REGULATION OF GONADOTROPIN SECRETION IN BEEF COWS BY GONADOTROPIN RELEASING HORMONE (GNRH) AND BODY ENERGY RESERVES

DAVID K. BISHOP

Master of Science

Oklahoma State University

Stillwater, Oklahoma

1991

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
December, 1993

REGULATION OF GONADOTROPIN SECRETION IN BEEF COWS BY GONADOTROPIN RELEASING HORMONE (GNRH) AND BODY ENERGY RESERVES

Thesis Approved:

Robert P. Wettemann
Thesis Adviser

Jim Bah

Keith L. Lusby

Russell C. Cl

Barbara J. Steeckel

Thomas C. Collins
Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to express appreciation to Dr. R. P. Wettemann for sharing a part of his expertise and a lot of his time in all phases of the research reported in this manuscript. I am especially thankful to him for friendship that he has shared during the extended period of my education in areas of animal sciences. I am also grateful to Drs. Keith Lusby and Archie Clutter of the Department of Animal Sciences, Dr. Tim Braden from Zoology and Dr. Barbara Stoecker from Nutritional Sciences for their advisement as members of my committee. Additionally, special thanks is extended to Dr. Leon Spicer for his help with parts of this research.

I also wish to thank Steve Welty, Mark Anderson, David Cox, Randy Jones and David Gay for the care and management of the research animals involved in these studies and for the extra effort involved in collection of data from those animals. Assistance in analysis of samples in the laboratory was provided by LaRuth Mackey, Karen Rogers and Brenita Randle. I am grateful to Paula Cinnamon for her friendship and assistance in preparation of the manuscripts from these data.

Many of my fellow graduate students have assisted with data collection and analysis. Special thanks are extended

to Cassie Scott and Jorge Vizcarra and their families for their friendship and assistance.

Finally, I am thankful for my wife and family for their love and understanding during this research. This manuscript is dedicated in loving memory to my grandmother, Mattie A. Bishop.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Beef cows experience periods of heightened sexual arousal prior to ovulation. This excitation, and the receptivity associated with it, is necessary for mating by natural service of a bull or for maximum success with artificial insemination. Cyclic patterns of estrus occur after a female reaches puberty and, if conditions are adequate, are maintained until the heifer becomes pregnant. After expulsion of the fetus the cow experiences a period of anestrus after which she initiates estrous cycles. Control of the timing of events necessary for puberty, and minimizing the interval from parturition to the onset of normal cycles is necessary to increase reproductive efficiency in beef females.

Although not all cows that are exposed to fertile bulls during estrus become pregnant (Short et al., 1990) or wean a calf (Bellows et al. 1979), failure of cows to initiate estrous cycles is the main reason for reduced pregnancy rates. Interactions in the hypothalamic-pituitary-ovarian axis control reproduction and determine the length of anestrous periods. Both gonadotropins,

luteinizing hormone (LH) and follicle stimulating hormone (FSH) are synthesized and released by the same gonadotropes in the pituitary (Childs et al., 1987) and are released in response to gonadotropin-releasing hormone (GnRH) which is secreted by the hypothalamus. The onset of estrous cycles at puberty in heifers (Kinder et al., 1987) or after parturition in beef cattle (Riley et al., 1981; Walters et al., 1982) occurs in response to increased pulsatile secretion of LH from the anterior pituitary.

Cows fed restricted diets release more LH in response to exogenous GnRH (Whisnant et al., 1985; Rasby et al., 1991) and have increased GnRH in the infundibular stalk-median eminence (Rasby et al., 1992). Pulsatile infusion of GnRH increased the concentration of LH in serum and initiated luteal activity in nutritionally anestrous beef cows (Bishop and Wettemann, 1993a). A proposed pulse generator, similar to that described in the hypothalamus of the Rhesus monkey (Krey et al., 1975), may regulate release of GnRH and therefore gonadotropins in cattle. Metabolic cues associated with nutrition and body energy reserves may influence the function of the pulse generator.

Two experiments were conducted to elucidate the mechanisms through which nutrition, body energy reserves and GnRH influence pulsatile secretion of gonadotropins in cattle. The specific objectives were: 1) to determine if body energy reserves have a direct effect on the pituitary to regulate gonadotropin secretion in response to an

analogue to GnRH, and 2) to determine if exogenous gonadotropins will stimulate follicular growth in heifers that are anestrus as a result of immunization against GnRH.

CHAPTER II

REVIEW OF LITERATURE

Endocrinology is the science of chemical messengers (hormones) which are produced by endocrine glands. The constant amount of hormone found most frequently in blood is defined as the basal concentration (Brinkley, 1981). A pulse of a hormone is a short-term increase in hormone concentration above the preceding concentration in blood which is usually of less amplitude and shorter duration than a surge of the hormone. A surge of hormone is a large increase in concentration which is detectable for an extended period of time. Pulses of reproductive hormones are integrative signals and surges are associated with dramatic changes in morphology of tissues such as ovulation and luteinization of follicles (Brinkley, 1981). GnRH is synthesized by neurons in the hypothalamus and is present in hypophyseal portal blood of Rhesus monkeys (Carmel et al., 1976), sheep (Clarke and Cummins, 1982; Clarke et al., 1987a), and cattle (Rodriques and Wise, 1989). The pattern of secretion of GnRH represents the integration of neural stimuli that are translated into endocrine signals (Knobil, 1980; Knobil, 1981).

Gonadotropin Releasing Hormone

GnRH is a decapeptide (Schally et al., 1971; Matsuo et al., 1971; Baba et al., 1971) with a similar structure (pyro-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) for virtually all domestic mammals. GnRH is synthesized in the hypothalamus. It binds specifically with its receptors on the plasma membrane of cells in the pituitary (Wise et al., 1984) which results in synthesis and secretion of LH and FSH (Naor and Childs, 1986). Both LH and FSH are synthesized and released by the same gonadotrope (Childs et al., 1987). The chemical nature of GnRH, some metabolically stable analogs and antagonists, and molecular events occurring after GnRH binds to its receptor on the plasma membrane have been reviewed (Conn, 1986; Conn et al., 1987; Braden and Conn, 1992). GnRH action is mediated through G-proteins, suggesting specific second messenger systems are associated with GnRH functions. Specifically, calcium-sensitive protein kinase C is involved in synthesis of specific subunits of gonadotropins. Protein kinase C and another calcium-activated calmodulin function in up-regulation of receptors for GnRH, and calmodulin alone mediates the release of gonadotropins from gonadotropes. Down-regulation of receptors for GnRH is independent of calcium regulation and is mediated through inositol phosphate pathways (Braden and Conn, 1992). However, the mechanisms of action of GnRH are not yet well defined

because desensitization of gonadotropes was later found to be independent of inositol phosphate production (Hawes and Conn, 1992). The structure of the GnRH receptor (Tsutsumi et al., 1992) is similar to other receptors which use G proteins in cellular responses. Using G protein activators (sodium fluoride, Waters et al., 1990; cholera toxin, Hawes et al., 1993) and an inhibitor (pertussis toxin, Hawes et al., 1993), it was concluded that multiple G proteins are involved in desensitization of GnRH receptors, inositol phosphate production and LH release from pituitary cells in culture.

Concentrations of GnRH in hypophyseal portal blood vary in a rhythmical pattern. Patterns of GnRH release were not highly correlated with pulses of LH in serum because a pulse of GnRH did not necessarily stimulate the release of enough LH to be identified as a pulse (Nett et al., 1974). Clarke and Cummins (1982) determined that the pulsatile secretion of LH is a direct result of pulsatile release of GnRH from the hypothalamus via the hypothalamic-hypophyseal portal vessels. Pulsatile secretion of LH, first observed in the Rhesus monkey (Dierschke et al., 1970), led to the description of a pulse generator for GnRH located in the medial basal hypothalamus (Knobil, 1981). The location of the pulse generator within GnRH secretory neurons in the arcuate nucleus-median eminence, and the identification of adrenergic and neuropeptide secretory

neurons that influence the release of GnRH in primates, were reviewed by Terasawa and Gore (1992).

The number of GnRH receptors and the amount of GnRH in circulation (Wise et al., 1984) are important for regulation of gonadotrophs. The frequency of GnRH stimulation is associated with the regulation of GnRH receptors in the pituitary (Katt et al., 1985) and influences the synthesis of specific ß mRNAs for LH or FSH (Hamernick et al.,1986; Dalkin et al., 1989). Without the continual pulsatile stimulation by GnRH, secretion of LH and FSH decrease rapidly (Clarke et al., 1983). Rhythmic secretions of GnRH into portal blood of ewes consists of large amplitude pulses which elicit the release of LH (Clarke and Cummins, 1982) and pulses of smaller amplitude which maintain gonadotropin synthesis, without release (Clarke and Cummins, 1987a). The smaller pulses may also modify the pituitary responsiveness to large pulses of GnRH (Clarke et al., 1984; Clarke and Cummins, 1985a).

Endocrine Regulation of GnRH Actions

Concentrations of LH in serum reflect basal or tonic secretions and pulses of LH from the anterior pituitary (Schwartz, 1968; Knobil, 1974). Regulation of LH pulses by GnRH is modulated by steroids from the ovary in rats and monkeys (Goodman and Knobil, 1981). In the monkey, GnRH pulses are essential to reproduction but the role is permissive as timing of ovulation is controlled by estrogen

from the ovary (pelvic control; Knobil, 1980). In addition to increasing the sensitivity of the pituitary to GnRH, estrogens act on the central nervous system of the rat to increase pulsatile secretion of GnRH and to cause the ovulatory surge of gonadotropins (Sarkar et al., 1976; Goodman and Knobil, 1981). Progesterone acts on the hypothalamus to suppress tonic LH secretion and on the central nervous system of the rat and monkey to inhibit the positive stimulus of estradiol (Goodman and Knobil, 1981). The actions of steroids in sheep (Goodman & Karsch, 1980; Clarke and Cummings, 1985b) and pigs (Kesner et al., 1987) are similar to those in the rat.

