al., 1991).

Implications

Body condition at parturition influences the intervals from parturition to first estrus, luteal activity, and conception. Duration of first postpartum luteal activity and the occurrence of estrous behavior associated with first normal luteal activity were not influenced by body condition score at parturition and postpartum weight change. Minimal increases in plasma concentrations of progesterone for one to nine days before estrus may be necessary for subsequent normal luteal function in postpartum beef cows.

Table 1. Influence of body condition score (BCS) at parturition on intervals to first estrus, normal luteal activity (LA) and conception in postpartum beef cows

Criteria	Body condition at parturition	
	Moderate	Thin
Cow no.	39	38
BCS at parturition	$4.7 \pm .1^{a}$ (39)	$3.6 \pm .1^{b}$ (38)
Parturition to first estrus, d	53 ± 4^{a} (30)	89 ± 6^{b} (32)
Parturition to LA[c], d	58 ± 3^{a} (39)	93 ± 5^{b} (38)
Parturition to conception, d	74 ± 5^{a} (30)	98 ± 6^{b} (33)

[ab]Means within a row without a common superscript differ ($P < .05$).
[c]Progesterone ≥ .5 ng/mL for at least 11 d.
( ) Number of cows.

Table 2. Least squares means for weight change during the first 60 days post partum of beef cows fed to gain (+ 11.4 kg), maintain (± 9.1 kg) or lose (- 11.4 kg) body condition

Criteria	n	Weight change (kg)
Gain	28	$+ 23.6 \pm 1.6^{a}$
Maintain	29	$+ 1.0 \pm 1.5^{b}$
Lose	20	$- 18.6 \pm 1.9^{c}$

[abc]Means without a common superscript differ ($P < .0001$).

Table 3. Classification of first luteal activity (LA) after parturition and after first estrus in postpartum beef cows

	Samples of progesterone ≥ .5 ng/mL				
Criteria	0	1	2	3	4
First LA[a], %	--	72	6	0	22
LA after first estrus[b], %	12	7	5	4	72

[a]77 cows had luteal activity.
[b]57 of 77 cows were detected in estrus.

Table 4. Influence of increased progesterone (P_4) prior to first estrus on subsequent normal luteal function in postpartum beef cows

P_4 increase in 1 or 2 samples prior to estrus	n	Normal luteal function (%)
Yes	46	81[a]
No	11	36[b]

[ab]Percentages differ ($P < .0001$).

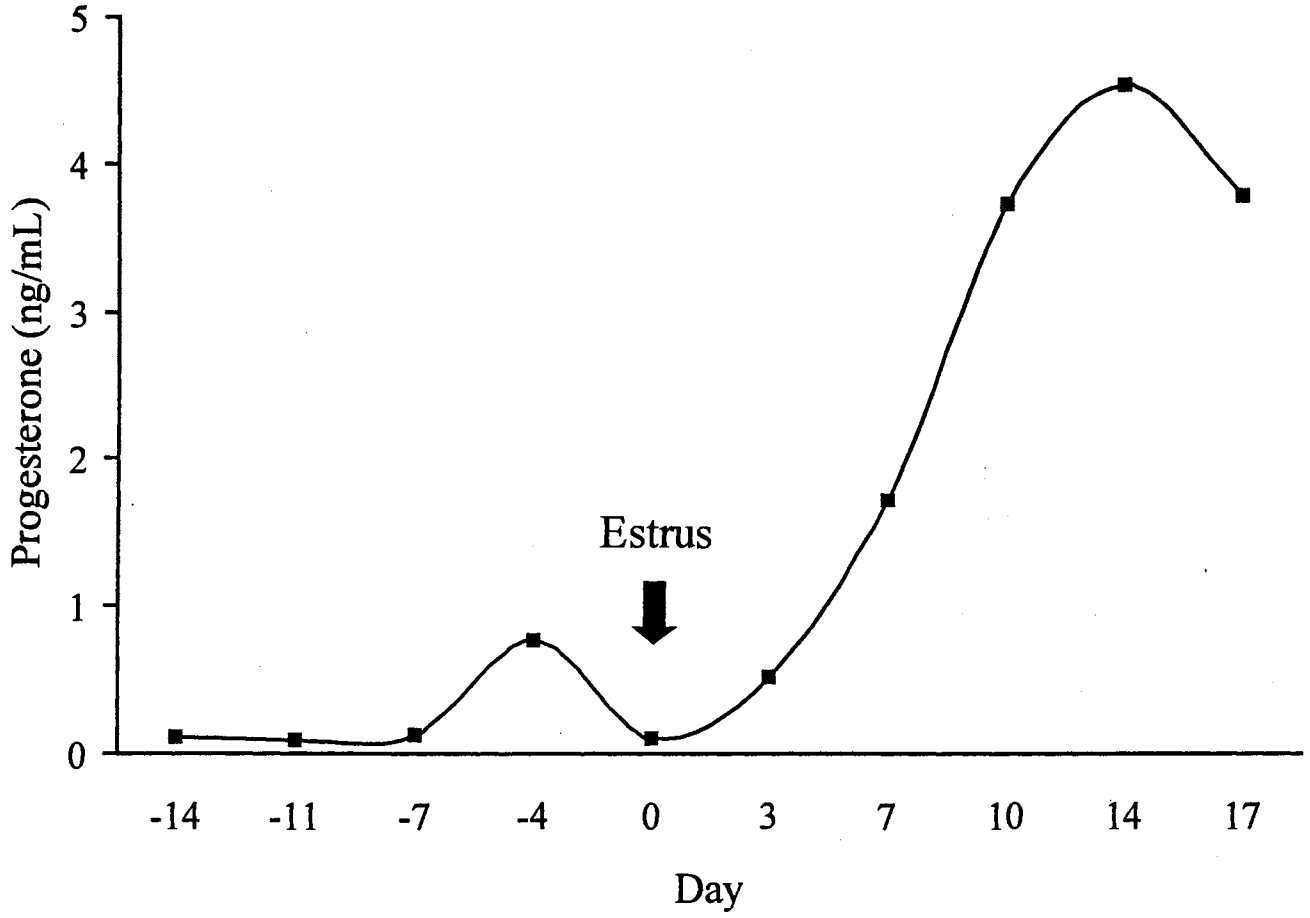

Figure 1. Progesterone concentrations in plasma typical of postpartum beef cows.

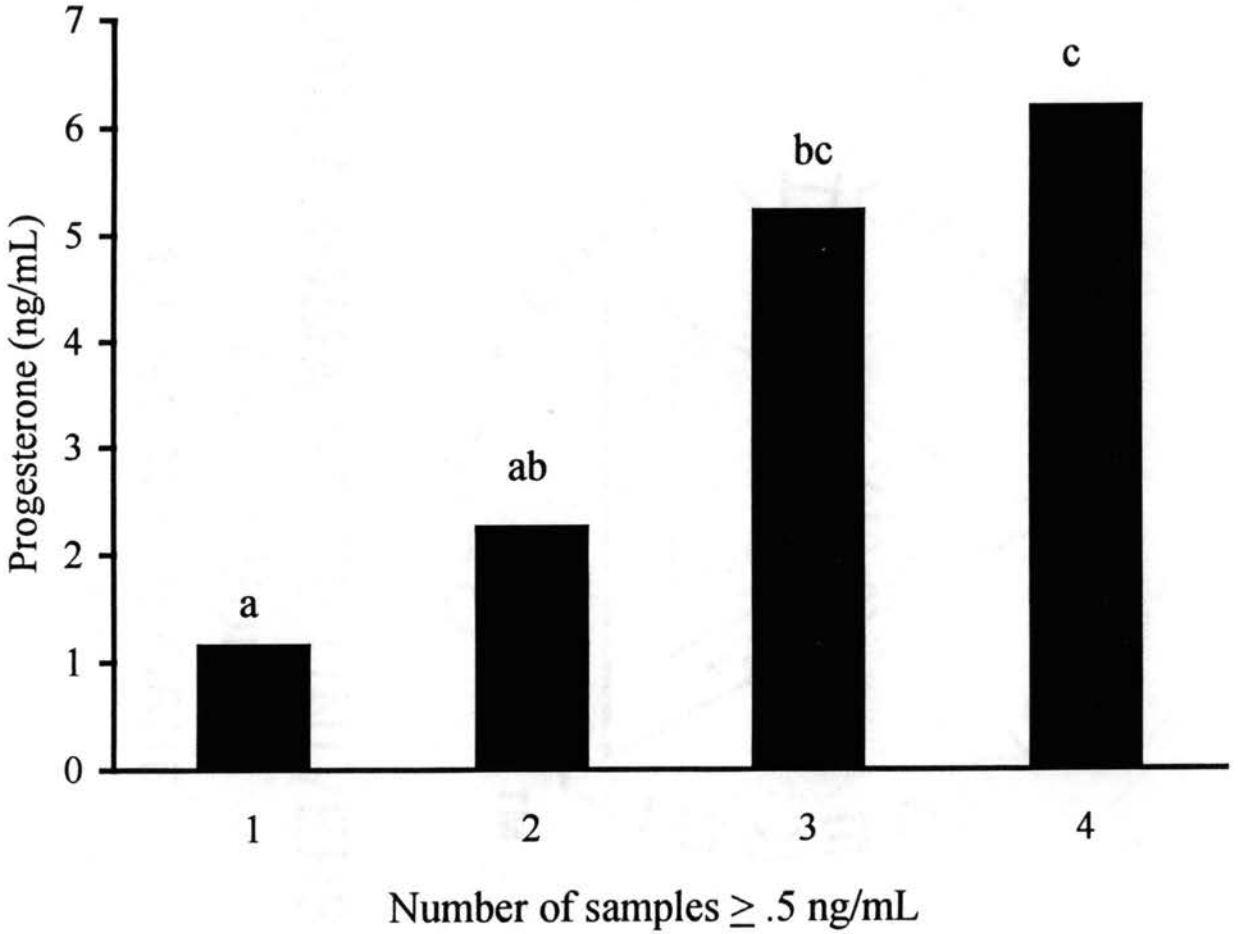

Figure 2. Least squares means for maximum concentrations of progesterone in postpartum beef cows with different durations of luteal activity. Duration effect ($P < .0001$; MSE = 4.2).

CHAPTER V

INFLUENCE OF NUTRITION AND STEROIDS ON PITUITARY GLAND GNRH RECEPTORS, GONADOTROPINS, AND GENE EXPRESSION FOR GNRH RECEPTORS AND GONADOTROPIN SUBUNITS IN BEEF COWS

Abstract: Nutritionally induced anovulatory cows (n = 28) and proestrous cows (n = 5) were used to determine the effect of steroids and reproductive state on regulation of synthesis and secretion of gonadotropins. Anovulatory cows were ovariectomized and received intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or a sham intravaginal device (C) for 6 d. Concentrations of LH and FSH were quantified in serum samples collected every 10 min for 4 h prior to treatment (d -1) and every 10 min for 8 h on d 6. Concentrations of E_2 and P_4 were quantified in daily plasma samples. Cows were exsanguinated within 2 h after removal of intravaginal devices and pituitary glands collected and stored at -80° C. Total RNA was isolated from one half of each pituitary gland, and mRNA for GnRH receptors (GnRH-R) and gonadotropin subunits were quantified. Pituitary content of GnRH-R (only anovulatory cows), LH and FSH were determined in the remaining half of each pituitary. Plasma concentrations of E_2 were greater ($P < .05$) in E_2-treated cows (1.6 pg/mL) than in nontreated cows (.6 pg/mL). Concentrations of P_4 were greater ($P < .05$) in cows treated with P_4 (3.1 ng/mL) than in nontreated cows (.3 ng/mL). Serum concentrations of LH and FSH were not

influenced by steroid treatments. Treatment of nutritionally induced anovulatory cows with E_2 increased frequency and amplitude of LH pulses in serum, decreased pituitary concentrations of FSH, and increased the number of GnRH-R in the pituitary gland. Treatment with P_4 increased amplitude of LH pulses in serum and gene expression for LHβ in anovulatory cows. Frequency of LH pulses was greater ($P < .05$) in E_2 than in non E_2-treated cows, and amplitude of LH pulses was greater ($P < .05$) in E_2- and P_4-treated than in nontreated cows. Concentrations of LH in the pituitary gland were not affected by treatments; however, pituitary concentrations of FSH were less ($P < .1$) in E_2 cows than in nontreated cows. Number of GnRH-R was increased ($P < .05$) in cows treated with E_2 but P_4 treatment did not influence the number of GnRH-R. Gene expression for GnRH-R, common α-subunit and FSHβ were not affected by treatments, while treatment with P_4 increased ($P < .05$) gene expression for LHβ. Pituitary concentrations of LH were greater ($P < .05$) and concentrations of FSH were less ($P < .05$) in proestrous cows than in ovariectomized, anovulatory cows treated with or without steroids. Gene expression for GnRH-R, common α-subunit, LHβ and FSHβ were similar for proestrous and anovulatory cows. We conclude that estradiol increases frequency of LH pulses in serum and GnRH-R in the pituitary gland, and treatment with progesterone increases synthesis of LHβ mRNA in the pituitary gland of ovariectomized, anovulatory cows.

Key Words: Beef Cow, Steroids, Gonadotropins

Introduction

Steroids regulate the synthesis and release of gonadotropins via positive and negative feedback on the hypothalamus and anterior pituitary gland. Increased

concentrations of estradiol and decreased progesterone are associated with the preovulatory LH surge (Wettemann et al., 1972; Chenault et al., 1975). Increased concentrations of estradiol in plasma during the follicular phase cause LH to be released in frequent pulses of minimal amplitude (Rahe et al., 1980). Frequency of LH pulses is minimal with maximum amplitude during the midluteal phase of the estrous cycle.

Estradiol has a biphasic effect on LH release. Serum concentrations of LH decrease initially after estradiol treatment followed by a preovulatory-like LH surge in steers and ovariectomized cows (Kesner et al., 1981; Schoenemann et al., 1985). Estradiol increases the sensitivity of the anterior pituitary gland to GnRH by increasing the number of GnRH-R in cows (Schoenemann et al., 1985). The number of GnRH-R in the pituitary gland of ovariectomized ewes was not altered by progesterone (Moss et al., 1981).

Removal of steroids by gonadectomy increases gene expression of gonadotropin subunits (common α-subunit, LHβ and FSHβ) in rats (Papavasiliou et al., 1986), sheep (Landefeld et al., 1984) and heifers (Roberson et al., 1992) compared with intact animals. The stimulatory effects of gonadectomy on mRNA for the gonadotropin subunits is inhibited by estrogen treatment (Gharib et al., 1987; Herring et al., 1991).

Reduced feed intake decreases concentration and pulse frequency of LH in rats (McClure and Saunders, 1985; Sisk and Bronson, 1986), monkeys (Dubey et al., 1986), ewes (Foster and Olster, 1985; Foster et al., 1989) and cattle (Day et al., 1986; Richards et al., 1989a; Bossis et al., 1999). Effects of nutrition on gonadotropin synthesis in cattle has not been elucidated. Severe undernutrition increases (Beckett et al., 1997a),

decreases (Landefeld et al., 1989; Kile et al., 1991) or does not influence (Thomas et al., 1990) mRNA for gonadotropin specific subunits in castrated sheep.

Effects of exogenous steroids on gene expression for gonadotropin synthesis in cows has not been established. The objectives of this experiment were: 1) to determine the effect of estradiol and progesterone on serum and pituitary concentrations of LH and FSH, concentrations of GnRH-R, gene expression for GnRH-R and gonadotropin subunits in pituitary glands of ovariectomized, nutritionally induced anovulatory beef cows, and 2) to determine the effect of nutritional deprivation on concentrations of LH and FSH, and gene expression for GnRH-R and gonadotropin subunits in pituitary glands of beef cows.

Materials and Methods

Animal Model and Experimental Design. Twenty-eight multiparous, nonlactating, Angus x Hereford beef cows (BW = 435 ± 10 kg) were fed a restricted diet of 2.7 kg of prairie hay (CP = 5.8%) and 35 g of a mineral mix (46.1% salt, 50.0% dicalcium phosphate, .4% copper sulfate, .5% zinc oxide and 3.0% mineral oil) daily to cause loss of 1% of the body weight per week until they became anovulatory. Body weight and body condition scores (BCS) were evaluated every 2 wk. Blood samples were collected weekly via tail venipuncture in 10 mL tubes containing EDTA (.1 mL of a 15% solution), placed on ice, and centrifuged (2,500 x g for 15 min) within 2 h. Plasma was decanted and stored at -20°C until progesterone was quantified. Cows were considered anovulatory when three consecutive plasma samples contained less than 1 ng/mL of progesterone.

Cows were ovariectomized and randomly assigned to treatment groups within 3 wk after the onset of anovulation. One week prior to the initiation of treatment, cows were confined in individual stalls in a barn at 21 ± 4°C and 14 h of light. A polyvinyl jugular cannula (id, 1.68 mm; od, 2.39 mm; BB 317 v11, Bolab, Lake Havasu City, AZ) was inserted two days prior to treatment, to allow collection of frequent blood samples.

On d 0, cows were assigned to receive one of four treatments (n = 7/treatment): 1) an intravaginal device containing progesterone (P_4; EAZI-BREED CIDR, InterAg, Hamilton, New Zealand; 1.9 g progesterone) designed to produce concentrations of progesterone similar to those during the early luteal phase of estrous cycles, 2) a modified intravaginal device containing 17β-estradiol (E_2; Sigma Chemical, St. Louis, MO) in a 60 mm silastic tube (id, 3.35 mm; od, 4.65 mm; Dow Corning Co., Midland, MI) attached to the CIDR device through a perforation, designed to produce plasma concentrations of estradiol similar to the early luteal phase of the estrous cycle, 3) an intravaginal device containing E_2 and P_4 (E_2P_4), or 4) a sham intravaginal device (C).