Growth of follicles within the ovary and the production of steroids from the follicle are regulated by concentrations of gonadotropins in blood (Fortune and Quirk, 1988), changes in blood flow to the ovary, and steroids and proteins within the follicle itself (Spicer and Echternkamp, 1986). Peptides, including inhibin, activin and follistatin (Findlay, 1993) and several transforming growth factors (Lobb and Dorrington, 1992), are produced in the follicle and are involved in local regulation of follicular growth. Inhibin, from bovine follicular fluid (Robertson et al., 1985), acts on the pituitary and selectively suppresses FSH synthesis and secretion (deKretser and Robertson, 1985). Concentrations of LH, and the frequency and amplitude of pulses of LH vary during the reproductive cycle of cows (Rahe et al., 1980;

Imakawa et al., 1986a) indicating effects of estradiol and progesterone on LH secretion.

The time required for the normal growth of medium-sized (5 mm) bovine follicles to ovulatory-sized (>10 mm) follicles is approximately 96 h (Staigmiller and England, 1982; Spicer and Echternkamp, 1986). Concentrations of estrogens in plasma are associated with the growth of the dominant follicle. During the luteal phase, concentrations of estradiol-17ß in plasma of cattle are minimal (≈ 5 pg/mL) and a two to three fold increase occurs at the time of estrus (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Dobson and Dean, 1974). Exogenous estradiol will increase concentrations of estradiol in blood of ovariectomized cattle and induce estrus (Allrich et al., 1984; Cook et al., 1986).

Estradiol receptors are present in cells of the anterior and medial basal hypothalamus of calves (Armstrong et al., 1977) and the anterior pituitary (Day et al., 1987) of prepuberal heifers. The number of estradiol receptors on the gonadotrophs may be related to the stage of the estrous cycle. Nett et al. (1987) hypothesized that the increase in estradiol receptors, prior to the ovulatory surge of LH, was associated with increased responsiveness to GnRH. Increased estradiol receptors in the anterior pituitary within 15 days after parturition were associated with increased synthesis of LH (Nett et al., 1988). Injections of estriol and estradiol increase receptors for

GnRH in the pituitary of ovariectomized cows prior to the ovulatory surge of LH (Schoenemann et al., 1985). Estrogens increase the responsiveness of the adenohypophysis to GnRH (Kesner, et al., 1981; Nett, 1990).

In addition to pituitary responsiveness, Kesner et al., (1981) suggested that increased GnRH release was necessary to induce the ovulatory surge in cows. Tortonese et al. (1990) induced an ovulatory surge in prepuberal heifers with repeated injections of a single dose (50 μg/2h) of LH during a 48 h period but could not separate the increase in LH due to injections from the effects of endogenous GnRH. Removal of the ovary containing the dominant follicle at 28 h after the onset of treatment decreased the number of heifers with an ovulatory surge of LH (Tortonese et al., 1990). Decreasing concentrations of estradiol are not necessary for ovulatory surges of LH in cows (Stumpf et al., 1991) because only cows with implants to maintain or increase estradiol concentrations after ovariectomy (d 16 of cycle) had ovulatory surges of LH. Increasing estradiol concentrations in ovariectomized ewes by giving estradiol implants result in a surge of GnRH followed by a surge of LH (Moenter et al., 1990). Estradiol receptors are present in cells of the anterior and medial basal hypothalamus of calves (Armstrong et al., 1977) but no attempts to actually quantify GnRH at the time of the ovulatory surge in cows have been reported.

Concentrations of progesterone in the plasma of cows are cyclic, with minimal amounts (< 1 ng/ml) at estrus, maximal concentration during the mid-luteal phase, and concentrations decrease abruptly during the 1-2 d prior to the onset of estrus (Stabenfeldt et al., 1969; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Humphrey et al., 1976). Secondary sources of progesterone in cattle include the adrenal gland (Wagner et al., 1979), nonpalpable luteal tissue within the ovary (Berradinelli et al., 1979) and luteinized-atretic follicles (Spicer et al., 1987).

Progestogens have been used to control reproductive cycles in beef females (Zimbelman, 1966; Roche et al., 1981). Progesterone decreases concentration of LH in serum by inhibiting synthesis and/or release of LH from the pituitary. Decreased concentrations of LH occur in cows during gestation (Little et al., 1982), during the luteal phase of the estrous cycle (Rahe et al., 1980; Clarke, 1989) and during treatment of cows with progestogens (Walters et al, 1982). Maximum concentrations of progesterone may allow for increased storage of LH in the pituitary by decreasing the frequency of GnRH release from the pituitary of cows. Weesner et al. (1993) observed decreased mRNA for GnRH in the brain of heifers killed during the mid-luteal phase of the cycle compared with those killed after ovulation.

Nutritional Control of Reproduction

The precise signal(s) necessary for the onset of reproductive cycles in females is unknown. Anestrus in beef cattle has been reviewed extensively (Wiltbank, 1970; Dunn and Kaltenbach, 1980; Wettemann, 1980; Lamming et al., 1981; Dziuk and Bellows, 1983; Peters, 1984; Short and Adams, 1988; Short et al., 1990; Williams, 1990) and was summarized by Bishop (1991). Factors including the availability of nutrients (body energy stores and nutrients in feed) to the cow and the effects of suckling have been suggested as the principal reasons for prolonged postpartum anestrus. Although puberty in heifers is dependent on both genetic and environmental factors (Kinder et al., 1987), the onset of fertile estrous cycles is related to nutrition (Short and Bellows, 1971) and body composition (Siebert and Field, 1975; Day et al., 1986; Yelich et al., 1991, 1993).

Nutritional Anestrus in Cows

Restriction of nutrient intake compromises hypothalamic-pituitary function in cattle (Echternkamp et al., 1982; McCann and Hansel, 1986; Imakawa et al., 1986c; Killen et al., 1989; Richards et al., 1989a). Synthesis and normal secretion of gonadotropins (Roberson et al., 1992), storage and release of GnRH from the hypothalamus (Rasby et al., 1992) and the amount of LH released from the pituitary in response to exogenous GnRH (Whisnant et al.,

1985; Rasby et al., 1991) differ in cows depending on the body condition score (BCS) of the animal.

Cows that become anestrus as a result of severe nutrient restriction and body weight loss (Imakawa et al., 1986b; Richards et al., 1989a; Vizcarra, 1991) were anestrus for extended periods, even after supplementation to achieve rapid weight gains. Re-initation of luteal activity does not occur in those cattle until they have greater body weight (BW, Imakawa et al., 1986c) and BCS (Richards et al., 1989a; Vizcarra, 1991) than at the onset of anestrus.

Anestrous cows have fewer pulses of LH (Imakawa et al., 1986b; Richards et al., 1989a). Pulsatile intravenous infusions of GnRH re-initiated luteal activity in nutritionally anestrous beef cows (Bishop and Wettemann, 1993a) and the frequency of pulses of GnRH influenced the response of the ovary. More nutritionally anestrous cows infused with GnRH had luteal activity compared with cows infused with saline and a greater percentage of cows infused with GnRH at a frequency of once per hour maintained luteal activity after treatment compared with cows infused with GnRH every fourth hour. Suppression of LH by severe nutrient restriction and loss of condition may be related to opioid activity (Bishop and Wettemann, 1992) because infusion of naloxone (.5 or 1.0 mg/kg BW) increased concentrations of LH and the amplitude of LH pulses in serum from nutritionally anestrous heifers. Control of LH

secretion in beef cows by body energy reserves is independent of the ovary (Richards et al., 1991).

The effects of dietary energy on ovarian function are difficult to distinguish from the influences of BCS. Insulin-like growth factor-1 (IGF-1) is an indicator of metabolic status and nutrient intake in beef animals (Breier et al., 1986; Rutter et al., 1989; Richards et al., 1989c; Spicer et al., 1990). Acute restriction of dietary intake to heifers decreases concentrations of IGF-1 in serum (Houseknecht et al., 1988; Spicer et al., 1992) but not in follicular fluid (Spicer et al., 1992). Differences in concentrations of IGF-1 in serum and intra-ovarian concentrations could suggest differential regulation of IGF-1 production (Spicer et al., 1992) or that the ovary was not responsive to fasting because body energy reserves in the heifers were adequate to counteract restricted nutrient intake. Restricted nutrient intake in dairy heifers and maintenance of a negative energy balance for four estrous cycles influenced hormones that indicate decreased metabolic status (Villa-Godoy et al., 1990), but did not influence secretory patterns of LH. Effects of energy balance on concentrations of progesterone, weights of luteal tissues, ratios of small to large luteal cells and steroid production per cell were confounded by body condition of heifers at the start of nutrient restriction. Thus, in the cow, nutrient availability should be

considered as the summation of body energy reserves available for mobilization and nutrients available in feed.

Nutritional Anestrus in Sheep

Season of the year (Karsch et al., 1984) and body condition of ewes (Tatman et al., 1990) should be considered in evaluating the influence of nutrition on gonadotropin secretion in ewes. Ovariectomized ewes that lost > 50% of their body energy stores (BCS 7.3 to 1.6; BW 71 kg to 37 kg) as a result of restricted nutrient intake became anestrus. Acyclic ewes had decreased LH concentrations in blood and in the pituitary, increased concentrations of GnRH in the median eminence and the total hypothalamus compared with ovariectomized ewes fed to maintain BCS and BW (Tatman et al., 1990). The concentration of GnRH in the preoptic area, number of receptors for GnRH in the pituitary and weight of the pituitary were not influenced by loss of body condition. Subsequent work with the same model (Kile et al., 1991) suggested that decreased concentrations of gonadotropins in serum of nutritionally restricted, ovariectomized ewes was due to decreased secretion of GnRH. Infusion of GnRH (250 ng/2 h) for 3 wk to restricted ewes restored mRNA for both gonadotropins and pituitary content of gonadotropins to similar concentrations as those in control ewes. Decreased concentrations of LH in ovariectomized sheep with insulin-induced hypoglycemia (Clarke et al., 1990) was due to

increased opioid activity as infusion of naloxone (40 mg/h for 3.5 to 5.5 h) counteracted the effects of insulin on LH secretion. Additionally, Clarke et al. (1990) injected corticotropin-releasing factor (CRF; 50 μg i.v.) and determined that decreased LH in hypoglycemic, ovariectomized ewes was not related to increased cortisol associated with stress.

The effects of another peptide associated with insulin regulation of energy balance, neuropeptide Y (NPY, Schwartz et al., 1992), was evaluated in ewe lambs (McShane et al., 1992). Concentrations of NPY in cerebral spinal fluid increased in ovariectomized lambs after an extended period (> 3 wk) of nutrient restriction (McShane et al., 1992). Increased concentrations of NPY in response to intracerebroventricular infusion of NPY decreased basal concentrations of LH compared with control lambs, but did not influence the concentrations of LH released in response to exogenous GnRH (McShane et al., 1992). Increased opioid activity and increased concentrations of NPY, associated with decreased nutrient availability, appear to decrease LH by decreasing the activity of neurons in the hypothalamus that release GnRH.

To evaluate the effects of nutrition separate from body energy stores on concentrations of LH and FSH, several studies have been conducted using growth-retarded, intact and ovariectomized ewe lambs (Foster and Olster, 1985; Foster et al., 1989). Ovariectomized prepuberal ewes fed

to maintain their weight at weaning (20 kg) have decreased concentrations of LH in serum compared with controls at puberty (30 wk of age, Foster and Olster, 1985). Concentrations of LH during restricted feeding were not different between lambs implanted with estradiol and unimplanted lambs. During ad libitum feeding of growth-retarded lambs the frequency of LH (Foster and Olster, 1985) and FSH pulses (Foster et al., 1989), concentrations of FSH (but not LH) in the pituitary, concentrations of mRNA for the α, LHß and FSHß subunits in the pituitary (Landefeld et al., 1989) and concentrations of LH and FSH in serum (Foster et al., 1989; Landefeld et al., 1989) were increased compared with ewe lambs maintained on the restricted diets.

The effects of nutrition on ovarian function of ewe lambs was evaluated by McShane and Kesler (1991). Chronic (wk 14-26 of age) feed restriction of intact ewe lambs followed by increased feed intake did not influence the inhibition of LH by photoperiod. The ability of estradiol infusion (.08 pg/mL for 56 h) into ovariectomized lambs to increase basal concentrations of LH and FSH or the ovulatory surge of gonadotropins (McShane and Kesler, 1991) was not influenced by nutrition. They concluded that the ovary is not directly responsible for the effects of nutrition on reproduction in ewes.

Nutritional Anestrus in Swine

Direct effects of nutrition on reproduction in monogastric animals can be evaluated as a combination of chronic and acute changes in nutrition. Severe nutrient restriction of gilts causes loss of BW and cessation of estrous cycles (Armstrong and Britt, 1987), but Cosgrove et al., (1992) blocked LH secretion with allyl trembolone (oral progestin; 15 mg/d) and found that the ovary of prepuberal gilts is influenced by short-term changes in feed intake which are independent of gonadotropin secretion. Increased concentrations of insulin, IGF-I or other hormones or metabolites may act with increased gonadotropin secretion to facilitate ovarian function in gilts (Cosgrove et al., 1992).

Nutritional Regulation of Reproduction in Primates

Regulation of body fat in humans is controlled by short- and long-term mechanisms (Le Magnen, 1983; Martin et al., 1991) and reproductive events depend on the availability of oxidizable fuels. Dietary restriction in humans (Frisch and Revelle, 1970; Frisch and McAuthur, 1974; Vigerski et al., 1977; Frisch, 1982; Wynn, 1982; Carlburg et al., 1983; Warren, 1983) and non-human primates (Lee, 1987) delays the onset of menstrual cycles and is associated with prolonged amenorrhea due to mobilization of fat to provide energy to the body. Malnutrition of women

in developing countries (Frisch, 1978; Reddy, 1981), self imposed restrictions of caloric intake (Pugliese et al., 1983), anorexia athletica (Smith, 1980) or anorexia nervosa (Button and Whitehouse, 1981) result in changes in endocrine function (Pugliese, 1990) which are manifested as reduced metabolic rate, decreased somatic tissue growth and loss of reproductive function. Correlations between body measurements and reproductive data in humans have been determined and models based on common body weight (Frisch and Revelle, 1970), body fat (Frisch, 1987) and weight to height ratios (Frisch 1988) have been evaluated. All of these models revolve around an evolutionary need of the female to delay the onset of reproductive events until such time as the body perceives that adequate nutrients are available to maintain pregnancy and the onset of lactation. However, after considering the endocrine changes discussed by Pugliese (1990) and variations in ability of body fat to influence ovulation (Bronson and Manning, 1991), it does not appear to be fat alone that signals the onset of reproductive events in humans.

Acute effects of nutrition on gonadotropin secretion can be evaluated in non-human primates because dietary input is a greater part of the total nutrient availability. Knobil (1993) emphasizes the importance of metabolic signals on GnRH secretory neurons. Pulsatile release of LH and the concentrations of LH and testosterone in blood from male Rhesus monkeys are influenced by the size of the meal

provided after fasting (Parfitt et al., 1991) and the time of feeding of the daily meal (Mattern et al., 1993). The effects of nutrition of Rhesus monkeys are due to metabolic cues and are not related to stress because intravenous feeding restored pulsatility of LH to a frequency similar to companions fed a normal meal, but infusion of nutrients did not decrease signs of behavioral stress compared with fasted monkeys (Schreihofer et al., 1993a). Additionally, the size of the meal provided on the day before fasting influenced pulsatility of LH on the day of fasting but not behavioral signs of stress associated with fasting (Schreihofer et al., 1993b). Pulsatile infusion of GnRH will increase concentrations of LH and testosterone in fasted monkeys (Bergendahl et al., 1991). NPY may be involved in regulation of the frequency of release of GnRH in Rhesus monkeys (Woller et al., 1992) and the effects of NPY may be mediated through steroid-sensitive cells in the hypothalamus that produce dopamine. The mechanism(s) through which nutrition influences the pulse generator of GnRH and reproduction are not yet clear (Knobil, 1993).

Techniques to Evaluate Regulation of Reproduction by GnRH

Removal of the gonad results in permanent infertility. Transection of the pituitary stalk abolishes the control of the pituitary by GnRH from the hypothalamus and results in decreased concentrations of luteinizing hormone (LH) in serum of ovariectomized monkeys (Plant et al., 1978), rats

(Kanematsu et al., 1979), calves (Anderson et al., 1981), ovariectomized ewes (Clarke and Cummins, 1982) and gilts (Kraeling et al., 1986). Surgical isolation of the pituitary from hypothalamic stimulation effectively blocks reproduction but is costly and is not reversible. Neutralization of endogenous hormones by the production of specific antibodies can be used to alter reproductive function (Reeves et al., 1989; Hage-van Noort et al., 1992) and the effects are reversed as antibody titers decrease with time.

Immunization Against GnRH

The ability to produce antibodies against GnRH in vivo have added to the understanding of the role of GnRH in reproduction (Jeffcoat et al., 1976;, Fraser, 1976; 1980). The effectiveness of immunization against GnRH to study reproductive endocrinology depends on the ability to break self-recognition of the hormone, individual variations in the immune systems among animals and differences in the effectiveness of immunizations within species (Fraser, 1980).

Rats. The female rat is a good model to use to evaluate the use of passive immunization against GnRH because the time of the ovulatory surge can be predicted (Fraser, 1980). Intravenous injection of antibodies against GnRH into cycling rats on the afternoon of

proestrus blocks the ovulatory surge of LH and FSH, thus inhibiting ovulation (Arimura et al., 1974; Fraser, 1977). Passive immunization of rats decreases concentrations of LH in serum within 1 h of injection of GnRH antibodies (Fraser, 1980).

Immunization of female rats against GnRH increased the length of the estrous cycle as antibody titers increased (wk 20 to wk 40 post-immunization; Takahashi et al., 1978), and as antibody titers against GnRH attained maximal concentrations (wk 50), cycles stopped and diestrous persisted (Fraser and Baker, 1978; Takahashi et al., 1978). During anestrus, induced by immunization against GnRH, female rats had decreased concentrations of LH and FSH in the blood and in the pituitary compared with rats during the estrous cycle (Fraser and Baker, 1978). The ovaries of immunized, anestrous females were smaller in size than those of cycling females, void of active luteal tissue and contained only small follicles (Fraser and Baker, 1978). Female rats that did not produce adequate titers against GnRH to cause persistent diestrous vaginal smears had normal basal concentrations of LH and FSH and normal growth of follicles (Fraser and Baker, 1978) but the surge of LH was inhibited and ovulation did not occur.

The ovaries of female rats immunized against GnRH remain responsive to gonadotropins. Injection of immunized rats with pregnant mare serum gonadotropin (PMSG) induced growth of follicles which could be induced to ovulate with

LH (Takahashi et al., 1978). Concentrations of LH released from the pituitary of rats immunized against GnRH in response to an analog of GnRH (not recognized by the antibodies, Fraser and Sandow, 1977), were less than concentrations of LH observed in the rats prior to ovulation (Fraser and Baker, 1978).

Ewes. Neutralization of endogenous GnRH by the production of specific antibodies causes anestrus in ewes (Clarke et al., 1978) and blocks the positive effects of estradiol benzoate on LH secretion in ovariectomized ewes (Fraser et al., 1977; Fraser and McNeilly, 1982). Different epitopes of GnRH used for active immunization were equally immunogenic (Goubou et al., 1989) but the antibody response to immunization was influenced by the carrier protein used in the conjugations.