On d 0 at 0800 h, treatments were initiated and continued through 0600 h on Day 7. Blood samples were collected at 10 min intervals for 4 h one day prior (d -1) to treatments and at 10 min intervals for 8 h on d 6 of steroid treatment. Samples were allowed to clot for 24 h at 4°C and then centrifuged at 2,500 x g for 20 min. Serum was decanted and stored at -20°C until concentrations of LH and FSH were quantified. Blood samples were collected from d -1 to d 6 into 10 mL tubes containing EDTA and plasma was decanted and stored at -20°C until progesterone and estradiol were determined.

Five Hereford x Angus cows with moderate body condition (BCS = 5) were used to compare gonadotropin synthesis in cows that lost body weight and became anovulatory

with cows with normal estrous cycles. Two days before exsanguination, cows were treated with $PGF_{2\alpha}$ (Lutalyse, 25 mg; The Upjohn Co., Kalamazoo, MI) once between days 8 and 12 of the estrous cycle to induce luteolysis. Ovaries were examined at exsanguination to assess CL regression.

Pituitary gland collection. On d 7 at 0700 h, anovulatory cows were exsanguinated within 2 h after removal of intravaginal devices. Cows with normal estrous cycles were exsanguinated 48 h after $PGF_{2\alpha}$ treatment. Pituitary glands were removed, placed on ice, trimmed, midsagitally sectioned and the posterior lobe was discarded. Anterior pituitary glands were weighed and frozen in liquid nitrogen (-72°C) within 40 min after exsanguination.

Hormone and receptor assays. Serum concentrations of LH were quantified by RIA (Bishop and Wettemann, 1993) using NIH LH-B9 as the standard. Intra- and interassay coefficients of variation were 19 and 25%, respectively (n = 6 assays). Concentrations of FSH were determined by RIA with USDA-bFSH-I-2 as the standard (Vizcarra et al., 1997). Intra- and interassay coefficients of variation were 12 and 22%, respectively (n = 6 assays).

Concentrations of LH and FSH in pituitary tissue were quantified by RIA. One-half of each pituitary gland was thawed, homogenized in buffer (10 mM Tris, 1mM $CaCl_2$, 0.25 M sucrose, pH 7.0) with three, 5 sec bursts in a Tissue Tearor (Biospec, Bartlesville, OK). The crude homogenate was then homogenized in a ground glass homogenizer (Kontez Tenbroeck Tissue Grinder, Fisher Scientific, Pittsburgh, PA) and

then rehomogenized in a dounce glass homogenizer (Kontez 7 mL Dounce Tissue Grinder, Fisher Scientific, Pittsburgh, PA). Tissue and buffer was maintained at 4°C. The homogenate was centrifuged at 16,000 x g for 15 min (4°C), and the supernatant was frozen and stored at -80°C until concentrations of LH and FSH were quantified. The remaining homogenate was resuspended in assay buffer [10 mM Tris, 1 mM $CaCl_2$, 0.3% (wt/vol) bovine serum albumin, pH 7.0] to determine concentrations of GnRH-R. Receptors for GnRH were quantified as described by Nett et al. (1987) with modifications (Vizcarra et al., 1997). Receptor assay results are expressed in femtomoles per mg protein.

Concentrations of progesterone in plasma were determined by solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997). Intraassay coefficient of variation was 4% (n = 1 assay). Concentrations of estradiol-17β were quantified by RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intraassay coefficient of variation was 9% (n = 1 assay).

Analyses of mRNA. Total RNA was isolated from pituitary tissue (.5 g) by homogenization in 5 mL of TRIZOL® reagent (Life Technologies, Inc., Gaithersburg, MD) in polypropylene tubes (Corning # 25319-15, Corning, NY) on ice with a VirTishear® homogenizer (Gardiner, NY). Homogenates were incubated at room temperature for 5 min. Chloroform (Molecular Biology Grade; Fisher Scientific BP 1145-1, Fair Lawn, NY) was added (1 mL) to each sample and vortexed for 15 sec. After centrifugation (5,000 x g for 30 min at 4°C), the aqueous phase was transferred to a new

polypropylene tube and the RNA was precipitated with 2.5 mL of isopropyl alcohol (99% purity; Amresco®, Solon, Ohio). Samples of RNA were incubated in polypropylene tubes at room temperature for 10 min and centrifuged at 5,000 x g for 25 min at 4°C. The supernatant was removed and the RNA pellet was washed with 5 mL of 75% ethanol and centrifuged (5,000 x g for 10 min at 4°C). The RNA pellet was dissolved in 200 μL of Tris buffer (10 mM Tris-Cl, 1 mM ETDA, pH 7.4) and quantified by spectrophotometry at absorbances of 260 and 280 nm. Purity of RNA was determined from calculations of 260/280 ratios. The extraction procedure yielded 260/280 ratios of 1.6-1.7. Total RNA (μg) was calculated by the following equation: A_{260} x 200 x refractory index (40) x 200 μL (Layne, 1957).

The integrity of RNA was verified by Northern blot analyses. Total RNA from one control and one steroid-treated (E_2P_4) cow were separated on an agarose/formaldehyde gel and transferred to a nylon membrane (ICN, Biotrans™ Nylon Membranes, Irvine, CA). Membranes were hybridized to radiolabled ovine GnRH receptor (GnRH-R; Turzillo et al., 1994), bovine common α-subunit (Erwin et al., 1983), bovine LHβ (Maurer, 1985) and bovine FSHβ (Kim et al., 1988) complementary DNA (cDNA). Each cDNA probe was radiolabled with ^{32}P using the random hexamer priming method (Prime-a-Gene® System, Promega Corp., Madison, WI). Three GnRH-R mRNA transcripts were evident on Northern blots at 5.2, 3.5 and 2.0 kilobases (kb). These findings are in good agreement with Vizcarra et al. (1997). Single transcripts were observed for common α-subunit, LHβ and FSHβ mRNA.

Steady state concentrations of pituitary mRNA were determined by dot blot analyses. Thirty-five and 17.5 μg of total RNA from each sample was applied in

duplicate to nylon membranes (ICN, Biotrans™ Nylon Membranes, Irvine, CA). Membranes were baked at 80°C for 2 h to cross-link RNA to the membrane. Dot blot membranes were hybridized at 42°C for 16-18 h to cDNAs encoding ovine GnRH-R, bovine α-subunit, bovine LHβ- and FSHβ-subunits. Hybridization buffer consisted of 0.5M Na_2PO_4, 7% SDS, 1% BSA, 1mM EDTA and 0.1 mg/mL denatured salmon sperm DNA (Church and Gilbert, 1984). Membranes were washed in 0.5 X SSC (standard sodium citrate) at 65°C for α-subunit, 0.5 X SSC at 42°C for FSHβ subunit, and 1 X SSC at 42°C for LHβ subunit and GnRH-R. To adjust for differences in RNA loading among samples, membranes were stripped of GnRH-R and subunit probes by washing in 0.2 M NaOH at room temperature for 2 h and reprobed with radiolabled 18s ribosomal DNA (pTRI RNA 18S, Ambion, Inc., Austin, TX). Membranes were hybridized with the 18s ribosomal DNA probe at 42°C for 16-18 h and washed with 0.1 X SSC at 65°C. Anaylsis of variance revealed similar ($P > .1$) amounts of radiolabled 18s hybridized to membranes indicating no differences in RNA loading. Membranes were exposed to film (Kodak Co., Inc.) for .5-5 days at -80°C and developed. Autoradiographs were quantified with a scanning densitometer and image analysis software (Image 1.60, NIH, Washington, DC). Results are expressed as arbitrary densitometric units.

Pulse analyses. Pulse frequency and amplitude of LH and FSH in serum samples were determined using the pulsar program (Merriam and Wachter, 1982). The G values for both LH and FSH were G1=99, G2=4.5, G3=4, G4=3.5 and G5=99. To determine if variations in hormone concentrations in serial samples are pulses in hormone secretion or just random variations in concentrations, appropriate G values were chosen to serve as

criteria for each experiment. The value for G1 is usually set at 99 to prevent identifying a one sample increase in concentration as a pulse, which by our definition of pulses can not occur due to the frequency at which samples were collected. The G5 value also was set at 99 to avoid the false positive determination of a small increase, followed by a return to baseline concentrations, as a pulse.

Statistical analyses. Control and P_4-treated cows, and control and E_2-treated cows were used in a 2 x 2 factorial arrangement. Analyses of variance were performed using the GLM procedure of SAS (1996) to determine the effects of treatment with progesterone or estradiol on concentrations and content of LH and FSH in the pituitary gland, pulse frequency and amplitude of LH and FSH in serum, concentration of GnRH-R and gene expression for GnRH-R, α-subunit, LHβ and FSHβ. Concentrations of progesterone and estradiol in daily plasma samples were analyzed as repeated measures using MIXED procedures of SAS (1996). Effects of steroid treatment on serum concentrations of LH and FSH were determined by the MIXED procedure of SAS (1996). Treatment means were compared using the PDIFF statement of SAS (1996) when protected by a significant ($P < .05$) treatment effect. Analysis of variance was used to determine differences between proestrous cows and anovulatory cows treated with steroids. Differences between proestrous cows and anovulatory cows with or without steroids were compared with Scheffe's multiple comparisons test (Steel and Torrie, 1980).

Results

Effect of steroid treatment on anovulatory cows. Treatment of ovariectomized, anovulatory cows with E_2 for 6 d resulted in greater ($P < .05$) plasma concentrations of estradiol (1.62 ± .10 pg/mL) compared with non E_2-treated cows (.56 ± .09 pg/mL) (Table 1). Concentrations of progesterone were greater ($P < .05$) in cows treated with P_4 (3.13 ± .22 ng/mL) than non P_4-treated cows (.31 ± .22 ng/mL) (Table 1).

Serum concentrations of LH and FSH on d -1 were similar for cows on all treatments ($P > .1$) and averaged 1.82 ± .06 and .58 ± .01 ng/mL, respectively (Table 2 and 3). Treatments did not influence ($P > .1$) concentrations of LH in serum of cows on d 6 (2.08 ± .04 ng/mL; Table 2). Similarly, FSH concentrations were not affected ($P > .1$) by steroid treatments for 6 d (.55 ± .02 ng/mL; Table 3).

Frequencies of LH and FSH pulses in serum were similar among treatments on d -1 and were 1.7 ± 1.1 and 1.4 ± 1.0 pulses/4 h, respectively (Table 2 and 3). Cows treated with E_2 or E_2P_4 for 6 d had increased ($P < .05$) LH pulse frequency compared with control and P_4-treated cows. Frequency of FSH pulses was not influenced by treatment (Table 3).

Amplitude of LH and FSH pulses in serum on d -1 averaged 1.01 ± .17 and .22 ± .02 ng/mL, respectively, and were not influenced by treatment (Table 2 and 3). Cows treated with E_2 or with P_4 had greater ($P < .05$) amplitudes of LH pulses than control and E_2P_4 cows, and the response to E_2 was not influenced by P_4 treatment ($E_2 \times P_4$; $P > .1$; Table 2). Amplitudes of FSH pulses were not influenced by treatments (Table 3).

Weight of anterior pituitary glands (1.86 ± .07 g) was not affected ($P > .1$) by treatment. Concentrations of LH in pituitary glands of anovulatory cows were not

influenced by treatment with E_2 and P_4. However, FSH concentrations in pituitary glands of cows treated with E_2 or E_2P_4 were reduced ($P = .08$) compared to nontreated cows or cows treated with P_4 (Figure 1). Content of LH and FSH in the pituitary gland was not influenced by treatment.

Concentrations of GnRH-R in pituitary glands were greater ($P < .05$) in cows treated with E_2 or E_2P_4 compared with P_4-treated or control cows (Figure 2). Steady state concentrations of GnRH-R mRNA in the pituitary gland were not influenced ($P > .1$) by steroid treatments (Figure 2).

Steady state concentrations of common α-subunit and FSHβ mRNA in the pituitary were not influenced ($P > .1$) by treatments (Figure 3). Steady state concentrations of LHβ mRNA were increased ($P < .05$) in cows treated with P_4 or E_2P_4 compared with E_2-treated or control cows (Figure 3).

Proestrous cows vs anovulatory cows with or without steroids. Weights of anterior pituitary glands were similar ($P > .1$) for proestrous cows ($1.64 \pm .14$ g) and ovariectomized, anovulatory cows with or without steroids ($1.86 \pm .07$ g). Concentration of LH in the pituitary gland was greater ($P < .05$) in proestrous cows than in anovulatory cows with or without steroid treatment (Figure 1). Pituitary concentration of FSH was less ($P < .05$) in proestrous cows than in ovariectomized, anovulatory cows (Figure 1). Similarly, content of LH in the pituitary gland was greater ($P < .05$), and pituitary content of FSH was less ($P < .05$) in proestrous cows than in ovariectomized, anovulatory cows with or without steroid treatment.

Steady state concentrations of mRNA for GnRH-R, common α-subunit, LHβ- and FSHβ-subunits did not differ ($P > .1$) between proestrous cows and ovariectomized, anovulatory cows with or without steroid treatment (Figure 2 and 3).

Discussion

Treatment of ovariectomized, nutritionally induced anovulatory cows with an intravaginal device containing P_4 for 6 d increased progesterone in plasma to concentrations observed during the early luteal phase of estrous cycles (Stabenfeldt et al., 1969; Wettemann et al., 1972). Concentrations of estradiol in plasma were increased in cows receiving an intravaginal device containing E_2 and concentrations were similar to values during the luteal phase of estrous cycles (Wettemann et al., 1972; Glencross et al., 1973; Bossis et al., 1999).

Treatment of ovariectomized, nutritionally induced anovulatory cows with E_2, P_4 or both E_2 and P_4 for 6 d did not influence concentrations of LH and FSH in serum. Removal of the regulatory control of the ovary increases concentrations of LH (Schallenberger and Peterson, 1982; Anderson et al., 1985) and FSH in serum (Ireland et al., 1983) in cattle with normal estrous cycles. However, Richards et al. (1991) found that concentrations of LH in serum were not influenced during the first 10 d after ovariectomy in nutritionally anestrous cows. Concentrations of LH in serum are reduced in anestrous cows (Richards et al., 1989a; Vizcarra et al., 1997) and feed restricted heifers (Kurz et al., 1990; Bossis et al., 1999). Steroid treatment for 6 d may not be sufficient to stimulate LH secretion from pituitary glands of anovulatory beef cows. Treatment of postpartum, lactationally anestrous cows with progesterone for 7 d followed by an

injection of estradiol benzoate 24 to 30 h after progesterone removal, increased the number of cows that ovulated and exhibited behavioral estrus (Fike et al., 1997). Lack of steroid effects on LH and FSH in serum may indicate that nutritionally anovulatory cows may be in a "deeper" state of anestrus and are not able to respond to steroid treatment as postpartum, anestrous cows can respond.

The number of pulses of LH in serum was greater in cows treated with E_2 or E_2P_4 for 6 d than in control or P_4 cows. Steroids act at the hypothalamus, anterior pituitary gland or at both sites to regulate gonadotropin release (Goodman and Karsch, 1980; Rahe et al., 1980). Treatment of anovulatory cows with E_2 or E_2P_4 for 6 d may stimulate GnRH release increasing pulsatile release of LH. Exposure of rats to concentrations of estradiol similar to those during proestrus and estrus increased concentrations of GnRH in the pituitary stalk blood (Sarkar and Fink, 1979). Similarly, estradiol induced a preovulatory-like surge of GnRH in the cerebrospinal fluid of ovariectomized cows, which was associated with a LH surge (Gazal et al., 1998). Progesterone treatment reduces LH secretion in cattle (Walters et al., 1982; Schallenberger et al., 1984) and sheep (Karsch et al., 1987). Goodman and Karsch (1980) proposed that progesterone has its effects on GnRH secretion. It is unlikely that P_4 influenced LH secretion in the anovulatory cows treated with E_2P_4 in the current study. Progesterone failed to block estradiol-induced LH release in GnRH-pulsed female monkeys with hypothalamic lesions that abolish endogenous GnRH release (Wildt et al., 1981b). Similarly, progesterone treatment did not influence estradiol-induced LH surge in hypothalamic-pituitary disconnected ewes treated with pulsatile GnRH (Clarke and Cummins, 1984). Increased pulse frequency of LH in anovulatory cows treated with E_2 may be due to the effects of

estrogens on the anterior pituitary gland. In vitro treatment of rat (Drouin et al., 1976) and cow (Padmanabhan et al., 1978) pituitary cells with estradiol increased LH release and synthesis. Estradiol also increases the sensitivity of the anterior pituitary gland to GnRH in cows (Kesner et al., 1981). The number of GnRH-R on the anterior pituitary gland of cows is increased after exogenous estrogen (Schoenemann et al., 1985).

Pulse amplitude of LH was greater in cows treated with P_4 or E_2. Progesterone reduces frequency of LH pulses and increases amplitude of pulses in cattle (Rahe et al., 1980) and sheep (Goodman and Karsch, 1980). Progesterone treatment decreases GnRH secretion in sheep (Karsch et al., 1987). Increased pulse amplitude is probably a result of E_2 increasing GnRH secretion and (or) increasing the number of GnRH-R on the anterior pituitary gland. Cows fed restricted diets released more LH in response to exogenous GnRH than did moderate condition cows (Beal et al., 1978; Rasby et al., 1991). Increased pulse amplitude of LH in anovulatory cows treated with P_4 or E_2 indicates that the pituitary gland is not depleted of gonadotropins in underfed cattle. The presence of either steroid, but not both, in the current study was sufficient to increase pulsatile release of LH.