LH and FSH responded differently to passive immunization against GnRH in ovariectomized ewes (Herman and Adams, 1990; Currie et al., 1993). Concentrations of LH in serum were reduced within 2 h of infusion of antisera to GnRH but FSH concentrations did not decrease until 76 h after treatment. Similarly, pulsatile administration of an analog of GnRH (des-gly^{10} GnRH ethylamide, 100 ng/h) increased concentrations of LH to values similar to those before immunization within 2 h of the initiation of treatment (Herman and Adams, 1990). Maintenance of pulses of the analog of GnRH for 90 h did not increase FSH

concentrations compared with immunized ewes that were not treated with the analog (Herman and Adams, 1990). Maintenance of a continuous amplitude pulsatile infusion of des-gly^{10} ethylamide (100 ng/h) increased LH and estradiol concentrations but did not cause ovulation in ewes passively immunized against GnRH (Sakurai et al., 1992). Administration of low-level (100 ng/h) pulses of a GnRH analog for 3 d, followed by an increase to 800 ng/h, induced increases in concentrations of LH and FSH resembling an ovulatory surge of these gonadotropins and induced ovulation in ewes passively immunized against GnRH (Sakurai et al., 1992).

Gilts. The importance of GnRH in gilts was reviewed by Esbenshade et al. (1990). Active immunization against GnRH causes anestrus in gilts (Esbenshade and Britt, 1985). Antibody titers increased within 1 wk after a booster immunization was given to gilts immunized against GnRH (Esbenshade and Britt, 1985). Increased antisera titers in gilts are related to decreased concentrations of LH, FSH and ovarian steroids and gilts become anestrus after termination of the luteal phase of the cycle (Esbenshade and Britt, 1985). Antibody titers against GnRH (50% binding of ^{125}I labeled GnRH at a 1:14,000 dilution of serum; Traywick and Esbenshade, 1988) in gilts actively immunized against GnRH are considerably greater than titers observed in other species.

Gilts actively immunized against GnRH have decreased ovarian and uterine weights compared with non-immunized gilts of a similar age (Esbenshade, 1987; Traywick and Esbenshade, 1988) and morphologically distinct structures are not present on the ovaries of immunized gilts. Passive immunization of gilts against GnRH early in the follicular phase (Esbenshade, 1991) retarded follicular growth and infusion of GnRH antibodies (Patton et al., 1991) into the ovary caused an increased incidence of follicular atresia. Growth of primary follicles in the pig is independent of gonadotropin stimulation (Foxcroft and Hunter, 1985). Histological evaluations of ovaries from gilts treated with antisera against GnRH had similar numbers of primary follicles as those observed in cyclic gilts but a greater incidence of atresia of secondary follicles with greater than 4 layers of granulosa cells occured in immunized gilts.

Ovaries of gilts immunized against GnRH are not responsive to stimulation by endogenous (Traywick and Esbenshade, 1988) or exogenous (Esbenshade, 1987) gonadotropins. Antibodies in gilts immunized against GnRH had low affinities for agonists to GnRH (Esbenshade and Britt, 1985), suggesting that antibodies were specific. Injections (100 ng/2h) of an analog of GnRH (D-ala^6, des-gly-NH_2 ethylamide) caused release of LH and FSH but maintenance of treatments for 72 or 144 hours failed to induce growth of follicles (Traywick and Esbenshade, 1988).

Administration of PMSG either as a single injection or as a series of injections to gilts immunized against GnRH did not cause follicular growth (Esbenshade, 1987). Neither treatment influenced the occurrence of atretic follicles on the ovaries.

Heifers. Several immunization techniques have been used to alter reproduction in beef heifers (Reeves et al., 1989). Immunization of heifers against estradiol and progesterone increased concentrations of LH in serum, frequency of LH pulses, weights of the ovaries and the number of follicles ≥ 15 mm, but did not influence secretion of FSH compared with control heifers (Chang et al., 1987). Active immunization of heifers against GnRH, human chorionic gonadotropin (hCG) or LH decreased the number of heifers observed in standing heat and blocked reproductive cycles (Johnson et al., 1988). Immunization against specific hormones, and the response observed after antibody titers against that hormone are produced, aid in the establishment of the relationship between that hormone and reproduction. The ability to create adequate titers against an endogenous hormone is related to the carrier protein and adjuvant used (Roberts et al., 1990), the technique used to link the hormone to the protein carrier (Grieger and Reeves, 1990), and the dosage and length of time of exposure of the antigen to the immune system (Greiger et al., 1990).

Immunoneutralization of GnRH separates the pituitary from stimulation by the hypothalamus and causes cessation of estrous cycles in heifers (Johnson et al., 1988; Adams and Adams, 1990; Wettemann and Castree, 1994). The reproductive response to antibodies against GnRH in heifers is mediated through decreased concentrations of LH in the anterior pituitary (Adams and Adams, 1990; Stumpf et al., 1992) and in serum collected weekly (Wettemann and Castree, 1994). When concentrations of LH were measured in frequent samples for 4 h, basal secretion of LH was not different between anestrous heifers that had been immunized against GnRH and cyclic controls (O'Connell, 1990). Heifers immunized against GnRH failed to ovulate probably due to the absence of an ovulatory surge of LH. Concentrations of FSH in serum of heifers immunized against GnRH have not been documented.

Immunization against GnRH decreased the concentrations of mRNA for the α subunit of LH and increased intra-pituitary concentrations of the particular isoform of LH that has the greatest biological activity (Stumpf et al., 1992). The number of receptors for GnRH in the pituitary were decreased in immunized heifers immunized against GnRH that were anestrus at slaughter compared with heifers that did not produce titers adequate to block estrous cycles or with control cyclic heifers (Adams and Adams, 1990). However, the affinity of receptors for GnRH was not influenced by immunization against GnRH. The ability to

decrease LH concentrations and block the ovulatory surge of gonadotropins may be related to the concentration of antibodies against GnRH produced in the heifer.

Ovarian and uterine weights of heifers immunized against GnRH, that were anestrus at slaughter, were decreased compared with cyclic (immunized or control) heifers (Adams and Adams, 1990). At anestrus, no follicles > 5 mm or luteal tissues were present on the ovaries of heifers immunized against GnRH (Johnson et al., 1988) and ovaries from heifers immunized against GnRH weighed less than ovaries from heifers immunized against hCG or LH. Serum concentrations of progesterone were decreased in heifers immunized against GnRH (Adams and Adams, 1990) compared with heifers given control immunizations. The typical response to immunization against GnRH during the luteal phase of the cycle is a continuance of normal luteal activity but an absence of ovulation after regression of the corpus luteum (CL, Wettemann and Castree, 1994). Immunization against GnRH during early gestation does not influence concentrations of progesterone in plasma (Loetz et al., 1990).

The onset of puberty in heifers may be delayed by immunization against GnRH (20 wk, O'Connell, 1990; 11 wk, Wettemann and Castree, 1994) but the ability to delay puberty may be related to the time of administration of primary and/or booster immunizations (Duggan et al., 1992) and the concentration of titers against GnRH near the

expected time of puberty (O'Connell, 1990). Antisera titers were greater in heifers that became anestrus after immunization against GnRH (Adams and Adams, 1990; Wettemann and Castree, 1994) but the ability to block reproductive cycles is decreased as antibody titers decrease with time after immunization (O'Connell, 1990, Wettemann and Castree, 1994). Immunization against GnRH does not alter estrous cyclicity after antisera titers are decreased.

Two analogs of GnRH [des-Gly^{10}(D-Ala^{6})-LH-RH and des-Gly^{10}-LH-RH] increased LH in anestrous heifers immunized against GnRH (Wettemann and Castree, 1994). Intravenous infusion of 3.5 μg of an analog of GnRH [GnRH-A, des-Gly^{10}(D-Ala^{6})-LH-RH] resulted in increased concentrations of LH in serum of heifers immunized against GnRH (O'Connell, 1990) but maximum concentrations of LH were greater in control heifers. Pulsatile infusion (2 μg/2 h) of GnRH-A (O'Connell, 1990) increased concentrations of LH and the amplitude of pulses of LH in serum compared with anestrous heifers infused with saline. However, maintenance of pulses of the analog for 14 d failed to establish luteal activity in anestrous heifers.

Actions of Antisera Against GnRH. Active immunization against GnRH causes cessation of reproductive cycles in all species that have been evaluated. The observation common to all reports is cessation of luteal activity as antibody titers against GnRH increase. GnRH has a short (8- 10 min,

Bennett and McMartin, 1979; Handelsman and Swerdloff, 1986) half-life and concentrations in the systemic circulation of sheep are minimal (< 10 pg/mL, Nett et al., 1974). Presumably, antibodies produced in response to immunization against GnRH alter reproductive cycles by acting in hypophyseal portal blood to bind GnRH so that gonadotrophin producing cells in the pituitary are not stimulated by the releasing hormone (Fraser, 1976; Schanbacher, 1984; Esbenshade, 1991). The ability to block secretion of LH and the duration of acyclicity depend on concentrations of antibodies against GnRH (O'Connell, 1990; Wettemann and Castree, 1993).

An alternative mechanism has been suggested in the pig (Esbenshade, 1991; Patton et al., 1991) to explain the results of infusion of antibodies against GnRH into blood or directly into the ovary. Since neither of the treatments altered gonadotropin concentrations, those authors suggested a direct effect of GnRH antibodies on the ovary. GnRH may directly inhibit progesterone production from luteal cells in pregnant rats (Bex and Corbin, 1981) and specific receptors, with an affinity for GnRH similar to that of the pituitary, have been reported in rat ovaries (Clayton et al., 1979; Brown and Reeves, 1983). Specific receptors for GnRH were isolated from pituitaries of cows, sheep and pigs using a radioreceptor assay (Brown and Reeves, 1983) but binding of the radio-labeled analog of

GnRH was not detected in ovarian tissues from these species.

Proteins which compete for binding sites for GnRH on luteal cells of rat ovaries have been isolated. (Aten et al., 1987). However, these proteins do not bind to GnRH antibodies and are not as heat stable as GnRH. These GnRH-like proteins have also been isolated in ovine (Aten et al., 1987) and bovine (Ireland et al., 1988) ovaries and may be involved in paracrine regulation of granulosa cells to decrease progesterone production. Because of low concentrations of GnRH in the systemic circulation, a short half-life of GnRH and the absence of receptors for GnRH on the ovary, a direct effect of immunization against GnRH at the ovary is doubtful at this time. The effect of antibodies against GnRH on GnRH-like proteins has not been addressed in the bovine so a direct effect of GnRH antibodies on the ovary cannot be ruled out.

Use of GnRH to Stimulate Gonadotropin Secretion

Continuous infusion of GnRH results in reduced synthesis and secretion of gonadotropins due to down regulation of GnRH receptors in monkeys (Knobil, 1980), and sheep (Nett et al., 1981). Clarke and Cummins (1985a) were unable to document the down regulation hypothesis in sheep, but determined that the frequency of GnRH pulses determined the amount of the releasable LH pool and the amplitude of LH pulses. The quantity of LH released from the

pituitaries of cows is increased with previous exposure to GnRH (Carruthers et al., 1980).

Down regulation of LH secretion by GnRH treatment has not been documented in cattle but may occur based on the short-lived increases in LH in suckled cows infused continuously with GnRH (Lamming and McLeod, 1988) and the transitory (4 h) increase followed by a decrease in LH concentrations in serum of postpartum anestrous cows given a subcutaneous implant containing GnRH (Britt et al., 1974). The decrease in LH release that occured after continuous treatment with GnRH could be due to down regulation of GnRH receptors in the anterior pituitary or to a decreased releasable pool of LH. Kesner et al., (1981) suggested that termination of the ovulatory surge of LH in estradiol treated ovariectomized cows pulsed with GnRH was due to the pituitary becoming refractory to GnRH stimulation. For a review of the use of GnRH to initiate cycles in beef females see Bishop (1991). The importance of frequency of pulses of GnRH administered to nutritionally anestrous cows on the re-initiation of luteal activity (Bishop and Wettemann, 1993a) was addressed earlier in this review. GnRH (Drost and Thatcher, 1992) and its analogs (Gordon and Hodgen, 1991; Mann et al., 1992) are available for therapeutic use to improve reproductive efficiency.

Clarke (1992) reviewed the relationship between pulses of GnRH and LH concentrations in sheep. Bolus injections

(250 ng, i.v.) of GnRH into ovariectomized ewes with severed hypothalamic-pituitary axes resulted in varying amounts of LH release from the pituitary depending on the rate of infusion and the interval between injections of GnRH. Variations in the amount of GnRH (50-500 ng), or the length of intervals between pulses (1, 2 or 4 h) of 100 ng of GnRH affected concentrations of both gonadotropins and testosterone concentrations in hypothalamo-pituitary disconnected rams (Wu et al., 1987). A dose of 100 ng of GnRH given at 2 hour intervals established pulses of LH and increased baseline concentrations of LH and FSH compared with noninfused rams, but the amplitude of gonadotropin pulses during the 7 d of treatment was decreased compared with amplitude prior to disconnection of the hypothalamus. Maximum concentrations of hormones and maximum amplitudes of gonadotropin pulses during the entire trial were present in rams treated with GnRH at a frequency of 100 ng per 2 h (Wu et al., 1987).

Similar observations of an inverse relationship between LH pulse frequency and amplitude have been demonstrated in ovariectomized, progesterone treated ewes (Kaynard and Karsch, 1988). Because of the variation in the amount of LH released in response to a constant amount of GnRH (5 ng/kg) given at 30 or 60 minute intervals, these authors suggested that progesterone directly affects the hypothalamus during the estrous cycle of ewes. Subcutaneous infusion (250 ng/h) of GnRH with an osmotic

mini pump increased concentrations of GnRH receptors in the pituitary of seasonally anestrous ewes within 24 h (Khalid et al., 1991a) but anestrous ewes given estradiol had greater concentrations of LH and GnRH receptors at a similar interval after infusions (Khalid et al., 1991b). Pulsatile infusion of GnRH increased mRNA for gonadotropins and pituitary content of LH and FSH in nutritionally anestrous, ovariectomized ewes (Kile et al., 1991). Variations in the frequency of pulses of GnRH (250 ng per 1 or 3 h) did not influence the number of GnRH receptors in the pituitary of ovariectomized ewes pretreated with GnRH (250 ng/2 h) for 2 wk after disconnection of the hypothalamic-pituitary axis (Clarke et al., 1987b). Variations in the frequency of pulses of an analog of GnRH are necessary to induce ovulation in sheep immunized against GnRH (Sakurai et al., 1992).

The frequency of exogenous pulses of GnRH also affects receptors for GnRH and concentrations of gonadotropins. The normal frequency of pulses of LH in castrated rats is one pulse every 30 min and administration of testosterone implants to castrated rats will inhibit pulses of LH and presumably GnRH (Steiner et al., 1982). Pulses of GnRH (25 ng) administered at 7.5, 15, 30, 60 or 120 minute intervals for 48 hours increased the concentration of GnRH receptors in castrated rats treated with testosterone (Katt et al., 1985). Maximum concentrations of GnRH receptors in the pituitary and maximum concentrations of LH and FSH in serum

were detected in rats receiving GnRH every 30 min. (Katt et al., 1985). The frequency of GnRH pulses differentially regulated the specific mRNA for the α, LHß and FSHß subunits in the pituitary of castrate male rat given testosterone to suppress endogenous pulses of GnRH (Dalkin et al., 1989; Haisenledger et al., 1991). Of the frequencies evaluated (8, 30 and 120 min, Haisenledger et al., 1991; 8, 30, 120, 240, 480 min, Dalkin et al., 1989), the α subunit responded the greatest to rapid pulses (8 or 30 min), LHß subunits responded maximally to a frequency of 30 min and FSHß subunits were most responsive to the slowest interval evaluated in each study (Dalkin et al., 1989; Haisenledger et al., 1991). Differences in the response of the FSHß subunit may be due to the technique used to quantify receptors, the length of time pulses were administered or the total amount of GnRH given during the treatment period. The regulation or gene expression in the pituitary by GnRH is the subject of a recent review (Marshall et al., 1992).

The precise mechanisms of action of exogenous pulses of GnRH in other species are not well defined. The LH response to continuous (Hyland et al., 1987) or pulsatile infusion (Becker and Johnson, 1992) of GnRH to anestrous mares is variable. Comparison of these with other studies in the mare (Johnson, 1986; Johnson and Becker, 1988) suggest a need for development of an optimum dose and

delivery system for GnRH as well as a better description of the anestrous mare.

Pulsatile infusion of 2.5 μg/2 h or 1.5 μg/1 h of GnRH to lactating sows increased LH concentrations to values similar to those in early-weaned sows and induced estrus in postpartum sows within 4 days of the onset of treatments (Cox and Britt, 1982). Hourly pulses of GnRH induced follicular growth and ovulation in chronically anestrous sows (Armstrong and Britt, 1985) and the same treatment regime (4.45 μg of GnRH/h) re-initiated follicular growth and ovulation in nutritionally anestrous gilts (Armstrong and Britt, 1987). Estradiol will not induce an ovulatory surge of LH in ovariectomized gilts given pulses of GnRH at a continuous frequency and concentration (Kesner et al., 1987).

Evaluation of procedures to initiate pulsatile secretion of gonadotropins and luteal activity will aid in determining the mechanisms involved in the onset and maintenance of fertile reproductive cycles in beef cows. Pulsatile infusion of GnRH will initiate luteal activity in nutritionally anestrous beef cows (Bishop and Wettemann, 1993a) and cows in thin condition have greater concentrations of GnRH in the hypothalamus (Rasby et al., 1992). These results suggest that decreased body energy reserves influence reproduction by inhibiting GnRH secretion from the hypothalamus but the possibility of direct effects of BCS on the pituitary can not be ignored.

Pulsatile infusion of an analog of GnRH into heifers immunized against GnRH (O'Connell, 1990) increased concentration of LH and the amplitude of LH pulses but did not initiate luteal activity. Administration of an analog of GnRH (Traywick and Esbenshade, 1988) or exogenous gonadotropins (Esbenshade, 1987) did not cause follicular growth in gilts immunized against GnRH. These results indicate that increased gonadotropins will not induce ovulation unless animals have a functional hypothalamus capable of responding to ovarian steroids. The following experiments were conducted to address these concerns.

CHAPTER III

DIRECT EFFECTS OF BODY ENERGY RESERVES ON GONADOTROPIN SECRETION

Abstract: Seventy beef cows at 30 d post partum (PP) were used to evaluate the effects of body condition score (BCS), immunization against gonadotropin-releasing hormone (GnRH) and pulsatile treatment with an analog of GnRH (GnRH-A) on secretion of LH and FSH, luteal activity, pregnancy rate and days from calving to conception. During each of two years, cows were assigned to a 2 (BCS = 4 or 6 at calving) by 3 (pulsed with saline or GnRH-A, or immunized against GnRH and pulsed with GnRH-A) factorial design. Cows were immunized against GnRH conjugated to human serum albumin (HSA) or HSA 3 wk prepartum and at 2 wk PP. Progesterone was quantified in daily plasma samples during treatment (d 30 - d 39 PP), every second day from d 40 to d 60 and weekly between 60 and 90 d PP. Pulses of saline or GnRH-A [des-gly^{10}, D-ala^{6})-LHRH; 675 ng/1.25 min] were given once per h for 198 h commencing on d 31 PP. LH was quantified in frequent serum samples collected on d 30 (prior to infusions) and on d 31, 33, 35, 37, and 39 PP. FSH was quantified in serum on d 30. Cows were exposed to fertile

bulls after d 39 PP. Concentrations of LH and FSH on d 30 PP averaged 4.3 ± .2 ng/mL and .4 ± .1 ng/mL, respectively, and were not influenced by immunization against GnRH or BCS. Prior to treatment, the frequency of LH pulses was greater ($P < .06$) in cows with BCS = 6 compared with thin (BCS = 4) cows. During the treatment period, infusion of GnRH-A increased LH concentrations and the amplitude of pulses of LH, but the response was dependent on the BCS of the cows (BCS x TRT x Day; $P < .01$). Maximum concentrations of LH in serum and the amplitude of LH pulses on d 31 (d 1 of treatment) were greater ($P < .05$) in cows treated with GnRH-A compared with those infused with saline. During year 2, HSA immunized cows treated with GnRH-A in good condition (BCS = 6; 18.0 ± 2.2 ng/mL) had greater ($P < .05$) concentrations of LH on d 31 compared with thin cows (BCS = 4; 10.6 ± 2.2 ng/mL). Mean concentrations of LH and the amplitude of LH pulses were less ($P < .01$) in serum collected on d 33 through d 39 of infusion compared with d 31 and were not influenced by treatment. Immunization of cows against GnRH did not influence the response of LH to GnRH-A during the infusion period. Cows with good BCS (6) at calving conceived in 86 d ($P<0.05$) PP compared with 96 d for cows with thin BCS (4). Body energy reserves of postpartum beef cows influence LH secretion, before and on the first day of treatment with a GnRH analog, but body energy reserves did not influenced LH secretion during continuous treatment

with an analog of GnRH. Body energy reserves did not influence ovarian response during treatment with an analog of GnRH, but BCS influenced ovarian response after treatment with an analog of GnRH. We conclude that body energy reserves influence the frequency of LH pulses but do not have a direct effect on the pituitary in the regulation of secretion of LH in postpartum beef cows.