Steroid treatment for 6 d did not influence concentration or content of LH in the anterior pituitary gland of anovulatory cows, but cows treated with E_2 had reduced pituitary FSH concentrations. Duration of steroid treatment may affect pituitary concentrations of LH and FSH. Treatment of ovariectomized cows with one injection of estradiol (1 mg) increased pituitary gland concentrations of LH and FSH 20 h after estradiol (Schoenemann et al., 1985). Steroids may influence LH and FSH synthesis and release differently. Estradiol treatment of ovariectomized cows caused a LH surge

without altering serum concentrations of FSH (Schoenemann et al., 1985). Amounts of mRNA in ovariectomized ewes for common α-subunit and LHβ decreased after 4 d of exposure to estradiol, and amounts of FSHβ mRNA were decreased after 8 d (Herring et al., 1991). Release of GnRH is reduced in underfed cattle (Rasby et al., 1991). Estradiol increases GnRH-R on the anterior pituitary gland, and reduced pulsatile secretion of GnRH in anovulatory cows may favor release of FSH over that of LH.

Concentrations of GnRH-R increased when anovulatory cows were treated with E_2 and E_2P_4 for 6 d. Estradiol increases concentrations of GnRH-R in the anterior pituitary gland of cattle with normal estrous cycles (Kaltenbach et al., 1974; Kesner et al., 1981). Schoenemann et al. (1985) found the number of GnRH-R increase prior to and during estrus in cows indicating estradiol up-regulation of receptors. Receptors for GnRH are influenced by GnRH secretion (Braden and Conn, 1991). Effects of E_2 on concentrations of GnRH-R in anovulatory cows are probably directed at the anterior pituitary gland. Estradiol administration of ewes in which the hypothalamus and pituitary gland were disconnected resulted in a 2.5 fold increase in the number of GnRH-R (Gregg and Nett, 1989). Estradiol may increase GnRH-R by increasing GnRH release from the hypothalamus and (or) increasing GnRH-R at the anterior pituitary gland.

Concentrations of GnRH-R mRNA were not influenced by steroid treatment for 6 d. Concentrations of GnRH-R mRNA are increased during the preovulatory period in sheep (Brooks et al., 1993; Turzillo et al., 1994) and rats (Bauer-Dantoin et al., 1993). Duration of exposure to steroids influences amounts of mRNA for GnRH-R. Acute treatment of ovariectomized ewes (Hamernik et al., 1995; Turzillo et al., 1995a) and wethers (Adams et al., 1996) with estradiol increased GnRH-R mRNA. However,

chronic treatment with estradiol for 7 d decreased GnRH-R mRNA in ovariectomized rats (Kaiser et al., 1993). Chronic exposure (7-8 d) to progesterone reduced concentrations of GnRH-R mRNA in wethers (Sakurai et al., 1997). Concentrations of GnRH-R mRNA in anovulatory cows in the current study could have increased after steroid treatment (~ 12 h) and returned to concentrations not different from control cows by d 6. Synthesis of mRNA for GnRH-R also is influenced by GnRH in normal cycling animals (Belchetz et al., 1978). However, pulsatile GnRH did not influence amounts of GnRH-R mRNA in the pituitary glands of anestrous cows (Vizcarra et al., 1997). Pituitary concentrations of GnRH-R mRNA increased 12 h after $PGF_{2\alpha}$ induced luteolysis in intact ewes (Turzillo et al., 1994). This increase in mRNA for GnRH-R occurred at a time when concentrations of progesterone were decreased and prior to significant increases in concentrations of estradiol, suggesting progesterone may mediate pretranslation of GnRH-R (Turzillo et al., 1994). Adequate concentrations of GnRH and the proper steroidal environment is needed to influence concentrations of mRNA for GnRH-R.

Concentrations of common α- and FSHβ-subunit mRNAs were not influenced by treatments. Pituitary concentrations of LHβ mRNA were greater in cows treated with P_4 and E_2P_4 than in E_2-treated and control cows. Withdrawal of exogenous progesterone increases LH secretion in postpartum anestrous cows (Garcia-Winder et al., 1987) and pubertal heifers (Anderson et al., 1996). Progesterone inhibits LH release in cattle (Walters et al., 1982) and sheep (Moss et al., 1981). Synthesis of LH in the pituitary gland may have adequate time to increase under the influence of progesterone. Progesterone treatment for 6 d increased the synthesis of LHβ mRNA without altering pulsatile release of LH in the current study. Similarly, steady state concentrations of

common α-subunit and LHβ mRNA are uncoupled from pulsatile LH release in the sexually maturing heifer (Roberson et al., 1992). Duration of steroid exposure also may influence amounts of mRNA for gonadotropins. Acute exposure to estradiol (12 h) decreased concentrations of mRNA for all three gonadotropin subunits followed by increases in common α- and LHβ mRNA subunits after 24 h with no affect on FSHβ mRNA in ovariectomized ewes (Herring et al., 1991). Further exposure to estradiol decreased concentrations of common α-subunit and LHβ mRNA after 4 d whereas concentrations of FSHβ subunit mRNA were decreased after 8 d (Herring et al., 1991).

Proestrous cows had greater concentration and content of LH in the pituitary gland than ovariectomized, anovulatory cows with or without steroids. Concentrations and content of LH in the current study are similar to those observed during proestrus in beef cows (Funston et al., 1995) and dairy cows (Hackett and Hafs, 1969) prior to LH release at ovulation. Concentrations and content of LH in the pituitary gland of ovariectomized, anovulatory cows were 25 and 27%, respectively, the concentrations and content in the pituitary gland of proestrous cows. Concentrations of estradiol are increasing while concentrations of progesterone are reduced during proestrus (Chenault et al., 1975). Pituitary concentrations of LH and FSH were increased 20 h after estradiol treatment of ovariectomized cows (Schoenemann et al., 1985).

Concentrations and content of FSH in the pituitary gland were reduced in proestrous cows compared with ovariectomized, anovulatory cows with or without steroids. Concentrations and content of FSH in the pituitary gland were decreased 65 and 70%, respectively, in proestrous cows compared with ovariectomized, anovulatory cows. Concentrations and content of FSH are less than those reported for proestrous dairy cows

(Hackett and Hafs, 1969). These differences could be due to cows in different stages of estrus. Increased pituitary content of FSH in ovariectomized cows is likely due to removal of ovarian peptides (i.e., inhibin) that regulate FSH (Ireland et al., 1983; Roberstson et al., 1985, 1988).

Steady state concentrations of mRNA for GnRH-R, common α-subunit, LHβ and FSHβ were similar for proestrous cows and ovariectomized, anovulatory cows. Increased steady state concentrations of GnRH-R mRNA during the preovulatory period is common in rats (Bauer-Dantoin et al., 1993) and sheep (Brooks et al., 1993; Turzillo et al., 1994). Acute exposure to estradiol increases common α-subunit and LHβ mRNA in ovariectomized ewes (Herring et al., 1991). Treatment with progesterone has no affect on pituitary concentrations of mRNA encoding for common α-subunit and LHβ subunit in rats (Counis et al., 1983) and mRNA for common α-subunit, LHβ and FSHβ subunits in ovariectomized sheep (Hamernik et al., 1987). Differences in animal model (intact vs castrated) as well as the physiological state of the animal at the time of pituitary harvest may account for differences in studies of steady state amounts of mRNA. Winters (1996) found a relationship between plasma concentrations of FSH and FSHβ mRNA in intact rats but not in castrated rats. Discrepancies when comparing amounts of mRNA between studies may be due to slight differences in the time at which pituitary glands were collected. Alexander and Miller (1982) proposed the half-life of the common α-subunit was 51 h while the β subunit is thought to be less stable with a half-life of 12-16 h (Hall and Miller, 1986; Hamernik and Nett, 1988; Di Gregorio and Nett, 1995).

Implications

Luteal phase concentrations of estradiol increased LH pulse frequency and GnRH-R without altering concentrations of LH and FSH in ovariectomized, anovulatory beef cows. Exposure to concentrations of progesterone in plasma similar to those during the estrous cycle increases synthesis of LHβ mRNA in the anterior pituitary gland of anovulatory beef cows. Increased synthesis of LHβ by progesterone would allow pituitary content of LH to increase, and estradiol may cause increased pulsatile release of LH in anovulatory cows.

Table 1. Least squares means for plasma concentrations of estradiol and progesterone in ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or with a sham intravaginal device (C) for 6 d

	Treatment				
Criteria	C	E_2	P_4	E_2P_4	MSE
Estradiol, pg/mL	.60[a]	1.79[b]	.52[a]	1.45[b]	.28
Progesterone, ng/mL	.16[a]	.45[a]	3.18[b]	3.08[b]	1.51

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

Table 2. Least squares means for concentration, pulse frequency and pulse amplitude of LH in serum on d -1 and d 6 in ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or with a sham intravaginal device (C) for 6 d

	Day -1					Day 6				
	Treatment					Treatment				
Criteria	C	E_2	P_4	E_2P_4	MSE	C	E_2	P_4	E_2P_4	MSE
Concentration, ng/mL	1.9[a]	1.8[a]	1.9[a]	1.6[a]	.5	1.8[a]	2.3[a]	2.5[a]	1.8[a]	.8
Pulse frequency, pulses/4 h	2.8[a]	1.3[a]	1.2[a]	1.4[a]	1.1	2.3[a]	4.0[b]	2.7[a]	3.2[b]	1.3
Pulse amplitude, ng/mL	1.6[a]	1.0[a]	.9[a]	.7[a]	1.1	1.0[a]	1.7[b]	1.7[b]	1.1[a]	1.6

[ab]Means within a day without a common superscript differ ($P < .05$).

Table 3. Least squares means for concentration, pulse frequency and pulse amplitude of FSH in serum on d -1 and d 6 in ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or with a sham intravaginal device (C) for 6 d

	Day -1					Day 6				
	Treatment					Treatment				
Criteria	C	E_2	P_4	E_2P_4	MSE	C	E_2	P_4	E_2P_4	MSE
Concentration, ng/mL	.61[a]	.57[a]	.52[a]	.63[a]	.05	.60[a]	.50[a]	.51[a]	.61[a]	.41
Pulse frequency, pulses/4 h	2.0[a]	1.0[a]	1.4[a]	1.2[a]	1.1	1.9[a]	2.3[a]	1.5[a]	2.7[a]	1.9
Pulse amplitude, ng/mL	.25[a]	.23[a]	.17[a]	.21[a]	.01	.27[a]	.21[a]	.31[a]	.23[a]	.03

[ab]Means within a day without a common superscript differ ($P < .05$).

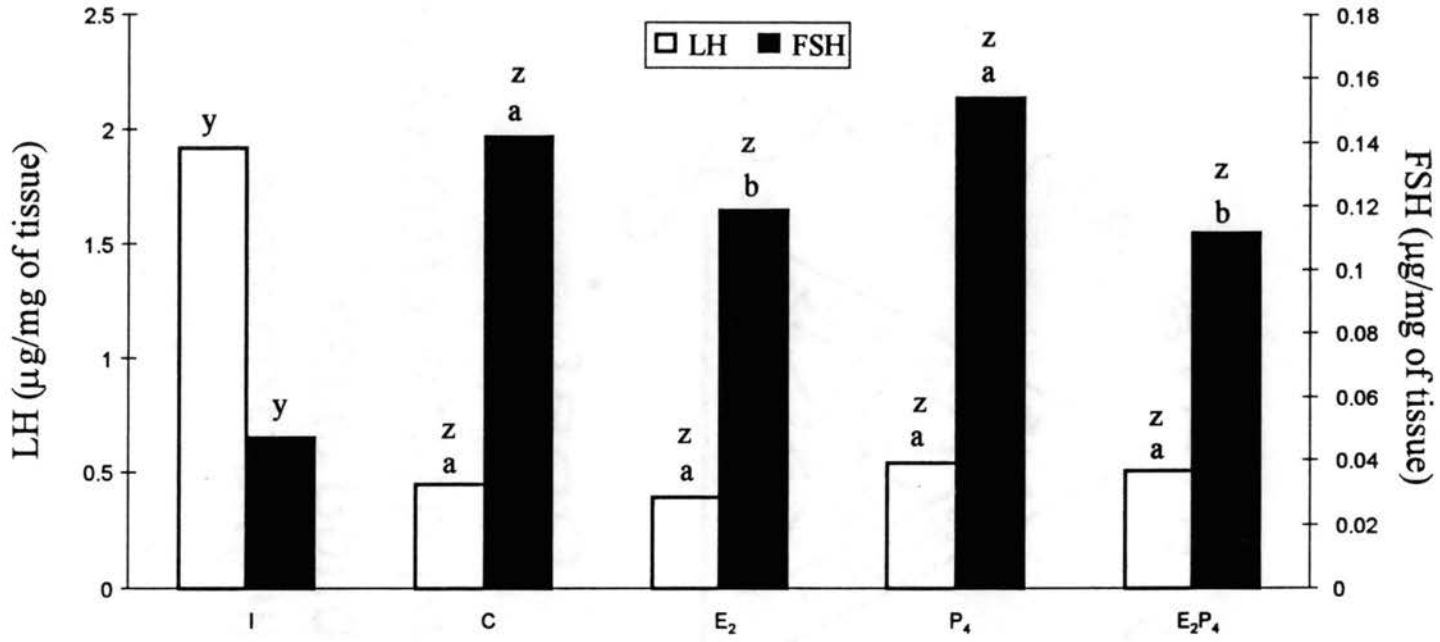

Figure 1. Least squares means for concentration of LH and FSH in the pituitary gland of proestrous cows (I) and ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or a sham intravaginal device (C) for 6 d. [ab]Different letters within a hormone differ for anovulatory cows ($P < .1$; LH MSE = .061; FSH MSE = .002). [yz]Different letters within a hormone differ for proestrous vs anovulatory cows ($P < .001$; LH MSE = .237; FSH MSE = .002).

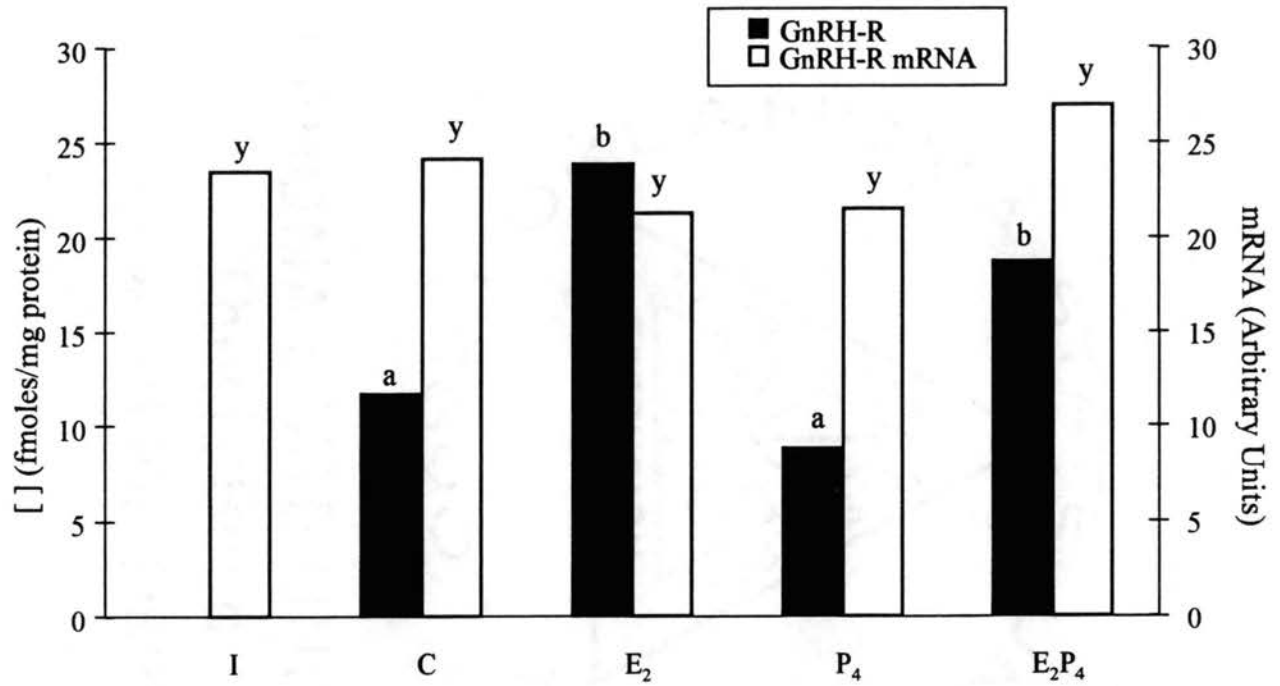

Figure 2. Least squares means for concentrations of GnRH-R and GnRH-R mRNA in the pituitary gland of proestrous cows (I) and ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or a sham intravaginal device (C) for 6 d. [ab]Different letters within GnRH-R and GnRH-R mRNA differ for anovulatory cows ($P < .05$; GnRH-R MSE = 75.5; GnRH-R mRNA MSE = 162.7). [yz]Different letters within GnRH-R mRNA differ for proestrous vs anovulatory cows ($P < .05$; MSE =158.4).