Introduction

Body energy reserves and weight loss influence the length of the postpartum anestrous interval (Dunn et al., 1980; Selk et al., 1988). The mechanisms by which energy availability influence postpartum fertility may include impaired ovarian response to LH, reduced pituitary responsiveness to GnRH and/or reduced pulsatile release of GnRH (Schillo, 1992).

While pituitary weights in cattle are not influenced by nutrient intake (Moss et al., 1982) or body energy reserves (Whisnant et al., 1985; Rasby et al., 1991), severe nutrient restriction and loss of body energy reserves cause cessation of estrous cycles in cattle (Richards et al., 1989; Bishop and Wettemann, 1993a). Anestrous cows have decreased concentrations and fewer pulses of LH in serum as they are initiating anestrus (Richards et al., 1989a) and pulsatile infusion of GnRH induces luteal activity in nutritionally anestrous beef cows (Bishop and Wettemann, 1993a).

Concentrations of GnRH in the infundibular stalk-median eminence are inversely related to body energy reserves (Rasby et al., 1992) and cows fed restricted diets release more LH in response to exogenous GnRH (Whisnant et al., 1985; Rasby et al., 1991). Separation of the hypothalamus and the pituitary by immunization of heifers against GnRH causes cessation of ovulatory surges of LH and estrous cycles (Wettemann and Castree, 1994) but basal concentrations of LH in serum were not reduced in anestrous heifers (O'Connell, 1990). Intravenous infusion of a GnRH analog, [des-Gly^{10}, (D-Ala^{6})-LHRH], increased mean concentrations of LH and the amplitude of LH pulses in heifers (O'Connell, 1990) but did not initiate luteal activity.

The objectives of this experiment were: 1) to determine if body energy reserves have direct effects on the pituitary of cows to regulate gonadotropin secretion, 2) to determine if the response of postpartum anestrous beef cows to an analog of GnRH is regulated by body energy reserves, 3) to determine if immunization against GnRH influences gonadotropin secretion in postpartum anestrous beef cows, and 4) to evaluate the direct effects of body energy reserves on gonadotropin secretion in beef cows between 30 and 39 days post partum.

Materials and Methods

Seventy mature anestrous Hereford and Hereford x Angus cows with a moderate to good body condition score (BCS=5 or 6) were randomly assigned to diets during gestation to achieve a BCS of 4 or 6 at calving. BCS were independently determined by two individuals using the system where 1 = emaciated and 9 = obese (Wagner et al., 1988). The experiment was conducted during two spring calving seasons. Thirty-eight cows were used in the first year and thirty-two cows were used in the second year. Cows were assigned to a 2 (BCS = 4 or 6 at calving) by 3 (pulsed with saline or GnRH-A, or immunized against GnRH and pulsed with GnRH-A) factorial design.

Primary immunizations were administered to cows at approximately 265 d of gestation and a booster immunization was given 14 ± 3 d post partum (PP). Immunizations, consisting of 2 mg of conjugate or HSA in 2 ml saline and 2 ml Freund's complete adjuvant (Difco Laboratories, Detroit, MI), were administered intradermally and subcutaneously at five locations in the posterior portion of the mammary gland (Wettemann and Castree, 1993). Conjugates were made by incubating GnRH (5 mg; Sigma Chemical Co., St. Louis, MO), HSA (5 mg; Sigma Chemical Co.) and 15 mg of 1-ethyl-3(3-Dimethylamino)propyl-carbodiimide hydrochloride (JBL Scientific, Inc., San Luis Obispo, CA) dissolved in 1.8 mL of distilled water in a glass tube (12 x 75) for 20 h at

21°C. The mixture was then transferred to dialysis tubing (Spectra/Por 3, mwco 3500, Fisher Scientific, Pittsburgh, PA) and dialized twice against distilled water (4°C) for 24 h each time (Wettemann and Castree, 1994).

Antibody titers against GnRH were quantified at 21 and 28 d PP (Wettemann and Castree, 1994). Briefly, dilutions of sera (1:100 and 1:1000) in phosphate (.01 M) buffered saline and ethylenediaminetetraacetate (PBS-EDTA; pH =7.0) were incubated at 4° C for 24 h with ^{125}I-labeled GnRH (15,000 cpms). After incubation, 1.5 mL of ETOH (4° C) was added and tubes were centrifuged at 2800 x g for 15 min. The supernatant was decanted and bound ^{125}I-labeled GnRH was determined in the precipitate. The radiolabeled GnRH was prepared using the chloramine-T procedure. GnRH (3 μg) in 20 μL of distilled water, 25 μL of phosphate (.05 M) buffered saline (pH 7.1) and .75 mCi ^{125}I (E.I. Du Pont de Nemours and Co., Inc., Wilmington, DE) in 7.5 μL water were combined in a reaction vial. Chloramine-T (10 μL of 2 mg/mL in water) was added and allowed to react for 45 seconds. The reaction was stopped by adding 10 μL of sodium metabisulfite (10 mg/mL). Free ^{125}I and ^{125}I-labeled GnRH were separated using a Sephadex LH-20 column swelled in .05 m phosphate buffer (pH 7.5) and eluted with phosphate (.05 M) buffered saline and .1% gelatin (pH 7.0). Only cows immunized against GnRH with ≥ 12% of ^{125}I-labeled GnRH bound (1:100 dilution of serum) to antisera at 25 d PP were used in the experiment. In addition to those

quantified prior to assignment to treatment, antibody titers (% ^{125}I-GnRH bound at 1:1000 dilution of serum) were quantified on d 30 and 39 PP.

Lactating cows and their calves were maintained in individual pens in a barn between d 24 and 39 PP. On d 29 PP polyvinyl cannulae (BB317-V 10, i.d. 1.57 mm, o.d. 2.08 mm, Bolab, Lake Havasu City, AZ) were inserted into the right and left external jugular veins of each cow to facilitate simultaneous infusion and collection of samples. Pulsatile (2 mL·1.25 $min^{-1}·h^{-1}$) infusions of saline or an analog of GnRH (GnRH-A; des-Gly^{10}, (D-Ala^{6})-LHRH; 675 ng; Sigma Chemical) were given for 198 h commencing on d 31 PP. Blood plasma samples were obtained daily between d 30 and 39 PP. Blood serum samples were collected at 10 min intervals for 6 h on d 30 (prior to infusions) and on d 31, 33, 35, 37 and 39 PP (during infusions).
After collection of the last blood sample on d 39, cows and their calves were returned to pastures and exposed to fertile bulls. Additional blood plasma samples were collected every second day between d 40 and 60, and weekly between days 60 and 90 PP. Concentrations of progesterone were quantified in all plasma samples by RIA (Bishop and Wettemann, 1993). Concentrations of less than 1 ng/ml of progesterone were used as the criterion for absence of luteal activity (Stabenfeldt et al., 1969; Wettemann et al., 1972). The number of consecutive samples with concentrations of progesterone > 2 ng/mL were used to

evaluate luteal activity during and after the infusion period. Date of conception after treatment was estimated from the subsequent calving date minus 280 d. Analyses of variance were used to determine the influence of BCS, immunization against GnRH and infusion of an analog to GnRH on luteal activity and reproductive function.

Concentrations of LH were determined in all serum samples (Bishop and Wettemann, 1993) and FSH (Vizcarra et al., 1994) concentrations were quantified in serum collected on d 30. A pulse of LH or FSH was defined as a value greater than 1 SD greater than the mean for a cow on a day, followed by two consecutive concentrations of lesser values (Bishop and Wettemann 1993). Pulse amplitude was the difference between the greatest value during a pulse and the nadir within 30 min before the pulse.

Mean LH, and amplitude and frequency of LH pulses were analyzed by split plot analyses of variance (SAS, 1982) with year, BCS and treatment and the two- and three-way interactions as the main plot and day of treatment and the interactions as the sub plot. The model for concentrations of FSH, and the amplitude and frequency of FSH pulses included assay block (1 cow from each treatment$\cdot$year$^{-1}\cdot$assay^{-1}), immunization against GnRH, BCS, year and the two- and three-way interactions of immunization, BCS and year as main effects. The influence of BCS, infusion of GnRH-A and immunization against GnRH on mean LH, pulse frequency and the amplitude of LH pulses on each day of

treatment were compared using Bonferroni t-statistics (α=.05; Gill, 1973).

Results

Body condition scores of cows were influenced by year (P <.001). Body condition scores of thin cows averaged 4.4 ± .1 in year one and 4.2 ± .1 in year 2. Cows in good condition had a BCS of 6 ± .1 in year 1 and 5.6 ± .1 in year 2. During the infusion period, antibody titers against GnRH (^{125}I-GnRH bound at 1:100 dilution) were 29.5 ± 2.2 % in cows immunized against GnRH and were non-detectable in cows immunized against HSA.