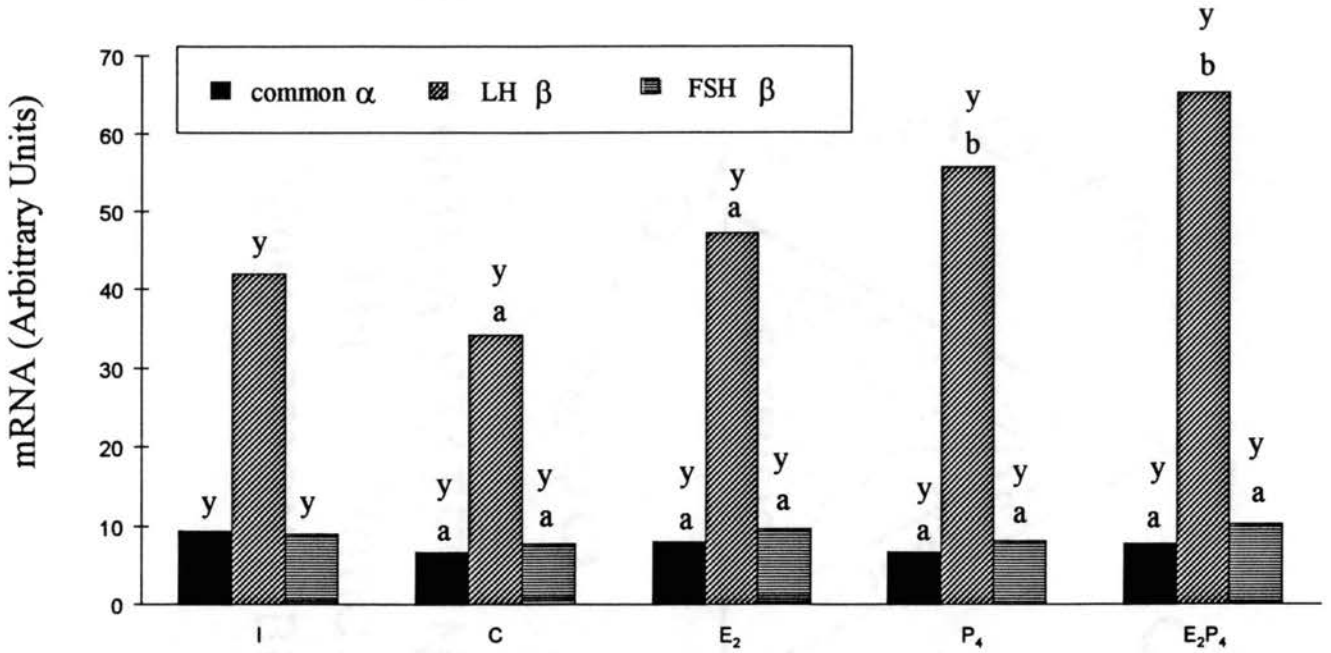

Figure 3. Least squares means for steady state concentrations of common α-, LHβ- and FSHβ-subunit mRNAs in the pituitary gland of proestrous cows (I) and ovariectomized, anovulatory cows treated with intravaginal devices containing estradiol (E_2), progesterone (P_4), E_2 and P_4 (E_2P_4), or a sham intravaginal device (C) for 6 d. [ab]Different letters within a subunit differ for anovulatory cows ($P < .05$; α-subunit MSE = 7.3; LHβ MSE = 622.2; FSHβ MSE = 17.1). [yz]Different letters within a subunit differ for proestrous vs anovulatory cows ($P < .05$; α-subunit MSE = 7.0; LHβ MSE = 628.9; FSHβ MSE = 14.8).

CHAPTER VI

SUMMARY AND CONCLUSIONS

Beef cattle are the least efficient domestic farm species usually producing a single offspring each year. A 10% increase in the annual calf crop would allow the production of the same kilograms of beef from 12% fewer cows. Understanding the physiological mechanisms responsible for resumption of ovarian activity in postpartum beef cows may allow increased reproductive efficiency. Three experiments were conducted to determine the mechanisms by which nutrition and steroids influence gonadotropin release in beef cows. The specific objectives of the current research were 1) to characterize effects of nutrition and body energy reserves on concentrations of LH and FSH in ovariectomized beef cows, 2) to evaluate endocrine and behavioral events associated with the first ovulation in postpartum beef cows, and 3) to evaluate the effects of nutrition and steroids on serum and pituitary gland concentrations of LH and FSH, mRNA for gonadotropin subunits and GnRH-R, and concentrations of GnRH-R in pituitary glands of beef cows.

In experiment I, ovariectomized Hereford and Angus x Hereford cows were used to determine the effects of chronic and acute nutritional deprivation on concentration, pulse frequency and amplitude of LH and FSH in serum. Anovulatory cows were ovariectomized and fed to either maintain or gain body weight for 4 months to determine the effects of chronic nutritional deprivation on gonadotropin secretion. Luteinizing hormone and FSH were quantified in serum at 10 min intervals for 8 h. Ovariectomized

cows in moderate body condition were assigned to receive either restricted or maintenance diets for 6 d to evaluate effects of acute nutritional deprivation on gonadotropin secretion and blood metabolites. Concentrations of LH and FSH were quantified in serum at 10 min intervals for 8 h from cows before treatment (d 0), and on d 3 and 6.

Anovulatory cows fed to gain weight had greater concentrations, pulse frequencies and amplitudes of LH and FSH than did anovulatory cows fed to maintain body weight. Concentrations of LH in serum of anovulatory cows fed maintenance diets were 52% of the concentrations in anovulatory cows fed to gain body weight, whereas FSH concentrations in maintenance fed, anovulatory cows were 76% of the concentrations in anovulatory cows fed to gain weight. This indicates that chronic nutritional deprivation may have less influence on FSH than LH in ovariectomized cows. Removal of ovarian regulatory peptides (i.e., inhibin) by ovariectomy may obscure effects of undernutrition by increasing concentrations of FSH in serum of cattle.

Feeding 30% of maintenance energy requirements for 6 d did not influence concentration, pulse frequency or amplitude of LH and FSH in ovariectomized cows in moderate condition. Concentrations of glucose and leptin in plasma were not altered during 6 d of feed restriction; however, concentrations of NEFA were increased and IGF-I in plasma were decreased. These results indicate that feed restriction for 6 d is not sufficient to decrease pulsatile release of LH and FSH. Concentrations of NEFA and IGF-I may be indicators of metabolic status in underfed animals.

Seventy-seven multiparous beef cows with moderate to good BCS were used in experiment II. Commencing approximately 3.5 mo prior to parturition, cows grazed

native grass range and were fed either .68 kg/d (L) or 1.36 kg/d (M) of a 40% crude protein supplement to either lose or maintain body weight until parturition. Blood samples were collected twice weekly after parturition to determine the occurrence of the first postpartum luteal activity (progesterone ≥ .5 ng/mL). Cows were exposed to bulls and observed twice daily for behavioral estrus. Luteal activity was classified as normal if four consecutive samples had ≥ .5 ng/mL of progesterone (LA for at least 11 d) or short if three or less consecutive samples had ≥ .5 ng/mL.

Body condition score of cows fed to lose weight was 3.6 at parturition, and cows fed to maintain condition had a BCS of 4.7. Postpartum intervals from parturition to first estrus, normal LA and conception were shorter for moderate condition (BCS ≥ 4.5) cows than for thin (BCS ≤ 4) cows. These results support the concept that BCS at parturition is the single most important factor affecting postpartum reproductive performance in beef cows (Selk et al., 1988).

Body condition score at parturition and postpartum weight change did not influence the duration of first luteal activity. Seventy-two percent of cows had a transient (progesterone ≥ .5 ng/mL in one sample) increase in progesterone preceding the first normal luteal activity, and 6% of cows had ≥ .5 ng/mL of progesterone for two samples. This indicates that short luteal activity at the first postpartum ovulation is a common occurrence in a majority of beef cows.

Body condition at parturition and postpartum weight change did not influence the duration of luteal activity associated with first estrus. Seventy-four percent of cows exhibited estrus and 26% did not express or were not detected in estrus with twice daily observation. After the first estrus, 72% of cows had normal luteal activity, 16% had short

luteal activity and 12% lacked luteal activity. Maximum concentrations of progesterone were greater in cows with four consecutive samples ≥ .5 ng/mL of progesterone than in cows with one or two sample[s] ≥ .5 ng/mL of progesterone.

Eighty-one percent of cows with a transient increase in progesterone before first estrus exhibited subsequent normal luteal activity, and only 36% of cows in which a transient increase in progesterone was not detected had normal luteal function after estrus. We speculate that a transient increase in progesterone in cattle during the postpartum period resensitizes the brain and possibly allows synthesis of LH in the pituitary gland. Transient increases in progesterone also may control timing of luteolysis by increasing progesterone receptors in the uterus.

The third experiment utilized twenty-eight nutritionally induced anovulatory cows and five proestrous cows to determine the effect of E_2 and P_4, and reproductive state on regulation of synthesis and secretion of LH and FSH. Anovulatory cows were ovariectomized and received intravaginal devices containing E_2, P_4, E_2 and P_4, or a sham intravaginal device (C) for 6 d. Concentrations of LH and FSH were quantified in serum samples collected every 10 min for 8 h on d 6. Cows were exsanguinated within 2 h after removal of intravaginal devices and pituitary glands collected and stored. Proestrous cows were exsanguinated and pituitary glands collected to compare concentrations of LH and FSH, and gene expression for gonadotropin subunits in anovulatory cows with cows with normal estrous cycles. Total RNA was isolated from one half of each pituitary gland, and mRNA for GnRH-R and gonadotropin subunits were quantified. Pituitary content of GnRH-R, LH and FSH were determined in the remaining half of each pituitary gland.

Cows treated with an intravaginal device containing E_2 had increased concentrations of E_2 in plasma similar to values during the early luteal phase of the estrous cycle. Concentrations of P_4 were increased in cows treated with intravaginal devices containing P_4, and were similar to values observed during the early luteal phase of the estrous cycle.

Frequency of LH pulses and number of GnRH-R were increased in anovulatory cows treated with E_2 and E_2P_4. Steady state concentrations of GnRH-R mRNA were not influenced by steroid treatments. Concentrations of LH and FSH in serum were not influenced by E_2 or P_4. Estradiol may increase LH secretion by increasing GnRH-R on the anterior pituitary gland.

Concentration and content of LH in pituitary glands was not influenced by steroid treatments; however, cows treated with E_2 had reduced pituitary FSH concentrations. Estradiol may regulate LH and FSH synthesis and release differently.

Pituitary concentrations of LHβ mRNA were greater in cows treated with P_4 and E_2P_4 than in E_2-treated and control cows. Concentrations of common α-subunit and FSHβ mRNA were not influenced by treatments. Progesterone may increase synthesis of LHβ in pituitaries of anovulatory cows.

Proestrous cows had greater pituitary concentration and content of LH and reduced concentration and content of FSH than did ovariectomized, anovulatory cows treated with or without steroids. Increased pituitary content of FSH in ovariectomized cows compared with proestrous cows may be due to removal of ovarian regulatory peptides.

Steady state concentrations of mRNA for GnRH-R, common α-subunit, LHβ and FSHβ in the pituitary were similar for proestrous cows and ovariectomized, anovulatory cows. The lack of an effect of physiological state on mRNAs may be because steady state concentrations of mRNA are a measurement of gene expression for a particular subunit at a single point in time.

Anestrous cows have decreased LH release from the anterior pituitary gland due to reduced GnRH secretion from the hypothalamus. Secretion of reduced amounts of gonadotropins suppresses synthesis and release of steroid hormones by the ovary, which influence secretion of gonadotropins. Reduced nutrient intake and BCS reduces secretion of LH, and LH is the limiting gonadotropin for reproductive performance of beef cows. Exposure of the hypothalamus and pituitary to increased concentrations of estradiol during gestation may reduce gonadotropin secretion during the early postpartum period, and anestrous is further attenuated by undernutrition. Increase in plasma concentrations of progesterone prior to the first ovulation in postpartum beef cows sensitizes the brain to the effects of estradiol. Progesterone regulates gonadotropin release by altering secretion of GnRH, and progesterone probably affects synthesis of LH in the anterior pituitary gland. Increased synthesis of LHβ would allow pituitary stores of LH to increase and be available for release. Minimal concentrations of estradiol from ovaries of anestrous cows could cause increased pulsatile release of LH by stimulation of GnRH release. Estradiol also increases the sensitivity of the pituitary gland to GnRH by increasing GnRH-R in the anterior pituitary gland. Increased GnRH-R may occur by increased GnRH release and (or) increased synthesis of GnRH-R in the pituitary. Pulsatile secretion of LH causes

increased production of estradiol in the ovary. Estradiol acts to induce a LH surge that causes ovulation and resumption of luteal function in anestrous cows.

LITERATURE CITED

Abbot, S. D., K. Docherty, J. L. Roberts, M. A. Tepper, W. W. Chin, and R. N. Clayton. 1985. Castration increases luteinizing hormone subunit messenger RNA levels in male rat pituitaries. J. Endocrinol. 107:R1-R4.

Adams, B. M., H. Sakurai, and T. E. Adams. 1996. Concentrations of gonadotropin-releasing hormone (GnRH) receptor messenger ribonucleic acid in pituitary tissue of orchidectomized sheep: Effect of estradiol and GnRH. Biol. Reprod. 54:407-412.

Adams, B. M., H. Sakurai, and T. E. Adams. 1997. Effect of oestradiol on mRNA encoding GnRH receptor in pituitary tissue of orchidectomized sheep passively immunized against GnRH. J. Reprod. Fertil. 111:207-212.

Adams, T. E., and H. G. Spies. 1981. Binding characteristics of gonadotropin-releasing hormone receptors throughout the estrous cycle of the hamster. Endocrinology 108:2245-2253.

Akbar, A. M., L. E. Reichert, Jr., T. G. Dunn, C. C. Kaltenbach, and G. D. Niswender. 1974. Serum levels of follicle-stimulating hormone during the bovine estrous cycle. J. Anim. Sci. 39:360-365.

Alexander, D. C., and W. L. Miller. 1982. Regulation of ovine follicle-stimulating hormone β-chain mRNA by 17β-estradiol in vivo and in vitro. J. Biol. Chem. 257:2282-2286.

Amoss, M., R. Burgus, R. Blackwell, W. Vale, R. Fellows, and R. Guillemin. 1971. Purification, amino acid composition and N-terminus of the hypothalamic hormone releasing factor (LRF) of ovine origin. Biochem. Biophys. Res. Comm. 44:205-210.

Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin-induced puberty and secretion of luteinizing hormone in heifers. Biol. Reprod. 54:1025-1031.

Anderson, L. L., D. L. Hard, L. S. Carpenter, E. K. Awotoni, and M. A. Diekman. 1981. Neuroendocrine regulation of luteinizing hormone in beef calves. Biol. Reprod. 24:795-800.

Anderson, W. J., D. W. Forrest, A. L. Schulze, D. C. Kramer, M. J. Bowen, and P. G. Harms. 1985. Ovarian inhibition of pulsatile luteinizing hormone secretion in prepuberal Holstein heifers. Dom. Anim. Endocrinol. 2:85-91.

Antunes, J. L., P. W. Carmel, E. M. Housepian, and M. D. Ferin. 1978. Luteinizing hormone-releasing hormone in human pituitary blood. J. Neurosurg. 49:382-386.

Arije, G. R., J. N. Wiltbank, and M. L. Hopwood. 1974. Hormone levels in pre- and post-parturient beef cows. J. Anim. Sci. 39:338-347.

Armstrong, D. T., and H. Papkoff. 1976. Stimulation of aromatization of exogenous and endogenous androgens in ovaries of hypophysectomized rats in vivo by follicle stimulating hormone. Endocrinology 99:1144-1151.

Armstrong, J. D., W. S. Cohick, R. W. Harvey, E. P. Heimer, and R. M. Campbell. 1993. Effect of feed restriction on serum somototrophin, insulin-like growth factor-I (IGF-I) and IGF binding proteins in cyclic heifers actively immunized against growth hormone releasing factor. Dom. Anim. Endocrinol. 10:315-324.

Asdell, S. A., J. de Alba, and S. J. Roberts. 1945. The levels of ovarian hormones required to induce heat and other reactions in the ovariectomized cow. J. Anim. Sci. 4:277-284.

Asdell, S. A., J. de Alba, and S. J. Roberts. 1949. Studies on the estrous cycle of dairy cattle: Cycle length, size of corpus luteum and endometrial changes. Cornell Vet. 39:389-402.

Atwell, W. J., and C. J. Marinus. 1918. A comparison of the activity of extracts of the pars tuberalis with extracts of other regions of the ox pituitary. Am. J. Physiol. 47:76-91.

Baird, D. T. 1977. Evidence in vivo for the two-cell hypothesis of ovarian estrogen synthesis by the sheep. J. Reprod. Fertil. 100:183-185.

Barash, I. A., C. C. Cheung, D. S. Weigle, H. Ren, E. B. Kabigting, J. L. Kuijper, D. K. Clifton, and R. A. Steiner. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144-3147.

Baratta, M., F. Grasselli, and C. Tamanini. 1994. Effects of gonadal steroids on tonic luteinizing hormone (LH) release and luteinizing hormone-releasing hormone-induced LH release from bovine pituitary cells cultured in vitro. Biol. Reprod. 50:1320-1327.

Barraclough, C. A., and R. A. Gorski. 1961. Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. Endocrinology 68:68-79.

Batra, S. K., and W. L. Miller. 1985. Progesterone inhibits basal production of follicle-stimulating hormone in ovine pituitary cell culture. Endocrinology 117:2443-2448.

Bauer-Dantoin, A. C., A. N. Hollenberg, and J. L. Jameson. 1993. Dynamic regulation of gonadotropin-releasing hormone receptor mRNA levels in the anterior pituitary gland during the rat estrous cycle. Endocrinology 133:1911-1914.

Bauer-Dantoin, A. C., J. Weiss, and J. L. Jameson. 1995. Roles of estrogen, progesterone, and gonadotropin-releasing hormone (GnRH) in the control of pituitary GnRH receptor gene expression at the time of the preovulatory gonadotropin surges. Endocrinology 136:1014-1019.

Beal, W. E., R. E. Short, R. B. Staigmiller, R. A. Bellows, C. C. Kaltenbach, and T. G. Dunn. 1978. Influence of dietary energy intake on bovine pituitary and luteal function. J. Anim. Sci. 46:181-188.

Beck, T. W., and E. M. Convey. 1977. Estradiol control of serum luteinizing hormone concentrations in the bovine. J. Anim. Sci. 45:1096-1101.

Beckett, J. L., H. Sakurai, B. M. Adams, and T. E. Adams. 1997a. Moderate and severe nutrient restriction has divergent effects on gonadotroph function in orchidectomized sheep. Biol. Reprod. 57:415-419.

Beckett, J. L., H. Sakurai, T. R. Famula, and T. E. Adams. 1997b. Negative feedback potency of estradiol is increased in orchidectomized sheep during chronic nutrient restriction. Biol. Reprod. 57:408-414.

Belchetz, P. E., T. M. Plant, Y. Nakai, E. G. Keogh, and E. Knobil. 1978. Hypophysial responses to continuous and intermittent delivery of hypothalamic GnRH. Science 202:631-633.

Bellows, R. A., and R. E. Short. 1978. Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. J. Anim. Sci. 46:1522-1528.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276-1280.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. 1980. Source of circulating progesterone in prepubertal ewes. Biol. Reprod. 22:233-236.

Bergendahl, M., A. Perheentupa, and I. Huhtaniemi. 1989. Effect of short-term starvation on reproductive hormone gene expression, secretion and receptor levels in male rats. J. Endocrinol. 121:409-417.

Bergendahl, M., and I. Huhtaniemi. 1994. The time since castration influences the effects of short-term starvation on gonadotrophin secretion in male rats. J. Endocrinol. 143:209-219.

Bishop, D. K., and R. P. Wettemann. 1993. Pulsatile infusion of gonadotropin-releasing hormone initiates luteal activity in nutritionally anestrous beef cows. J. Anim. Sci. 71:2714-2720.

Blache, D., C. J. Fabre-Nys, and G. Venier. 1991. Ventromedial hypothalamus as a target for oestradiol action on proceptivity, receptivity and luteinizing hormone surge of the ewe. Brain Res. 546:241-249.

Bossis, I. 1997. Nutritionally induced anovulation in the bovine: Ovarian and endocrine events. Ph.D. Dissertation. Oklahoma State Univ., Stillwater.

Bossis, I., R. P. Wettemann, S. D. Welty, J. A. Vizcarra, L. J. Spicer, and M. G. Diskin. 1999. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function preceding cessation of ovulation. J. Anim. Sci. (In press).

Boyar, R. M., J. Katz, J. W. Finkelstein, S. Kapen, H. Weiner, E. D. Weitzman, and L. Hellman. 1974. Anorexia nervosa. Immaturity of the 24-hour luteinizing hormone secretory pattern. New England J. Med. 291:861-865.

Braden, T. D., M. E. King, K. G. Odde, and G. D. Niswender. 1989. Development of preovulatory follicles expected to form short-lived corpora lutea in beef cows. J. Reprod. Fertil. 85:97-104.

Braden, T. D., and P. M. Conn. 1991. Gonadotropin-releasing hormone and its actions. Can. J. Physiol. Pharmacol. 69:445-458.

Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotrophic axis in young steers: Influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor-I. J. Endocrinol. 111:209-215.

Britt, J. H., R. G. Scott, J. D. Armstrong, and M. D. Whitacre. 1986. Determinants of estrous behavior in lactating Holstein cows. J. Dairy Sci. 69:2195-2202.

Brooks, J., P. L. Taylor, P. T. K. Saunders, K. A. Eidne, W. J. Struthers, and A. S. McNeilly. 1993. Cloning and sequencing of the sheep pituitary gonadotropin-releasing hormone receptor and changes in expression of its mRNA during the estrous cycle. Mol. Cell. Endocrinol. 94:R23-R27.

Cagampang, F. R. A., K. I. Maeda, H. Tsukamura, S. Ohkura, and K. Ota. 1991. Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. J. Endocrinol. 129:321-328.

Cameron, J. L., and C. Nosbisch. 1991. Suppression of pulsatile luteinizing hormone and testosterone secretion during short term food restriction in the adult male rhesus monkey (*Macaca mulatta*). Endocrinology 128:1532-1540.

Cameron, J. L., D. J. Koerker, and R. A. Steiner. 1985. Metabolic changes during maturation of male monkeys: Possible signals for onset of puberty. Am. J. Physiol. 249:E385-E391.

Cameron, J. L., T. E. Weltzin, C. McConaha, D. L. Helmreich, and W. H. Kaye. 1991. Slowing of pulsatile luteinizing hormone secretion in men after forty-eight hours of fasting. J. Clin. Endocrinol. Metab. 73:35-41.

Campbell, G. A., M. Kurcz, S. Marshall, and J. Meites. 1977. Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin; response to LH-releasing hormone and thyrotropin-releasing hormone. Endocrinology 100:580-587.

Caraty, A., A. Locatelli, and G. B. Martin. 1989. Biphasic response in the secretion of gonadotrophin-releasing hormone in ovariectomized ewes injected with oestradiol. J. Endocrinol. 123:375-382.

Carrick, M. J., and J. N. Shelton. 1969. Oestrogen-progesterone relationships in the induction of oestrous in spayed heifers. J. Endocrinol. 45:99-109.

Chamley, W. A., H. A. Jonas, and R. A. Parr. 1976. Content of LH, FSH, and growth hormone in the pituitaries of pregnant and anestrous sheep. Endocrinology 98:1535-1538.

Chandrasheker, V., and A. Bartke. 1993. Induction of endogenous IGF-I secretion alters the hypothalamic-pituitary-testicular function in growth hormone-deficient adult dwarf mice. Biol. Reprod. 48:544-551.

Chenault, J. R., W. W. Thatcher, P. S. Kalra, R. M. Abrams, and C. J. Wilcox. 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:709-717.

Chi, L., W. Zhou, A. Prikhozhan, C. Flanagan, J. S. Davidson, M. Golembo, N. Illing, R. P. Millar, and S. C. Sealfon. 1993. Cloning and characterization of the human GnRH receptor. Mol. Cell. Endocrinol. 91:R1-R6.

Childs, G. V., J. M. Lloyd, G. Unabia, S. D. Gharib, M. E. Weirman, and W. W. Chin. 1987. Detection of luteinizing hormone β messenger ribonucleic acid (RNA) in individual gonadotropes after castration: Use of a new in situ hybridization method with a photobiotinylated complementary RNA probe. Mol. Endocrinol. 1:926-932.

Chin, W. W., H. M. Kronenberg, P. C. Dee, F. Maloof, and J. F. Habener. 1981. Nucleotide sequence of the mRNA encoding the pre-α subunit of mouse thyrotropin. Proc. Natl. Acad. Sci. 78:5329-5333.

Church, G. M. and W. Gilbert. 1984. Genomic sequencing. Proc. Natl. Acad. Sci. 81:1991-1995.

Clarke, I. J., and J. T. Cummins. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 111:1737-1739.

Clarke, I. J., and J. T. Cummins. 1984. Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. Neuroendocrinol. 39:267-274.

Clarke, I. J., and J. T. Cummins. 1985. Increased gonadotropin-releasing hormone pulse frequency associated with estrogen-induced luteinizing hormone surges in ovariectomized ewes. Endocrinology 116:2376-2383.

Clayton, R. N., A. R. Solano, A. Garcia-Vela, M. L. Dufau, and K. J. Catt. 1980. Regulation of pituitary receptors for gonadotropin-releasing hormone during the rat estrous cycle. Endocrinology 107:699-706.

Clegg, M. T., J. A. Santolucito, J. D. Smith, and W. F. Ganong. 1958. The effect of hypothalamic lesions on sexual behavior and estrous cycles in the ewe. Endocrinology 62:790-797.

Coe, B. L., and R. D. Allrich. 1989. Relationships between endogenous estradiol-17β and estrous behavior in heifers. J. Anim. Sci. 67:1546-1551.

Conn, P. M. 1986. The molecular basis of gonadotropin-releasing hormone action. Endocr. Rev. 7:3-10.

Cook, D. L., T. A. Winters, L. A. Horstman, and R. D. Allrich. 1986. Induction of estrus in ovariectomized cows and heifers: Effects of estradiol benzoate and gonadotropin releasing hormone. J. Anim. Sci. 63:546-550.

Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia, and E. K. Inskeep. 1991. Effects of progestogen treatment on concentrations of prostaglandins and oxytocin in plasma from the posterior vena cava of post-partum beef cows. J. Reprod. Fertil. 91:411-421.

Copelin, J. P., M. F. Smith, D. H. Keisler, and H. A. Garverick. 1989. Effect of active immunization of pre-partum and post-partum cows against prostaglandin F-2α on lifespan and progesterone secretion of short-lived corpora lutea. J. Reprod. Fertil. 87:199-207.

Copelin, J. P., M. F. Smith, H. A. Garverick, and R. S. Youngquist. 1987. Effect of the uterus on subnormal luteal function in anestrous beef cows. J. Anim. Sci. 64:1506-1511.

Corah, L. R., A. P. Quealy, T. G. Dunn, and C. C. Kaltenbach. 1974. Prepartum and postpartum levels of progesterone and estradiol in beef heifers fed two levels of energy. J. Anim. Sci. 39:380-385.

Counis, R., M. Corbani, and M. Jutisz. 1983. Estradiol regulates mRNAs encoding precursors to rat lutropin (LH) and follitropin (FSH) subunits. Biochem. Biophys. Res. Comm. 114:65-72.

Cupp, A. S., F. N. Kojima, M. S. Roberson, T. T. Stumpf, M. W. Wolfe, L. A. Werth, R. J. Kittok, H. E. Grotjan, and J. E. Kinder. 1995. Increasing concentrations of 17β-estradiol has differential effects on secretion of luteinizing hormone and follicle-stimulating hormone and amounts of mRNA for gonadotropin subunits during the follicular phase of the bovine estrous cycle. Biol. Reprod. 52:288-296.

Cushing, H. 1930a. Neurohypophysial mechanisms from a clinical standpoint. Part I. Lancet 2:119-127.

Cushing, H. 1930b. Neurohypophysial mechanisms from a clinical standpoint. Part II. Lancet 2:175-184.

Dalkin, A. C., D. J. Haisenleder, G. A. Ortolano, T. R. Ellis, and J. C. Marshall. 1989. The frequency of gonadotropin-releasing-hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrinology 125:917-924.

Davidge, S. T., J. L. Wiebold, P. L. Senger, and J. K. Hillers. 1987. Influence of varying levels of blood progesterone upon estrous behavior in cattle. J. Anim. Sci. 64:126-132.

Day, M. L., K. Imakawa, D. D. Zalesky, R. J. Kittok, and J. E. Kinder. 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J. Anim. Sci. 62:1641-1648.

Dieleman, S. J., and D. M. Blankenstein. 1984. Changes in oestrogen-synthesizing ability of preovulatory bovine follicles relative to the peak of LH. J. Reprod. Fertil. 72:487-494.

Di Gregorio, G. B., and T. M. Nett. 1995. Estradiol and progesterone influence the synthesis of gonadotropins in the absence of gonadotropin-releasing hormone in the ewe. Biol. Reprod. 53:166-172.

Dobson, H. 1978. Plasma gonadotrophins and oestradiol during oestrus in the cow. J. Reprod. Fertil. 52:51-53.

Dohlman, H. G., J. Thorner, M. G. Caron, and R. J. Lefkowitz. 1991. Model systems for the study of seven-transmembrane-segment receptors. Ann. Rev. Biochem. 60:653-688.

Dong, Q., B. Li, H. Rintala, S. Blair, J. Spaliviero, and J. Handelsman. 1993. LH pulsatility, biopotency, and clearance during undernutrition in orchidectomized mature rats. Am. J. Physiol. 265:E304-E313.

Dorrington, J. H., Y. S. Moon, and D. T. Armstrong. 1975. Estradiol-17β biosynthesis in cultured granulosa cells from hypophysectomized immature rats; stimulation by follicle-stimulating hormone. Endocrinology 97:1328-1331.

Dransfield, M. B. G., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

Drouin, J., L. Lagace, and F. Labrie. 1976. Estradiol-induced increase of the LH responsiveness to LH releasing hormone (LHRH) in rat anterior pituitary cells in culture. Endocrinology 99:1477-1481.

Dubey, A. K., J. L. Cameron, R. A. Steiner, and T. M. Plant. 1986. Inhibition of gonadotropin secretion in castrated male rhesus monkeys (*Macaca mulatta*) induced by dietary restriction: Analogy with the prepubertal hiatus of gonadotropin release. Endocrinology 118:518-525.

Duello, T. M., and T. M. Nett. 1980. Uptake, localization and retention of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs in rat gonadotrophs. Mol. Cell. Endocrinol. 19:101-112.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman, and J. N. Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci. 29:719-726.

Dyer, R. G., S. Mansfield, H. Corbet, and A. D. P. Dean. 1985. Fasting impairs LH secretion in female rats by activating an inhibitory opioid pathway. J. Endocrinol. 105:91-97.

Echternkamp, S. E., L. J. Spicer, K. E. Gregory, S. F. Canning, and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. Biol. Reprod. 43:8-14.

Echternkamp, S. E., and W. Hansel. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Anim. Sci. 37:1362-1370.

Edqvist, L. E., L. Ekman, B. Gustafsson, and E. D. B. Johansson. 1973. Peripheral plasma levels of oestrogens and progesterone during late bovine pregnancy. Acta Endocrinol. 72:81-88.

Erickson, G. F., D. A. Magoffin, C. A. Dyer, and C. Hofeditz. 1985. The ovarian androgen producing cells: A review of structure/function relationships. Endocr. Rev. 6:371-399.

Erwin, C. R., M. L. Croyle, J. E. Donelson, and R. A. Maurer. 1983. Nucleotide sequence of cloned complementary deoxyribonucleic acid for the α subunit of bovine pituitary glycoprotein hormones. Biochemistry 22:4856-4860.

Espey, L. L. 1967. Ultrastructure of the apex of the rabbit Graafian follicle during the ovulatory process. Endocrinology 81:267-276.

Espey, L. L. 1994. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol. Reprod. 50:233-238.

Espey, L. L., and H. Lipner. 1963. Measurement of intrafollicular pressures in the rabbit ovary. Am. J. Physiol. 205:1067-1072.

Evans, N. P., G. E. Dahl, D. T. Mauger, V. Padmanabhan, L. A. Thrun, and F. J. Karsch. 1995. Does estradiol induce the preovulatory gonadotropin-releasing hormone (GnRH) surge in the ewe by inducing a progressive change in the mode of operation of the GnRH neurosecretory system? Endocrinology 136:5511-5519.

Falck, B. 1959. Site of production of oestrogen in rat ovary as studied in micro-transplants. Acta Physiol. Scand. (Suppl. 163) 47:1-101.

Fetherston, J., and I. Boime. 1982. Synthesis of bovine lutropin in cell-free lysates containing pituitary microsomes. J. Biol. Chem. 257:8143-8147.

Fevold, H. L., F. L. Hisaw, and S. L. Leonard. 1931. The gonad stimulating and the luteinizing hormones of the anterior pituitary lobe of the hypophysis. Am. J. Physiol. 97:291-301.

Fiddes, J. C., and H. M. Goodman. 1979. Isolation, cloning and sequence analysis of the cDNA for the α-subunit of human chorionic gonadotropin. Nature 281:351-356.

Fike, K. E., M. L. Day, E. K. Inskeep, J. E. Kinder, P. E. Lewis, R. E. Short, and H. D. Hafs. 1997. Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. J. Anim. Sci. 75:2009-2015.

Fogwell, R. L., C. W. Weems, G. S. Lewis, R. L. Butcher, and E. K. Inskeep. 1978. Secretion of steroids after induced luteal regression in beef heifers: Effects of $PGF_{2\alpha}$ and removal of corpora lutea. J. Anim. Sci. 46:1718-1723.

Fortune, J. E. 1986. Bovine theca and granulosa cells interact to promote androgen production. Biol. Reprod. 35:292-299.

Fortune, J. E., and D. T. Armstrong. 1977. Androgen production by theca and granulosa isolated from proestrous rat follicles. Endocrinology 100:1341-1347.

Fortune, J. E., and D. T. Armstrong. 1978. Hormonal control of 17β-estradiol biosynthesis in proestrous rat follicles: Estradiol production by isolated theca versus granulosa. Endocrinology 102:227-235.

Foster, D. L., and D. H. Olster. 1985. Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge. Endocrinology 116:375-381.

Foster, D. L., F. J. P. Ebling, A. F. Micka, L. A. Vannerson, D. C. Bucholtz, R. I. Wood, J. M. Suttie, and D. E. Fenner. 1989. Metabolic interfaces between growth and reproduction. I. Nutritional modulation of gonadotropin, prolactin, and growth hormone secretion in the growth-limited female lamb. Endocrinology 125:342-350.

Funston, R. N., G. E. Moss, and A. J. Roberts. 1995. Insulin-like growth factor-1 (IGF-1) and IGF-binding proteins in bovine sera and pituitaries at different stages of the estrous cycle. Endocrinology 136:62-68.

Garcia-Winder, M., P. E. Lewis, D. R. Deaver, V. G. Smith, G. S. Lewis, and E. K. Inskeep. 1986. Endocrine profiles associated with life span of induced corpora lutea in postpartum beef cows. J. Anim. Sci. 62:1353-1362.

Garcia-Winder, M., P. E. Lewis, E. C. Townsend, and E. K. Inskeep. 1987. Effects of norgestomet on follicular development in postpartum beef cows. J. Anim. Sci. 64:1099-1109.

Garmendia, J. C. 1986. Energy metabolites in blood, luteinizing hormone secretion and reproductive performance of range beef cows. Ph.D. Dissertation. Oklahoma State Univ., Stillwater.

Garverick, H. A., J. R. Parfet, C. N. Lee, J. P. Copelin, R. S. Youngquist, and M. F. Smith. 1988. Relationship of pre- and post-ovulatory gonadotropin concentrations to subnormal luteal function in postpartum beef cattle. J. Anim. Sci. 66:104-111.

Garverick, H. A., W. G. Zollers, and M. F. Smith. 1992. Mechanisms associated with corpus luteum lifespan in animals having normal or subnormal luteal function. Anim. Reprod. Sci. 28:111-124.