Concentrations of LH before treatment (d 30 PP) were greater (P<.001) in year 1 (5.7 ± .2 ng/mL) compared with concentrations in year 2 (2.9 ± .2 ng/mL). During body years, concentrations of LH were not influenced by BCS or immunization against GnRH. Mean concentrations of LH were also greater (P<.001) during the infusion period in year 1 compared with year 2.

Concentrations of LH in HSA immunized cows treated with saline averaged 6.0 ± .8 ng/mL during the infusion period of year 1 (Figure 1) and were not influenced by BCS. Infusion of cows immunized against HSA or GnRH with GnRH-A increased concentrations of LH (Trt x Day, P<.001) compared with saline infused cows. Maximum concentrations of LH in serum were detected during the first day of infusions (d 31

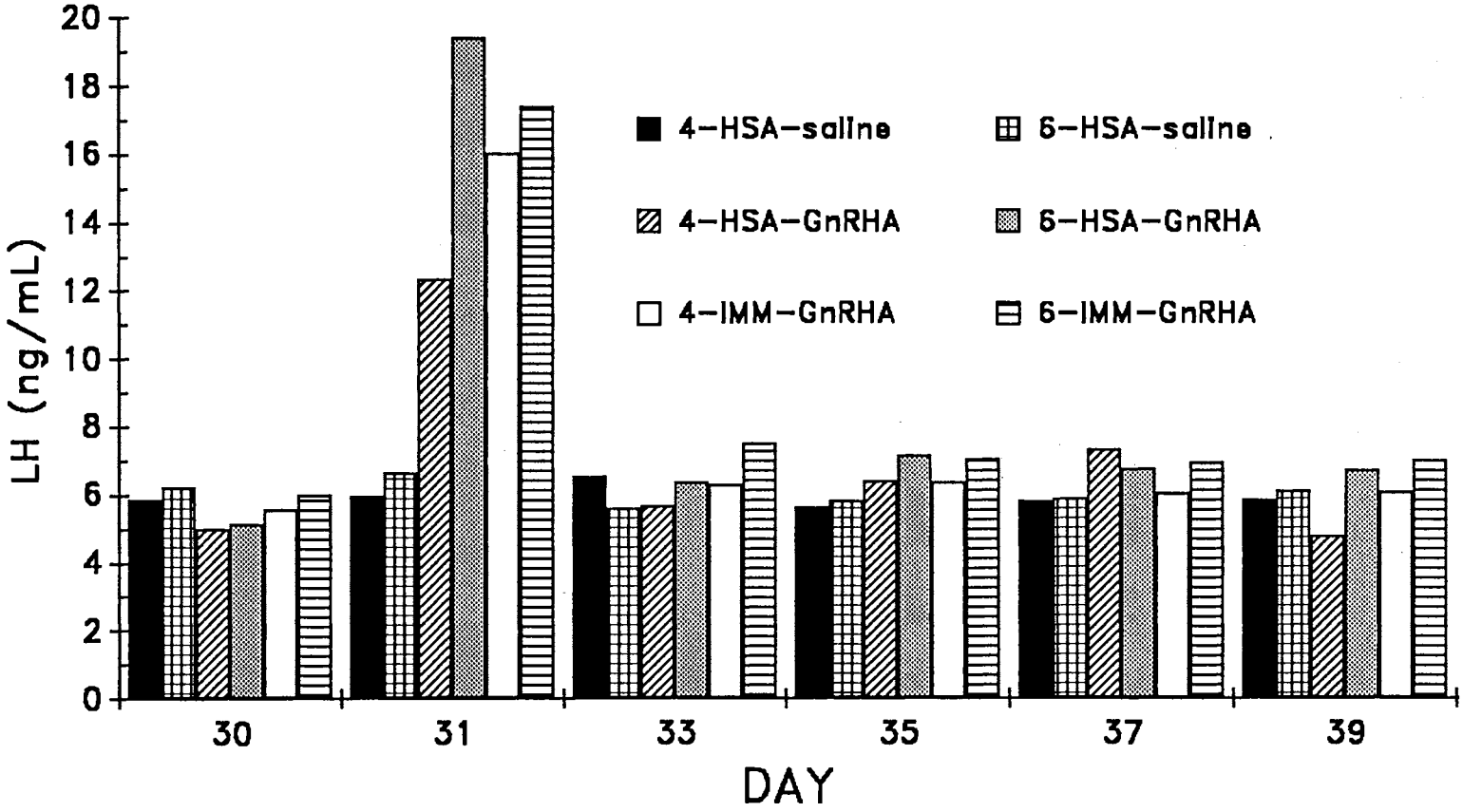

Figure 1. Least squares mean concentrations of LH in serum of postpartum beef cows infused with an analog of GnRH or saline (year 1)

PP). Concentrations of LH in cows treated with GnRH-A averaged 16.3 ± .7 ng/mL on d 31 PP and were not influenced by BCS or immunization against GnRH. Concentrations of LH were less (P<.05) in cows infused with GnRH-A on d 33, 35, 37 and 39 compared with d 31 and were not significantly influenced by treatment or BCS.

Concentrations of LH in serum of cows during year 2 (Figure 2) were increased by infusion of GnRH-A (Trt x Day, P<.001), and the response was influenced by the condition of the cows at calving (Trt x BCS, P<.04). During the treatment period, control (HSA) immunized cows with BCS=4 treated with saline tended (P<.07) to have greater concentrations of LH compared with cows in good (BCS=6) condition on the same treatment. Cows in good condition (BCS=6) immunized against HSA and treated with GnRH-A had greater (P< .05) concentrations of LH on d 31 PP (17.8 ± 1.7 ng/mL) compared with thin (BCS=4; HSA immunized; 8.9 ± 1.7 ng/mL) cows. Maximum concentrations of LH in cows on d 31 were not influenced by immunization against GnRH. Similar to year 1, concentrations of LH during year 2 were less (P<.05) in cows infused with GnRH-A on d 33, 35, 37 and 39 compared with d 31.

The frequency of pulses of LH (number/6 h) on d 30 was greater (P<.05) in cows in good condition (BCS=6; 2.2 ± .1) compared with thin (BCS=4; 1.7 ± .1) cows in both years 1 and 2 (Figure 3 and 4). The year x treatment x day interaction was significant during the infusion period. In

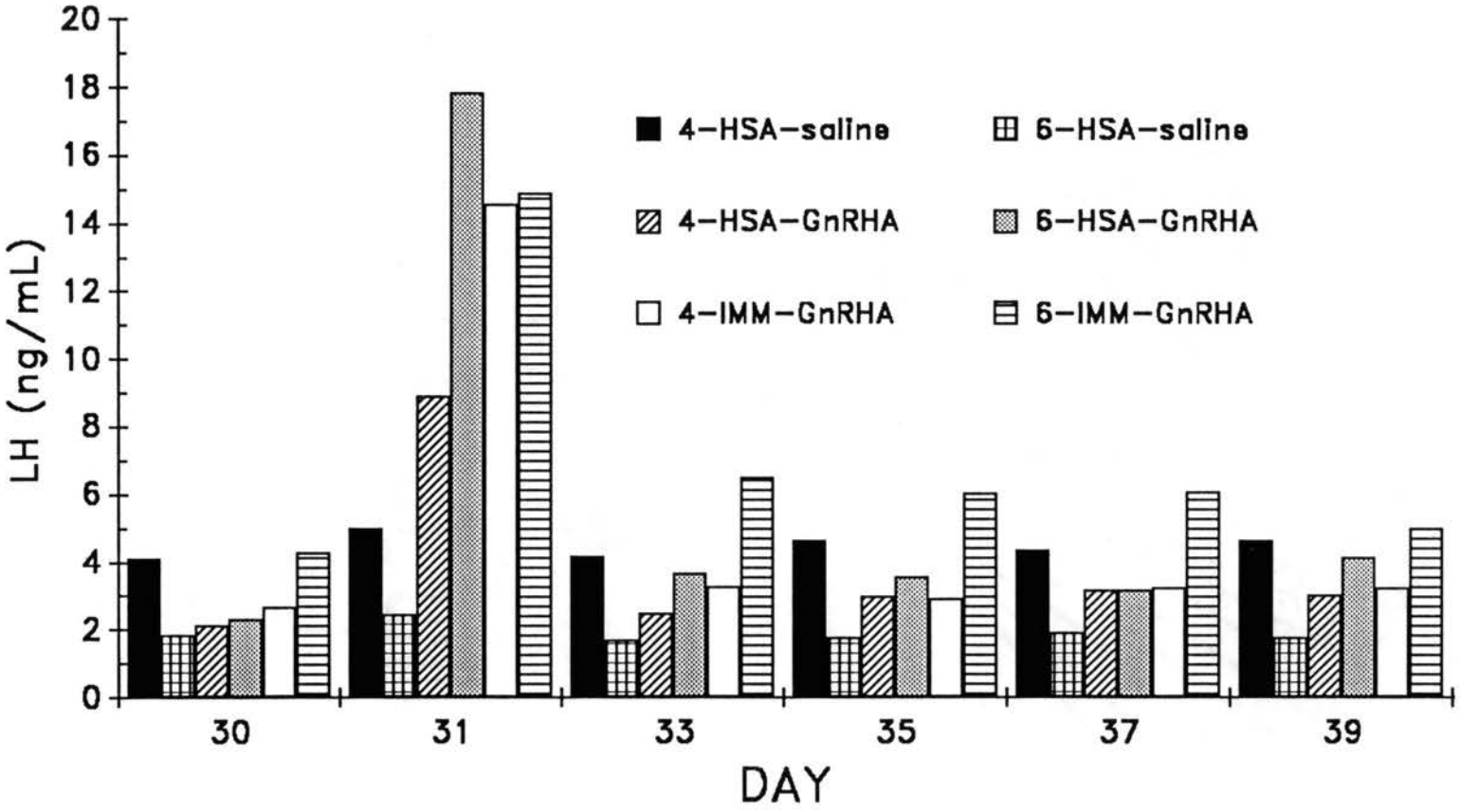

Figure 2. Least squares mean concentrations of LH in serum of postpartum beef cows infused with an analog of GnRH or saline (year 2)

year 1, cows in good condition (HSA immunized, BCS = 6; 2.4 ± .2 pulses/6 h) had a greater ($P < .02$) frequency of LH pulses (Figure 3) compared with thin (HSA immunized, BCS = 4; 1.7 ± .2 pulses/6 h) cows. Immunization against GnRH did not influence the number of pulses detected in cows infused once per h with GnRH-A during year 1.