Gazal, O. S., L. S. Leshin, R. L. Stanko, M. G. Thomas, D. H. Keisler, L. L. Anderson, and G. L. Williams. 1998. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: Correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. Biol. Reprod. 59:676-683.

Gharib, S. D., A. Roy, M. E. Wierman, and W. W. Chin. 1989. Isolation and characterization of the gene encoding the β-subunit of rat follicle-stimulating hormone. DNA 5:339-349.

Gharib, S. D., M. E. Wierman, M. A. Shupnik, and W. W. Chin. 1990. Molecular biology of the pituitary gonadotropins. Endocr. Rev. 11:177-199.

Gharib, S. D., M. E. Wierman, T. M. Badger, and W. W. Chin. 1987. Sex steroid hormone regulation of follicle-stimulating hormone subunit messenger ribonucleic acid (mRNA) levels in the rat. J. Clin. Invest. 80:294-299.

Glencross, R. G., I. B. Munro, B. E. Senior, and G. S. Pope. 1973. Concentrations of oestradiol-17β, oestrone and progesterone in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta Endocrinol. 73:374-384.

Godine, J. E., W. W. Chin, and J. F. Habener. 1980. Luteinizing and follicle-stimulating hormones. Cell-free translations of messenger RNAs coding for subunit precursors. J. Biol. Chem. 255:8780-8783.

Godine, J. E., W. W. Chin, and J. F. Habener. 1982. α subunit of rat pituitary glycoprotein hormones. J. Biol. Chem. 257:8368-8371.

Gomes, W. R., and R. E. Erb. 1965. Progesterone in bovine reproduction: A review. J. Dairy Sci. 48:314-330.

Gonzalez-Padilla, E., J. N. Wiltbank, and G. D. Niswender. 1975. Puberty in beef heifers. I. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091-1104.

Goodman, R. L., and F. J. Karsch. 1980. Pulsatile secretion of luteinizing hormone: Differential suppression by ovarian steroids. Endocrinology 107:1286-1290.

Goodman, R. L., L. E. Reichert, Jr., S. J. Legan, K. D. Ryan, D. L. Foster, and F. J. Karsch. 1981. Role of gonadotropins and progesterone in determining the preovulatory estradiol rise in the ewe. Biol. Reprod. 25:134-142.

Graves, W. E., J. W. Lauderdale, E. R. Hauser, and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows. Univ. Wisconsin Res. Bull. 270:23-26.

Green, J. D. 1951. The comparative anatomy of the hypophysis, with special reference to its blood supply and innervation. Am. J. Anat. 88:225-311.

Green, J. D., and G. W. Harris. 1947. The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol. 5:136-146.

Green, J. D., and G. W. Harris. 1949. Observation of the hypophysio-portal vessels of the living rat. J. Physiol. 108:359-361.

Greep, R. O. 1935. Separation of a thyrotropic from the gonadotropic substances of the pituitary. Am. J. Physiol. 110:692-699.

Gregg, D. W., and T. M. Nett. 1989. Direct effects of estradiol-17β on the number of gonadotropin-releasing hormone receptors in the ovine pituitary. Biol. Reprod. 40:288-293.

Grimard, B., P. Humblot, A. A. Ponter, J. P. Mialot, D. Sauvant, and M. Thibier. 1995. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. J. Reprod. Fertil. 104:173-179.

Guilbault, L. A., W. W. Thatcher, D. B. Foster, and D. Canton. 1984. Relationship of 15-keto-13, 14-dihydroprostaglandin $F_{2\alpha}$ concentrations in peripheral plasma with uterine production in F series prostaglandins and changes in uterine blood flow during the early postpartum period of cattle. Biol. Reprod. 31:870-878.

Gust, C. M., D. R. Deaver, R. A. Dailey, and E. K. Inskeep. 1984. Relationships between LH and estradiol-17β after removal of luteal progesterone in the ewe. J. Anim. Sci. 58:396-400.

Gwazdauskas, F. C. 1985. Effects of climate on reproduction in cattle. J. Dairy Sci. 68:1568-1578.

Gwazdauskas, F. C., J. A. Lineweaver, and M. L. McGilliard. 1983. Environmental and management factors affecting estrous activity in dairy cattle. J. Dairy Sci. 66:1510-1514.

Hackett, A. J., and H. D. Hafs. 1969. Pituitary and hypothalamic endocrine changes during the bovine estrous cycle. J. Anim. Sci. 28:531-536.

Haisenleder, D. J., A. C. Dalkin, G. A. Ortolano, J. C. Marshall, and M. A. Shupnik. 1991. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes: Evidence for differential regulation of transcription by pulse frequency in vivo. Endocrinology 128:509-517.

Haisenleder, D. J., A. L. Barkan, S. Papavasiliou, S. M. Zmeili, C. Dee, M. L. Jameel, G. A. Ortolano, M. R. El-Gewely, and J. C. Marshall. 1988. LH subunit mRNA concentrations during LH surge in ovariectomized estradiol-replaced rats. Am. J. Physiol. 254:E99-E103.

Haisenleder, D. J., G. A. Ortolano, A. C. Dalkin, T. R. Ellis, S. J. Paul, and J. C. Marshall. 1990. Differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone pulse amplitude in female rats. Endocrinology 127:2869-2875.

Halasz, B., and L. Pupp. 1965. Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. Endocrinology 77:553-562.

Halasz, B., L. Pupp, and S. Uhlarik. 1962. Hypophysiotrophic area in the hypothalamus. J. Endocrinol. 25:147-154.

Hall, J. B., K. K. Schillo, S. M. Hileman, and J. A. Boling. 1992. Does tyrosine act as a nutritional signal mediating the effects of increased feed intake on luteinizing hormone patterns in growth-restricted lambs? Biol. Reprod. 46:573-579.

Hall, S. H., and W. L. Miller. 1986. Regulation of ovine pituitary glycoprotein hormone alpha subunit mRNA by 17β-estradiol in cell culture. Biol. Reprod. 34:533-542.

Hamernik, D. L. 1995. Molecular biology of gonadotropins. J. Reprod. Fertil. (Suppl. 49):257-269.

Hamernik, D. L., C. M. Clay, A. Turzillo, E. A. Van Kirk, and G. E. Moss. 1995. Estradiol increases amounts of messenger ribonucleic acid for gonadotropin-releasing hormone receptors in sheep. Biol. Reprod. 53:179-185.

Hamernik, D. L., K. E. Kim, R. A. Maurer, and T. M. Nett. 1987. Progesterone does not affect the amount of mRNA for gonadotropins in the anterior pituitary gland of ovariectomized ewes. Biol. Reprod. 37:1225-1232.

Hamernik, D. L., M. E. Crowder, J. H. Nilson, and T. M. Nett. 1986. Measurement of messenger ribonucleic acid for luteinizing hormone β-subunit, α-subunit, growth hormone, and prolactin after hypothalamic pituitary disconnection in ovariectomized ewes. Endocrinology 119:2704-2710.

Hamernik, D. L., and T. M. Nett. 1988. Measurement of the amount of mRNA for gonadotropins during an estradiol-induced preovulatory-like surge of LH and FSH in ovariectomized ewes. Dom. Anim. Endocrinol. 5:129-139.

Hansel, W., and E. M. Convey. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57(Suppl. 2):404-423.

Hansel, W., and J. P. Dowd. 1986. New concepts of the control of corpus luteum function. J. Reprod. Fertil. 78:755-768.

Hansel, W., P. W. Concannon, and J. H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222-245.

Hauger, R. L., F. J. Karsch, and D. L. Foster. 1977. A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. Endocrinology 101:807-817.

Henricks, D. M., J. F. Dickey, and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346-351.

Henricks, D. M., J. F. Dickey, and J. R. Hill. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. Endocrinology 89:1350-1355.

Herbison, A. E., J. E. Robinson, and D. C. Skinner. 1993. Distribution of oestrogen receptor-immunoreactive cells in the preoptic area of the ewe: Co-localization with glutamic acid decarboxylase but not luteinizing hormone-releasing hormone. Neuroendocrinol. 57:751-759.

Herbison, A. E., T. L. Horvath, F. Naftolin, and C. Leranth. 1995. Distribution of oestrogen receptor-immunoreactive cells in monkey hypothalamus: Relationship to neurones containing luteinizing hormone-releasing hormone and tyrosine hydroxylase. Neuroendocrinol. 61:1-10.

Herring, P. T. 1908a. The development of the mammalian pituitary and its morphological significance. Quart. J. Exp. Physiol. 1:161-185.

Herring, P. T. 1908b. The histological appearances of the mammalian pituitary body. Quart. J. Exp. Physiol. 1:121-159.

Herring, R. D., D. L. Hamernik, J. P. Kile, M. E. Sousa, and T. M. Nett. 1991. Chronic administration of estradiol produces a triphasic effect on serum concentrations of gonadotropins and messenger ribonucleic acid for gonadotropin subunits, but not on pituitary content of gonadotropins, in ovariectomized ewes. Biol. Reprod. 45:151-156.

Houseknecht, K. L., C. A. Baile, R. L. Matteri, and M. E. Spurlock. 1998. The biology of leptin: A review. J. Anim. Sci. 76:1405-1420.

Hu, Y., J. D. H. Sanders, S. G. Kurz, J. S. Ottobre, and M. L. Day. 1990. In vitro prostaglandin production by bovine corpora lutea destined to be normal or short-lived. Biol. Reprod. 42:801-807.

Humphrey, W. D., C. C. Kaltenbach, T. G. Dunn, D. R. Koritnik, and G. D. Niswender. 1983. Characterization of hormonal patterns in the beef cow during postpartum anestrus. J. Anim. Sci. 56:445-453.

Hunter, M. G. 1991. Characteristics and causes of the inadequate corpus luteum. J. Reprod. Fertil. 43:91-99.

Hunter, M. G., J. A. Southee, B. J. McLeod, and W. Haresign. 1986. Progesterone pre-treatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes. J. Reprod. Fertil. 76:349-363.

Hunter, M. G., J. A. Southee, and G. E. Lamming. 1988. Function of abnormal corpora lutea in vitro after GnRH-induced ovulation in the anoestrous ewe. J. Reprod. Fertil. 84:139-148.

Hunter, M. G., V. J. Ayad, C. L. Gilbert, J. A. Southee, and D. C. Wathes. 1989. Role of prostaglandin F-2α and oxytocin in the regression of GnRH-induced abnormal corpora lutea in anoestrous ewes. J. Reprod. Fertil. 85:551-561.

Hurnik, J. F., and G. J. King. 1987. Estrous behavior in confined beef cows. J. Anim Sci. 65:431-438.

Hurnik, J. F., G. J. King, and H. A. Robertson. 1975. Estrous and related behavior in postpartum Holstein cows. Appl. Anim. Ethol. 2:55-68.

Ibrahim, S. N., S. M. Moussa, and G. V. Childs. 1986. Morphometric studies of rat anterior pituitary cells after gonadectomy: Correlation of changes in gonadotropes with the serum levels of gonadotropins. Endocrinology 119:629-637.

Illing, N., G. F. M. Jacobs, I. I. Becker, C. A. Flanagan, J. S. Davidson, A. Eales, W. Zhou, S. C. Sealfon, and R. P. Millar. 1993. Comparative sequence analysis and functional characterization of the cloned sheep gonadotropin-releasing hormone receptor reveal differences in primary structure and ligand specificity among mammalian receptors. Biochem. Biophys. Res. Comm. 196:745-751.

Imakawa, K, M. L. Day, D. D. Zalesky, A. Clutter, R. J. Kittock, and J. E. Kinder. 1987. Effects of 17 β-estradiol and diets varying in energy on secretion of luteinizing hormone in beef heifers. J. Anim. Sci. 64:805-815.

Imakawa, K., M. L. Day, D. D. Zalesky, M. Garcia-Winder, R. J. Kittok, and J. E. Kinder. 1986. Regulation of pulsatile LH secretion by ovarian steroids in the heifer. J. Anim. Sci. 63:162-168.

Inskeep, E. K., T. D. Braden, P. E. Lewis, M. Garcia-Winder, and G. D. Niswender. 1988. Receptors for luteinizing hormone and follicle-stimulating hormone in largest follicles of postpartum beef cows. Biol. Reprod. 38:587-591.

Ireland, J. J., A. D. Curato, and J. Wilson. 1983. Effect of charcoal-treated bovine follicular fluid on secretion of LH and FSH in ovariectomized heifers. J. Anim. Sci. 57:1512-1516.

Ireland, J. J., and J. F. Roche. 1982a. Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology 111:2077-2086.

Ireland, J. J., and J. F. Roche. 1982b. Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers. J. Reprod. Fert. 64:295-302.

Ireland, J. J., and J. F. Roche. 1983. Development of nonovulatory antral follicles in heifers: Changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 112:150-156.

Ireland, J. J., R. L. Fogwell, W. D. Oxender, K. Ames, and J. L. Cowley. 1984. Production of estradiol by each ovary during the estrous cycle of cows. J. Anim. Sci. 59:764-771.

Irvine, C. H. G., and S. L. Alexander. 1994. The dynamics of gonadotrophin-releasing hormone, LH and FSH secretion during the spontaneous ovulatory surge of the mare as revealed by intensive sampling of pituitary venous blood. J. Endocrinol. 140:283-295.

Jameson, J. L., C. B. Becker, C. M. Lindell, and J. F. Habener. 1988. Human follicle-stimulating hormone β-subunit gene encodes multiple messenger ribonucleic acids. Mol. Endocrinol. 2:806-815.

Jirikowski, G. F., I. Merchenthaler, G. E. Rieger, and W. E. Stumpf. 1986. Estradiol target sites immunoreactive for β-endorphin in the arcuate nucleus of the rat and mouse hypothalamus. Neurosci. Lett. 65:121-126.

Kaiser, U. B., A. Jakubowiak, A. Steinberger, and W. W. Chin. 1993. Regulation of rat pituitary gonadotropin-releasing hormone receptor mRNA levels in vivo and in vitro. Endocrinology 133:931-934.

Kaiser, U. B., A. Jakubowiak, A. Steinberger, and W. W. Chin. 1997. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels in vitro. Endocrinology 138:1224-1231.

Kaiser, U. B., D. Zhao, G. R. Cardona, and W. W. Chin. 1992. Isolation and characterization of cDNAs encoding the rat pituitary gonadotropin-releasing hormone receptor. Biochem. Biophys. Res. Comm. 189:1645-1652.

Kaiser, U. B., E. Sabbagh, R. A. Katzenellenbogen, P. M. Conn, and W. W. Chin. 1995. A mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone. Proc. Natl. Acad. Sci. 92:12280-12284.

Kakar, S. S., C. H. Rahe, and J. D. Neill. 1993. Molecular cloning, sequencing, and characterizing the bovine receptor for gonadotropin releasing hormone (GnRH). Dom. Anim. Endocrinol. 10:335-342.

Kaltenbach, C. C., T. G. Dunn, T. E. Kiser, L. R. Corah, A. M. Akbar, and G. D. Niswender. 1974. Release of FSH and LH in beef heifers by synthetic gonadotropin releasing hormone. J. Anim. Sci. 38:357-362.

Karsch, F. J., J. T. Cummins, G. B. Thomas, and I. J. Clarke. 1987. Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. Biol. Reprod. 36:1207-1218.

Kasa-Vubu, J. Z., G. E. Dahl, N. P. Evans, L. A. Thrun, S. M. Moenter, V. Padmanabhan, and F. J. Karsch. 1992. Progesterone blocks the estradiol-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 131:208-212.

Kesler, D. J., T. R. Troxel, and D. L. Hixon. 1980. Effect of days postpartum and exogenous GnRH on reproductive hormone and ovarian changes in postpartum suckled beef cows. Theriogenology 13:287-296.

Kesner, J. S., E. M. Convey, and C. R. Anderson. 1981. Evidence that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release. Endocrinology 108:1386-1391.

Kile, J. P., B. M. Alexander, G. E. Moss, D. M. Hallford, and T. M. Nett. 1991. Gonadotropin-releasing hormone overrides the negative effect of reduced dietary energy on gonadotropin synthesis and secretion in ewes. Endocrinology 128:843-849.

Kim, K. E., D. F. Gordon, and R. A. Maurer. 1988. Nucleotide sequence of the bovine gene for follicle-stimulating hormone β-subunit. DNA 7:227-233.

Klibanski, A., I. Z. Beitins, T. Badger, R. Little, and J. W. McArthur. 1981. Reproductive function during fasting in men. J. Clin. Endocrinol. Metab. 53:258-263.

Knobil, E. 1980. The neuroendocrine control of the menstrual cycle. Rec. Prog. Horm. Res. 36:53-88.

Kobayashi, Y., H. Kitai, R. Santulli, K. H. Wright, and E. E. Wallach. 1984. Influence of calcium and magnesium deprivation on ovulation and ovum maturation in the perfused rabbit ovary. Biol. Reprod. 31:287-295.

Krishna, A., K. Beesley, and P. F. Terranova. 1989. Histamine, mast cells and ovarian function. J. Endocrinol. 120:363-371.

Kurz, S. G., R. M. Dyer, Y. Hu, M. D. Wright, and M. L. Day. 1990. Regulation of luteinizing hormone secretion in prepubertal heifers fed an energy-deficient diet. Biol. Reprod. 43:450-456.

Lacroix, E., W. Eechaute, and I. Leusen. 1974. The biosynthesis of estrogens by cow follicles. Steroids 23:337-356.

Landefeld, T. D., F. J. P. Ebling, J. M. Suttie, L. A. Vannerson, V. Padmanabhan, I. Z. Beitins, and D. L. Foster. 1989. Metabolic interfaces between growth and reproduction. II. Characterization of changes in messenger ribonucleic acid concentrations of gonadotropin subunits, growth hormone, and prolactin in nutritionally growth-limited lambs and the differential effects of increased nutrition. Endocrinology 125:351-356.

Landefeld, T. D., J. Kepa, and F. Karsch. 1984. Estradiol feedback effects on the α-subunit mRNA in the sheep pituitary gland: Correlation with serum and pituitary luteinizing hormone concentrations. Proc. Natl. Acad. Sci. 81:1322-1326.

Layne, E. 1957. Spectrophotometric and Turbidimetric Methods for Measuring Protein. In: S. P. Colowick and N. O. Kaplan (Ed.) Methods in Enzymology, (Vol. III). pp 447-454. Academic Press, New York.

Lasley, J. F., and R. Bogart. 1943. Some factors influencing reproductive efficiency of range cattle under artificial and natural breeding conditions. Missouri Ag. Exp. Sta. Res. Bull. 376:1-56.

Lee, L. R., D. J. Haisenleder, J. C. Marshall, and M. S. Smith. 1989. Effects of progesterone on pulsatile luteinizing hormone (LH) secretion and LH subunit messenger ribonucleic acid during lactation in the rat. Endocrinology 124:2128-2134.

Lehman, M., J. E. Robinson, F. J. Karsch, and A. J. Silverman. 1986. Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) pathways in the sheep brain during anestrus and the mid-luteal phase of the estrous cycle. J. Comp. Neurol. 244:19-35.

Lehman, M. N., and F. J. Karsch. 1993. Do gonadotropin-releasing hormone, tyrosine hydroxylase- and β-endorphin-immunoreactive neurons contain oestrogen receptors? A double-label immunocytochemical study in the Suffolk ewe. Endocrinology 133:887-895.

Lemon, M., J. Pelletier, J. Saumande, and J. P. Signoret. 1975. Peripheral plasma concentrations of progesterone, oestradiol-17β and luteinizing hormone around oestrus in the cow. J. Reprod. Fertil. 42:137-140.

Leshin, L. S., L. A. Rund, J. W. Crim, and T. E. Kiser. 1988. Immunocytochemical localization of luteinizing hormone-releasing hormone and proopiomelanocortin neurons within the preoptic area and hypothalamus of the bovine brain. Biol. Reprod. 39:963-975.

Lishman, A. W., S. M. J. Allison, R. L. Fogwell, R. L. Butcher, and E. K. Inskeep. 1979. Follicular development and function of induced corpora lutea in underfed postpartum anestrous beef cows. J. Anim. Sci. 48:867-875.

Liu, J., P. D. Carriere, M. Dore, and J. Sirois. 1997. Prostaglandin G/H synthase-2 is expressed in bovine preovulatory follicles after the endogenous surge of luteinizing hormone. Biol. Reprod. 57:1524-1531.

Liu, Y. C., Y. Kato, K. Inoue, K. Tanaka, and K. Kurosumi. 1988. Co-localization of LHβ and FSHβ mRNAs in the porcine anterior pituitary by in situ hybridization with biotinylated probes. Biochem. Biophys. Res. Comm. 154:80-84.

Looper, M. L., R. P. Wettemann, T. Prado, and G. L. Morgan. 1998. Estrous behavior and time of ovulation of beef cows in summer and winter. J. Anim. Sci. 76(Suppl. 1):215 (Abstr.).

Loucks, A. B., E. M. Heath, T. Law, Sr., M. Verdun, and J. R. Watts. 1994. Dietary restriction reduces luteinizing hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. J. Clin. Endocrinol. Metab. 78:910-915.

Louis, T. M., J. N. Stellflug, H. A. Tucker, and H. D. Hafs. 1974. Plasma prolactin, growth hormone, luteinizing hormone and glucocorticoids after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 147:128-133.

Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74:473-482.

Marian, J., and P. M. Conn. 1983. Subcellular localization of the receptor for gonadotropin-releasing hormone in pituitary and ovarian tissue. Endocrinology 112:104-112.

Martin, T. E., D. M. Henricks, J. R. Hill, Jr., and N. C. Rawlings. 1978. Active immunization of the cow against oestradiol-17β. J. Reprod. Fertil. 53:173-178.

Maurer, R. A. 1985. Analysis of several bovine lutropin β subunit cDNAs reveals heterogeneity in nucleotide sequence. J. Biol. Chem. 260:4684-4687.

McCann, J. P., and W. Hansel. 1986. Relationships between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. Biol. Reprod. 34:630-641.

McCaughey, W. P., L. M. Rutter, and J. G. Manns. 1988. Effect of glucose infusion on metabolic and reproductive function in postpartum beef cows. Can. J. Anim. Sci. 68:1079-1087.

McClure, T. J., and J. Saunders. 1985. Effect of withholding food for 0-72 h on mating, pregnancy rate and pituitary function in female rats. J. Reprod. Fertil. 74:57-64.

McCracken, J. A., W. Schroamm, and W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of PGF $_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31-55.

McCutcheon, S. N., and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44-51.

McLeod, B. J., and W. Haresign. 1984. Evidence that progesterone may influence subsequent luteal function in the ewe by modulating preovulatory follicle development. J. Reprod. Fertil. 71:381-386.

McLeod, B. J., W. Haresign, and G. E. Lamming. 1982. Response of seasonally anoestrous ewes to small-dose multiple injections of GnRH with and without progesterone pretreatment. J. Reprod. Fertil. 65:223-230.

McShane, T. M., and D. H. Keisler. 1991. Effects of dietary energy on ovarian function, estrogen suppression of luteinizing hormone and follicle-stimulating hormone, and competency of the gonadotropin surge. Biol. Reprod. 45:486-492.

Mercer, J. E., J. A. Clements, J. W. Funder, and I. J. Clarke. 1988. Luteinizing hormone-β mRNA levels are regulated primarily by gonadotropin-releasing hormone and not by negative estrogen feedback on the pituitary. Neuroendocrinol. 47:563-566.

Merriam, G. R., and K. W. Wachter. 1982. Algorithms for the study of episodic hormone secretion. Am. J. Physiol. 243:E310-E318.

Moenter, S. M., A. Caraty, and F. J. Karsch. 1990. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 127:1375-1384.

Moss, G. E., M. E. Crowder, and T. M. Nett. 1981. GnRH-receptor interaction. VI. Effect of progesterone and estradiol on hypophyseal receptors for GnRH, and serum and hypophyseal concentrations of gonadotropins in ovariectomized ewes. Biol. Reprod. 25:938-944.

Moss, G. E., T. E. Adams, G. D. Niswender, and T. M. Nett. 1980. Effects of parturition and suckling on concentrations of pituitary gonadotropins, hypothalamic GnRH and pituitary responsiveness to GnRH in ewes. J. Anim. Sci. 50:496-502.

Murdoch, W. J., R. A. Dailey, and E. K. Inskeep. 1981. Preovulatory changes in prostaglandins E_2 and $F_{2\alpha}$ in ovine follicles. J. Anim. Sci. 53:192-205.

Murdoch, W. J., T. A. Peterson, E. A. Van Kirk, D. L. Vincent, and E. K. Inskeep. 1986. Interactive roles of progesterone, prostaglandins, and collagenase in the ovulatory mechanism of the ewe. Biol. Reprod. 35:1187-1194.

Nagatani, S., H. Tsukamura, and K. Maeda. 1994. Estrogen feedback needed at the paraventricular nucleus or A2 to suppress pulsatile luteinizing hormone release in fasting female rats. Endocrinology 135:870-875.

Nett, T. M. 1990. Regulation of genes controlling gonadotropin secretion. J. Anim. Sci. 68(Suppl. 2):3-17.

Nett, T. M., D. Cermak, T. Braden, J. Manns, and G. Niswender. 1987. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. I. Changes during the estrous cycle. Dom. Anim. Endocrinol., 4:123-132.

Nett, T. M., J. A. Flores, F. Carnevali, and J. P. Kile. 1990. Evidence for a direct negative effect of estradiol at the level of the pituitary gland in sheep. Biol. Reprod. 43:554-558.

Nikitovitch-Winer, M., and J. W. Everett. 1958. Functional restitution of pituitary grafts re-transplanted from kidney to median eminence. Endocrinology 63:916-930.

Nikitovitch-Winer, M., and J. W. Everett. 1959. Histocytologic changes in grafts of rat pituitary on the kidney and upon re-transplantation under the diencephalon. Endocrinology 65:357-368.

Nilson, J. H., A. R. Thomason, M. T. Cserbak, C. L. Moncman, and R. P. Woychik. 1983. Nucleotide sequence of a cDNA for the common α subunit of the bovine pituitary glycoprotein hormones. J. Biol. Chem. 258:4679-4682.

NRC. 1984. Nutrient requirements of beef cattle (6^{th} Ed.). National Academy Press, Washington, DC.

Odde, K. G., H. S. Ward, G. H. Kiracofe, R. M. McKee, and R. J. Kittok. 1980. Short estrous cycles and associated serum progesterone levels in beef cows. Theriogenology 14:105-112.

Ojeda, A. J., R. P. Wettemann, J. A. Vizcarra, and F. W. Owens. 1996. The effect of acute nutrient deprivation on LH secretion and blood components in steers. J. Anim. Sci. 74(Suppl 1):218 (Abstr.).

Oklahoma Agricultural Statistics. 1997. Central printing division, Oklahoma Department of Agriculture, Oklahoma City, OK.

O'Shea, J. D., R. J. Rodgers, and P. J. Wright. 1984. Morphometric analysis and function in vivo and in vitro of corpora lutea from ewes treated with LHRH during seasonal anoestrus. J. Reprod. Fertil. 72:75-85.

Padmanabhan, V., and E. M. Convey. 1981. Progesterone inhibits the ability of estradiol to increase basal and luteinizing hormone-releasing hormone-induced luteinizing hormone release from bovine pituitary cells in culture: Neither progesterone nor estradiol affects follicle-stimulating hormone release. Endocrinology 109:1091-1096.

Padmanabhan, V., J. S. Kesner, and E. M. Convey. 1978. Effects of estradiol on basal and luteinizing hormone releasing hormone (LHRH)-induced release of luteinizing hormone (LH) from bovine pituitary cells in culture. Biol. Reprod. 18:608-613.

Papavasiliou, S. S., S. Zmeili, L. Herbon, J. Duncan-Weldon, J. C. Marshall, and T. D. Landefeld. 1986. α and luteinizing hormone β messenger ribonucleic acid (RNA) of male and female rats after castration: Quantitation using an optimized RNA dot blot hybridization assay. Endocrinology 119:691-698.

Pau, K. Y. F., M. Berria, D. L. Hess, and H. G. Spies. 1993. Preovulatory gonadotropin-releasing hormone surge in ovary-intact rhesus macaques. Endocrinology 133:1650-1656.

Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540-543.

Pendergrass, P. B., and P. Talbot. 1979. The distribution of contractile cells in the apex of the preovulatory hamster follicle. Biol. Reprod. 20:205-213.

Perheentupa, A., and I. Huhtaniemi. 1990. Gonadotropin gene expression and secretion in gonadotropin-releasing hormone antagonist-treated male rats: Effect of sex steroid replacement. Endocrinology 126:3204-3209.

Perry, R. C., L. R. Corah, G. H. Kiracofe, J. S. Stevenson, and W. E. Beal. 1991. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. J. Anim. Sci. 69:2548-2555.

Peter, A. T., W. T. K. Bosu, R. M. Liptrap, and E. Cummings. 1989. Temporal changes in serum prostaglandin $F_{2\alpha}$ and oxytocin in dairy cows with short luteal phases after the first postpartum ovulation. Theriogenology 32:277-284.

Phillips, C. L., L. W. Lin, J. C. Wu, K. Guzman, A. Milsted, and W. L. Miller. 1988. 17β-estradiol and progesterone inhibit transcription of the genes encoding the subunits of ovine follicle-stimulating hormone. Mol. Endocrinol. 2:641-649.

Pierce, J. G., and T. F. Parsons. 1981. Glycoprotein hormones: Structure and function. Ann. Rev. Biochem. 50:465-495.

Pinski, J., N. Lamharzi, G. Halmos, K. Groot, A. Jungwirth, M. Vadillo-Buenfil, S. S. Kakar, and A. V. Schally. 1996. Chronic administration of the luteinizing hormone-releasing hormone (LHRH) antagonist Cetrorelix decreases gonadotrope responsiveness and pituitary LHRH receptor messenger ribonucleic acid levels in rats. Endocrinology 137:3430-3436.

Pomerantz, L., and M. G. Mulinos. 1939. Pseudo-hypophysectomy produced by inanition. Am. J. Physiol. 126:P601 (Abstr.).

Pratt, B. R., J. G. Berardinelli, L. P. Stevens, and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestogen and estrogen. J. Anim. Sci. 54:822-829.

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton, and P. G. Harms. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: Dependence upon the period of the cycle. Endocrinology 107:498-503.

Rajamahendran, R., P. C. Lague, and R. D. Baker. 1979. Estrus and LH release in ovariectomized heifers following vaginal devices containing ovarian steroids. J. Anim. Sci. 49:554-559.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittock. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 15:613-623.

Ramirez-Godinez, J. A., G. H. Kiracofe, R. R. Schalles, and G. D. Niswender. 1982. Endocrine patterns in the postpartum beef cow associated with weaning: A comparison of the short and subsequent normal cycles. J. Anim. Sci. 55:153-158.

Rasby, R. J., R. P. Wettemann, R. D. Geisert, J. J. Wagner, and K. S. Lusby. 1991. Influence of nutrition and body condition on pituitary, ovarian, and thyroid function of nonlactating beef cows. J. Anim. Sci. 69:2073-2080.

Rawlings, N. C., L. Weir, B. Todd, J. Manns, and J. H. Hyland. 1980. Some endocrine changes associated with the post-partum period of the suckling beef cow. J. Reprod. Fertil. 60:301-308.

Reich, R., A. Tsafriri, and G. L. Mechanic. 1985. The involvement of collagenolysis in ovulation in the rat. Endocrinology 116:522-527.

Reinhart, J., L. M. Mertz, and K. J. Catt. 1992. Molecular cloning and expression of cDNA encoding the murine gonadotropin-releasing hormone receptor. J. Biol. Chem. 267:21281-21284.

Rhodes, F. M., K. W. Entwistle, and J. E. Kinder. 1996. Changes in ovarian function and gonadotropin secretion preceding the onset of nutritionally induced anestrus in Bos indicus heifers. Biol. Reprod. 55:1437-1443.

Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989a. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520-1526.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989b. Nutritional anestrus in beef cows: Concentrations of glucose and nonesterified fatty acids in plasma and insulin in serum. J. Anim. Sci. 67:2354-2362.

Roberson, M. S., M. W. Wolfe, T. T. Stumpf, D. L. Hamernik, A. S. Cupp, L. A. Werth, N. Kojima, R. J. Kittok, H. E. Grotjan, and J. E. Kinder. 1992. Steady-state amounts of α- and luteinizing hormone (LH) β-subunit messenger ribonucleic acids are uncoupled from pulsatility of LH secretion during sexual maturation of the heifer. Biol. Reprod. 46:435-441.

Robertson, D. M., C. G. Tsonis, R. I. McLachlan, D. J. Handelsman, R. Leask, D. T. Baird, A. S. McNeilly, S. Hayward, D. L. Healy, J. K. Findlay, H. G. Burger, and D. M. de Kretser. 1988. Comparison of inhibin immunological and in vitro biological activities in human serum. J. Clin. Endocrinol. Metab. 67:438-443.

Robertson, D. M., L. M. Foulds, L. Leversha, F. J. Morgan, M. T. W. Hearn, H. G. Burger, R. E. H. Wettenhall, and D. M. de Kretser. 1985. Isolation of inhibin from bovine follicular fluid. Biochem. Biophys. Res. Comm. 126:220-226.

Rodgers, R. J., M. R. Waterman, and E. R. Simpson. 1986. Cytochromes P-450_{scc}, P-$450_{17\alpha}$, adrenodoxin, and reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase in bovine follicles and corpora lutea. Changes in specific contents during the ovarian cycle. Endocrinology 118:1366-1374.

Rodriguez, R. E., and M. E. Wise. 1989. Ontogeny of pulsatile secretion of gonadotropin-releasing hormone in the bull calf during infantile and pubertal development. Endocrinology 124:248-256.

Rutter, L. M., R. D. Randel, G. T. Schelling, and D. W. Forrest. 1983. Effect of abomasal infusion of propionate on the GnRH-induced luteinizing hormone release in prepubertal heifers. J. Anim. Sci. 56:1167-1173.

Rutter, L. M., R. Snopek, and J. G. Manns. 1989. Serum concentrations of IGF-I in postpartum beef cows. J. Anim. Sci. 67:2060-2066.

Rutter, L. M., T. D. Carruthers, and J. G. Manns. 1985. The postpartum induced corpus luteum: Functional differences from that of cycling cows and the effects of progesterone pretreatment. Biol. Reprod. 33:560-568.

Saade, G., D. R. London, and R. N. Clayton. 1989. The interaction of gonadotropin-releasing hormone and estradiol on luteinizing hormone and prolactin gene expression in female hypogonadal (hpg) mice. Endocrinology 124:1744-1753.

Sakurai, H., B. M. Adams, and T. E. Adams. 1997. Concentrations of GnRH receptor and GnRH receptor mRNA in pituitary tissue of orchidectomized sheep: Effect of oestradiol, progesterone, and progesterone withdrawal. J. Endocrinol. 152:91-98.

Sarkar, D. K., and G. Fink. 1979. Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. J. Endocrinol. 80:303-313.

Sarkar, D. K., S. A. Chiappa, and G. Fink. 1976. Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature 264:461-463.

SAS. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Inst., Inc. Cary, NC.

Savoy-Moore, R. T., N. B. Schwartz, J. A. Duncan, and J. C. Marshall. 1980. Pituitary gonadotropin-releasing hormone receptors during the rat estrous cycle. Science 209:942-944.

Schallenberger, E. D., and A. J. Peterson. 1982. Effect of ovariectomy on tonic gonadotropin secretion in cyclic and post-partum dairy cows. J. Reprod. Fertil. 64:47-52.

Schallenberger, E., D. Schams, B. Bullermann, and D. L. Walters. 1984. Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fertil. 71:493-501.

Schally, A. V., A. Arimura, Y. Baba, R. M. G. Nair, H. Matsuo, T. W. Redding, L. Debeljuk, and W. F. White. 1971. Isolation and properties of the FSH and LH-releasing hormone. Biochem. Biophys. Res. Comm. 43:393-399.

Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70:1271-1282.

Schoenemann, H. M., W. D. Humphrey, M. E. Crowder, T. M. Nett, and J. J. Reeves. 1985. Pituitary luteinizing hormone-releasing hormone receptors in ovariectomized cows after challenge with ovarian steroids. Biol. Reprod. 32:574-583.

Schreihofer, D. A., D. B. Parfitt, and J. L. Cameron. 1993a. Suppression of luteinizing hormone secretion during short-term fasting in male rhesus monkeys: The role of metabolic versus stress signals. Endocrinology 132:1881-1889.

Schreihofer, D. A., J. A. Amico, and J. L. Cameron. 1993b. Reversal of fasting-induced suppression of luteinizing hormone (LH) secretion in male rhesus monkeys by intragastric nutrient infusion: Evidence for rapid stimulation of LH by nutritional signals. Endocrinology 132:1890-1897.

Sealfon, S. C., S. C. Laws, J. C. Wu, B. Gillo, and W. L. Miller. 1990. Hormonal regulation of gonadotropin-releasing hormone receptors and messenger RNA activity in ovine pituitary culture. Mol. Endocrinol. 4:1980-1987.

Self, D. A., A. R. Koch, and P. C. Schroeder. 1989. Relaxation-induced contraction of smooth muscle surrounding hamster ovarian follicles. J. Reprod. Fertil. 85:593-603.

Selk, G. E., R. P. Wettemann, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby, and J. C. Garmendia. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153-3159.

Shipka, M. P., C. B. Campbell, T. L. Wierenga, K. E. Panter, and R. C. Lamb. 1998. Use of radiotelemetry, visual observation and progesterone assay to determine true silent ovulation. J. Dairy Sci. 81(Suppl.1):216 (Abstr.).

Shivers, B. D., R. E. Harlan, J. I. Morrell, and D. W. Pfaff. 1983. Absence of oestradiol concentration in cell nuclei of GnRH-immunoreactive neurons. Nature 304:345-347.

Short, R. E., B. E. Howland, R. D. Randel, D. S. Christensen, and R. A. Bellows. 1973. Induced LH release in spayed cows. J. Anim. Sci. 37:551-557.

Shupnik, M. A., S. D. Gharib, and W. W. Chin. 1988. Estrogen suppresses rat gonadotropin gene transcription in vivo. Endocrinology 122:1842-1846.

Sirois, J., and M. Dore. 1997. The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process. Endocrinology 138:4427-4434.

Sisk, C. L., and F. H. Bronson. 1986. Effects of food restriction and restoration on gonadotropin and growth hormone secretion in immature male rats. Biol. Reprod. 35:554-561.

Sisson, S. and J. D. Grossman. 1953. The Anatomy of the Domestic Animals. W. B. Saunders Co., Philadelphia.

Smith, G. D., H. R. Sawyer, M. A. Mirando, M. D. Griswold, A. Sadhu, and J. J. Reeves. 1996. Steady-state luteinizing hormone receptor messenger ribonucleic acid levels and endothelial cell composition in bovine normal- and short-lived corpora lutea. Biol. Reprod. 55:902-909.

Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. J. Anim. Sci. 36:391-396.

Spicer, L. J., and C. C. Francisco. 1997. The adipose obese gene product, leptin: Evidence of a direct inhibitory role in ovarian function. Endocrinology 138:3374-3379.

Spicer, L. J., and C. C. Francisco. 1998. Adipose obese gene product, leptin, inhibits bovine ovarian thecal cell steroidogenesis. Biol. Reprod. 58:207-212.

Spicer, L. J., E. Alpizar, and S. E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. J. Anim. Sci. 71:1232-1241.

Spicer, L. J., M. A. Crowe, D. J. Prendiville, D. Goulding, and W. J. Enright. 1992. Systemic but not intraovarian concentrations of insulin-like growth factor-I are affected by short-term fasting. Biol. Reprod. 46:920-925.

Spicer, L. J., and S. E. Echternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Dom. Anim. Endocrinol. 12:223-245.

Spitzer, J. C., D. G. Morrison, R. P. Wettemann, and L. C. Faulkner. 1995. Reproductive responses and calf birth weight and weaning weights as affected by body condition at parturition and postpartum weight gain in primiparous beef cows. J. Anim. Sci. 73:1251-1257.

Stabenfeldt, G. H., L. L. Ewing, and L. E. McDonald. 1969. Peripheral plasma progesterone levels during the bovine oestrous cycle. J. Reprod. Fertil. 19:433-442.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. LeFever. 1996. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. J. Anim. Sci. 74:729-735.

Stojilkovic, S. S., J. Reinhart, and K. J. Catt. 1994. Gonadotropin-releasing hormone receptors: Structure and signal transduction pathways. Endocr. Rev. 15:462-499.

Stojilkovic, S.S., T. Iida, M. Cesnjaj, and K. J. Catt. 1992. Differential actions of endothelin and gonadotropin-releasing hormone in pituitary gonadotrophs. Endocrinology 131:2821-2828.

Stumpf, T. T., M. L. Day, M. W. Wolfe, A. C. Clutter, J. A. Stotts, P. L. Wolfe, R. J. Kittok, and J. E. Kinder. 1989. Effect of estradiol on secretion of luteinizing hormone during the follicular phase of the bovine estrous cycle. Biol. Reprod. 41:91-97.

Stumpf, T. T., M. W. Wolfe, M. S. Roberson, G. Caddy, R. J. Kittok, B. D. Schanbacher, H. E. Grotjan, and J. E. Kinder. 1992. Bovine luteinizing hormone (LH) isoforms and amounts of messenger ribonucleic acid for alpha- and LH beta-subunits in pituitaries of cows immunized against LH-releasing hormone. Biol. Reprod. 47:776-81.

Swanson, L. V., and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038-1041.

Swanson, L. V., H. D. Hafs, and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoid from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284-293.

Swanson, L. V., K. T. Kirton, A. J. Hackett, and H. D. Hafs. 1971. Pituitary and blood plasma levels of gonadotropin after ovariectomy of heifers. J. Anim. Sci. 32:678-681.

Swerdloff, R. S., M. Peterson, A. Vera, R. A. Batt, D. Hever, and G. A. Bray. 1978. The hypothalamic-pituitary axis in genetically obese (ob/ob) mice: Response to luteinizing hormone-releasing hormone. Endocrinology 103:542-547.

Talmadge, K., N. C. Vamvakopoulos, and J. C. Fiddes. 1984. Evolution of the genes for the β subunits of human chorionic gonadotropin and luteinizing hormone. Nature 307:37-40.

Tanaka, N., L. L. Espey, and H. Okamura. 1989. Increase in ovarian blood volume during ovulation in the gonadotropin-primed immature rat. Biol. Reprod. 40:762-768.

Tepper, M. A., and J. L. Roberts. 1984. Evidence for only one β-luteinizing hormone and no β-subunit chorionic gonadotropin gene in the rat. Endocrinology 115:385-391.

Thomas, G. B., J. E. Mercer, T. Karalis, A. Rao, J. T. Cummins, and I. J. Clarke. 1990. Effect of restricted feeding on the concentrations of growth hormone (GH), gonadotropins, and prolactin (PRL) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotropin subunits, and PRL in the pituitary glands of adult ovariectomized ewes. Endocrinology 126:1361-1367.

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Univ. Nebraska Ag. Exp. Sta. Res. Bull. 153:3-26.

Troxel, T. R., and D. J. Kesler. 1984. Ability of indomethacin to alter prostaglandin metabolite concentrations and to enhance the function of corpora lutea induced in postpartum suckled beef cows. J. Anim. Sci. 59:177-181.

Tsutsumi, M., W. Zhou, R. P. Millar, P. L. Mellon, J. L. Roberts, C. A. Flanagan, K. Dong, B. Gillo, and S. C. Sealfon. 1992. Cloning and functional expression of a mouse gonadotropin-releasing hormone receptor. Mol. Endocrinol. 6:1163-1169.

Turzillo, A. M., C. E. Campion, C. M. Clay, and T. M. Nett. 1994. Regulation of gonadotropin-releasing hormone (GnRH) receptor messenger ribonucleic acid and GnRH receptors during the early preovulatory period in the ewe. Endocrinology 135:1353-1358.

Turzillo, A. M., G. B. Di Gregorio, and T. M. Nett. 1995a. Messenger ribonucleic acid for gonadotropin-releasing hormone receptor and numbers of gonadotropin-releasing hormone receptors in ovariectomized ewes after hypothalamic-pituitary disconnection and treatment with estradiol. J. Anim. Sci. 73:1784-1788.

Turzillo, A. M., J. L. Juengel, and T. M. Nett. 1995b. Pulsatile gonadotropin-releasing hormone (GnRH) increases concentrations of GnRH receptor messenger ribonucleic acid and numbers of GnRH receptors during luteolysis in the ewe. Biol. Reprod. 53:418-423.

USDA. 1986. Livestock and meat situation. Economic research service. Government Printing Office, Washington, DC.

USDA. 1987. Livestock and meat situation. Economic research service. Government Printing Office, Washington, DC.

USDA. 1988. Livestock and meat situation. Economic research service. Government Printing Office, Washington, DC.

USDA Census of Agriculture. 1997. USDA National Agricultural Statistics Service home page. Available at: http://www.usda.gov/nass/. Accessed March 23, 1999.

Vailes, L. D., and J. H. Britt. 1990. Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. J. Anim. Sci. 68:2333-2339.

Vallet, J. L., G. E. Lamming, and M. Batten. 1990. Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe. J. Reprod. Fertil. 90:625-634.

Veldhuis, J. D., A. Iranmanesh, W. S. Evans, G. Lizarralde, M. O. Thorner, and M. L. Vance. 1993. Amplitude suppression of the pulsatile mode of immunoradiometric luteinizing hormone release in fasting-induced hypoandrogenemia in normal men. J. Clin. Endocrinol. Metab. 76:587-593.

Virgin, J. B., J. B. Silver, A. R. Thomason, and J. H. Nilson. 1985. The gene for the β subunit of bovine luteinizing hormone encodes a gonadotropin mRNA with an unusually short 5′-untranslated region. J. Biol. Chem. 260:7072-7077.

Vizcarra, J. A., R. P. Wettemann, J. C. Spitzer, and D. G. Morrison. 1998. Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and nonesterified fatty acids in plasma of primiparous beef cows. J. Anim. Sci. 76:927:936.

Vizcarra, J. A., R. P. Wettemann, T. D. Braden, A. M. Turzillo, and T. M. Nett. 1997. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology 138:594-601.

Wade, N. 1981. The Nobel Dual. Anchor Press Doubleday, New York, NY.

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603-612.

Walker, W. L., R. L. Nebel, and M. L. McGillard. 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci. 79:1555-1561.

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn, and C. C. Kaltenbach. 1982. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod. 26:647-654.

Wang, Q., C. Bing, K. Al-Barazanji, D. E. Mossakowaska, X. Wang, D. L. McBay, W. A. Neville, M. Taddayon, L. Pickavance, S. Dryden, M. E. A. Thomas, M. T. McHale, I. S. Gloyer, S. Wilson, R. Buckingham, J. R. S. Arch, P. Trayhurn, and G. Williams. 1997. Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. Diabetes 46:335-341.

Werth, L. A., J. C. Whittier, S. M. Azzam, G. H. Deutscher, and J. E. Kinder. 1996. Relationship between circulating progesterone and conception at the first postpartum estrus in young primiparous beef cows. J. Anim. Sci. 74:616-619.

Wettemann, R. P., T. W. Beck, E. J. Turman, and R. L. Hintz. 1982. Endocrine response of postpartum anestrous beef cows to GnRH or PMSG. Theriogenology 18:599-613.

Wettemann, R. P., E. J. Turman, R. D. Wyatt, and R. Totusek. 1978. Influence of suckling intensity on reproductive performance of range cows. J. Anim. Sci. 47:342-346.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton, and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020-1024.

Whisnant, C. S., T. E. Kiser, F. N. Thompson, and J. B. Hall. 1985. Effect of nutrition on the LH response to calf removal and GnRH. Theriogenology 24:565-573.

Wildt, L., A. Hausler, G. Marshall, J. S. Hutchison, T. M. Plant, P. E. Belchetz, and E. Knobil. 1981a. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. Endocrinology 109:376-385.

Wildt, L., J. S. Hutchison, G. Marshall, C. R. Pohl, and E. Knobil. 1981b. On the site of action of progesterone in the blockade of the estradiol-induced gonadotropin discharge in the rhesus monkey. Endocrinology 109:1293-1294.

Williams, G. L., and D. E. Ray. 1980. Hormonal and reproductive profiles of early postpartum beef heifers after prolactin suppression or steriod-induced luteal function. J. Anim. Sci. 50:906-918.

Windle, J. J., R. I. Weiner, and P. L. Mellon. 1990. Cell lines of the pituitary gonadotrope lineage derived by target oncogenesis in transgenic mice. Mol. Endocrinol. 4:597-603.

Winters, S. J. 1996. Relationship between gonadotropin subunit messenger ribonucleic acid levels and plasma gonadotropin concentrations in intact and orchidectomized adult rats. Biol Reprod. 55:828-832.

Wise, M. E., J. H. Nilson, M. T. Nejedlik, and T. M. Nett. 1985. Measurement of messenger RNA for luteinizing hormone β-subunit and α-subunit during gestation and the postpartum period in ewes. Biol. Reprod. 33:1009-1015.

Wislocki, G. B., and L. S. King. 1936. The permeability of the hypophysis and hypothalamus to vital dyes, with a study of the hypophyseal vascular supply. Am. J. Anat. 58:421-472.

Wu, J. C., S. C. Sealfon, and W. L. Miller. 1994. Gonadal hormones and gonadotropin-releasing hormone (GnRH) alter messenger ribonucleic acid levels for GnRH receptors in sheep. Endocrinology 134:1846-1850.

Yasin, M., A. C. Dalkin, D. J. Haisenleder, J. R. Kerrigan, and J. C. Marshall. 1995. Gonadotropin-releasing hormone (GnRH) pulse pattern regulates GnRH receptor gene expression: Augmentation by estradiol. Endocrinology 136:1559-1564.

Yelich, J. V., R. P. Wettemann, H. G. Dolezal, K. S. Lusby, D. K. Bishop, and L. J. Spicer. 1995. Effects of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like growth factor I, insulin, and metabolites before puberty in beef heifers. J. Anim. Sci. 73:2390-2405.

Yu, W. H., M. Kimura, A. Walczewska, S. Karanth, and S. M. McCann. 1997. Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci. 94:1023-1028.

Zmeili, S. M., S. S. Papavasiliou, M. O. Thorner, W. S. Evans, J. C. Marshall, and T. D. Landefeld. 1986. Alpha- and LH beta subunit mRNAs during the rat estrous cycle. Endocrinology 119:1867-1869.

Zollers, W. G., Jr., H. A. Garverick, and M. F. Smith. 1989. Oxytocin-induced release of prostaglandin $F_{2\alpha}$ in postpartum beef cows; comparison of short versus normal luteal phases. Biol. Reprod. 41:262-267.

Zollers, W. G., Jr., H. A. Garverick, M. F. Smith, R. J. Moffatt, B. E. Salfen, and R. S. Youngquist. 1993. Concentrations of progesterone and oxytocin receptors in endometrium of postpartum cows expected to have a short or normal oestrous cycle. J. Reprod. Fertil. 97:329-337.

Zolman, J., E. M. Convey, and J. H. Britt. 1974. Relationships between luteinizing hormone response to gonadotropin releasing hormone and endogenous steroids. J. Anim. Sci. 39:355-359.

Zolman, J., E. M. Convey, J. H. Britt, and H. D. Hafs. 1973. Release of bovine luteinizing hormone by purified porcine and synthetic gonadotropin releasing hormone. Proc. Soc. Exp. Biol. Med. 142:189-193.

VITA

Michael L. Looper

Candidate for the Degree of

Doctor of Philosophy

Thesis: BODY ENERGY RESERVES AND STEROIDS REGULATE GONADOTROPINS AND REPRODUCTION IN BEEF COWS

Major Field: Animal Breeding and Reproduction

Biographical:

Personal Data: Born in Fort Smith, Arkansas, July 31, 1969; the son of Jim and Sue Looper.

Education: Graduated from Greenwood High School, Greenwood, Arkansas in May, 1987; received Bachelor of Science degree in Animal Science from University of Arkansas, Fayetteville, Arkansas in December, 1992; received Master of Science degree from University of Arkansas, Fayetteville, Arkansas in December, 1994. Completed the requirements for the Doctor of Philosophy degree at Oklahoma State University, Stillwater, Oklahoma, in July, 1999.

Experience: Graduate research and teaching assistant, University of Arkansas, Department of Animal Science, 1993 to 1994. Graduate research and teaching assistant, Oklahoma State University, 1995 to May, 1999.

Professional Organizations: American Society of Animal Science, American Dairy Science Association, Society for the Study of Reproduction, Society for Experimental Biology and Medicine, American Registry of Professional Animal Scientists, The Endocrine Society, American Association for the Advancement of Science, Sigma Xi Scientific Research Society, Gamma Sigma Delta, Phi Kappa Phi, Alpha Zeta.