During the second year, the frequency of LH pulses (Figure 4) was influenced by treatment but the response was dependent on the condition of cows at parturition (BCS x Treatment, $P<.05$) and the day of infusion of GnRH-A (Treatment x Day, $P<.07$). Cows with BCS=6 given control (HSA) immunizations and infused with saline had more (1.8 ± .2; $P<.06$) pulses per 6 h than thin (HSA immunized, BCS = 4; 1.3 ± .2 pulses/6 h) cows infused with saline. Infusion of GnRH-A did not influence the number of pulses of LH in HSA immunized cows with BCS=6 compared with saline treated cows, but thin (BCS=4) cows given control immunizations and infused with GnRH-A had more (2.0 ± .2 pulses/6 h; $P<.02$) pulses per 6 h than HSA immunized cows with BCS=4 that were infused with saline (1.4 ± .2 pulses/6 h).

The amplitudes of LH pulses on d 30 PP (prior to treatment) averaged 3.0 ± .4 ng/mL and were not influenced by BCS or immunization against GnRH. During the treatment periods (d 31-39) of year 1 (Figure 5) and year 2 (Figure 6), infusion of GnRH-A increased the amplitude of LH pulses but the response was dependent on the body energy reserves of the cow (BCS x Trt x Day, $P<.05$). Maximum amplitudes of

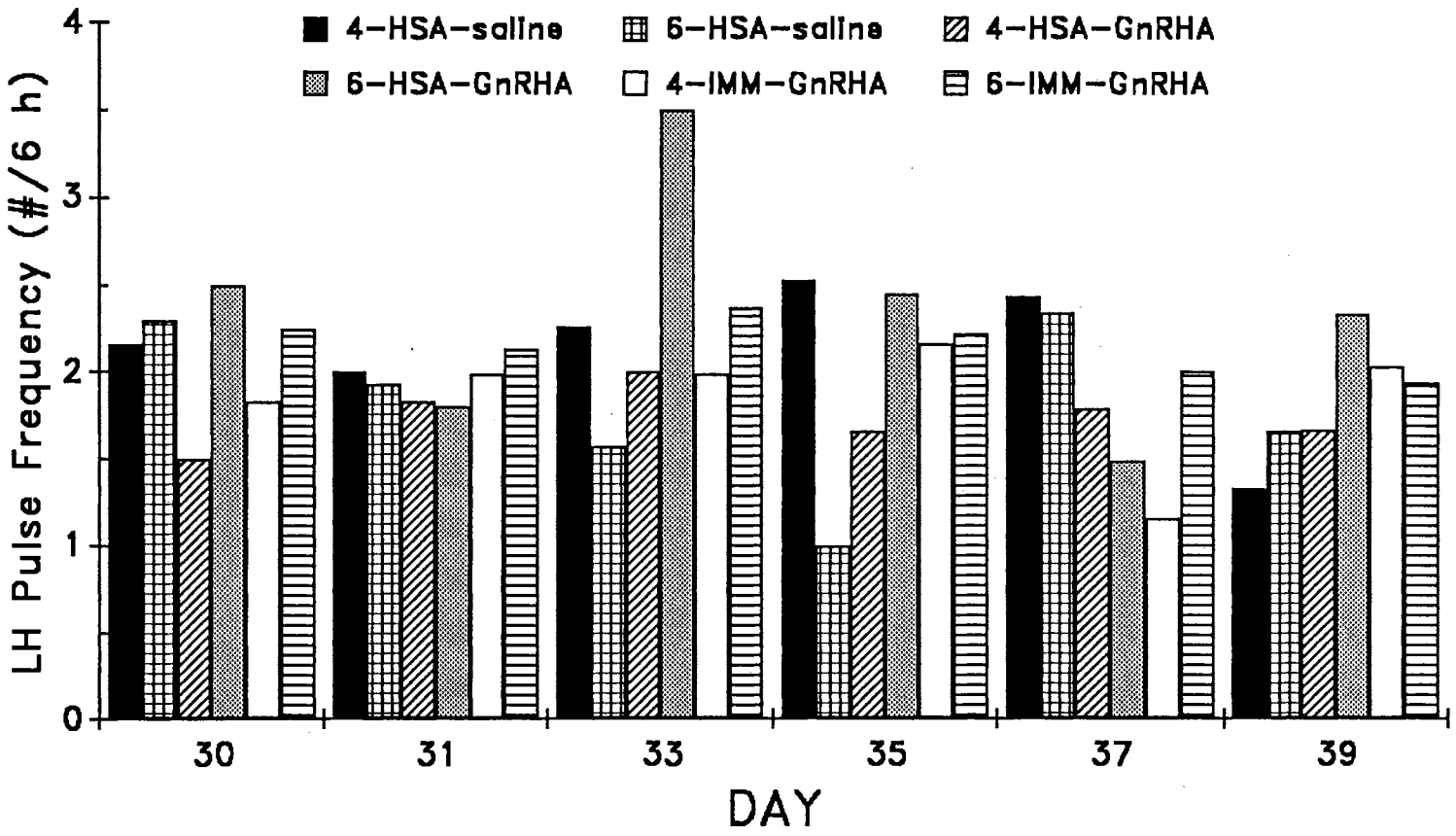

Figure 3. Least squares mean frequencies of LH in serum of postpartum beef cows infused with an analog of GnRH or saline (year 1)

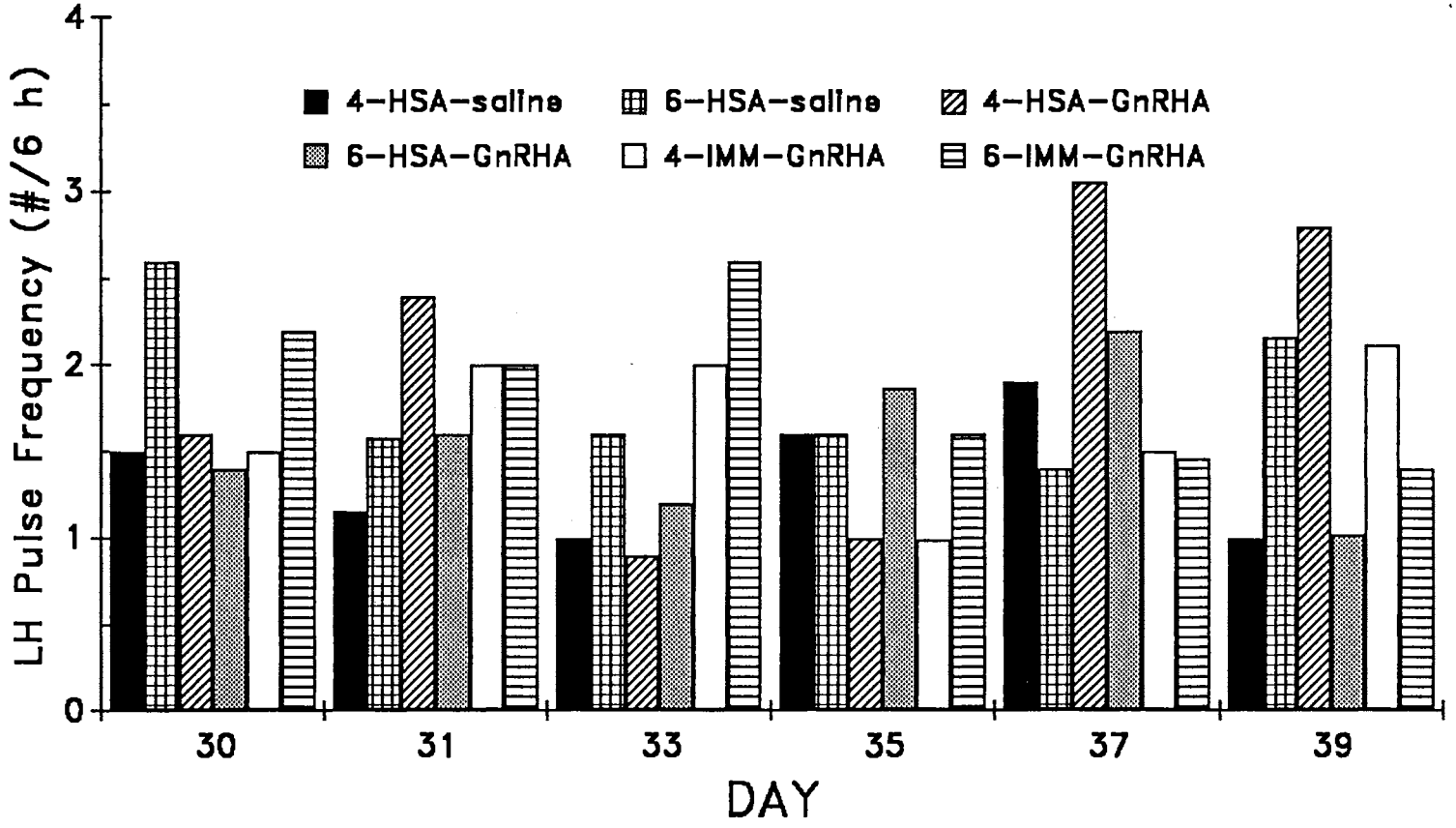

Figure 4. Least squares mean frequencies of LH in serum of postpartum beef cows infused with an analog of GnRH or saline (year 2)

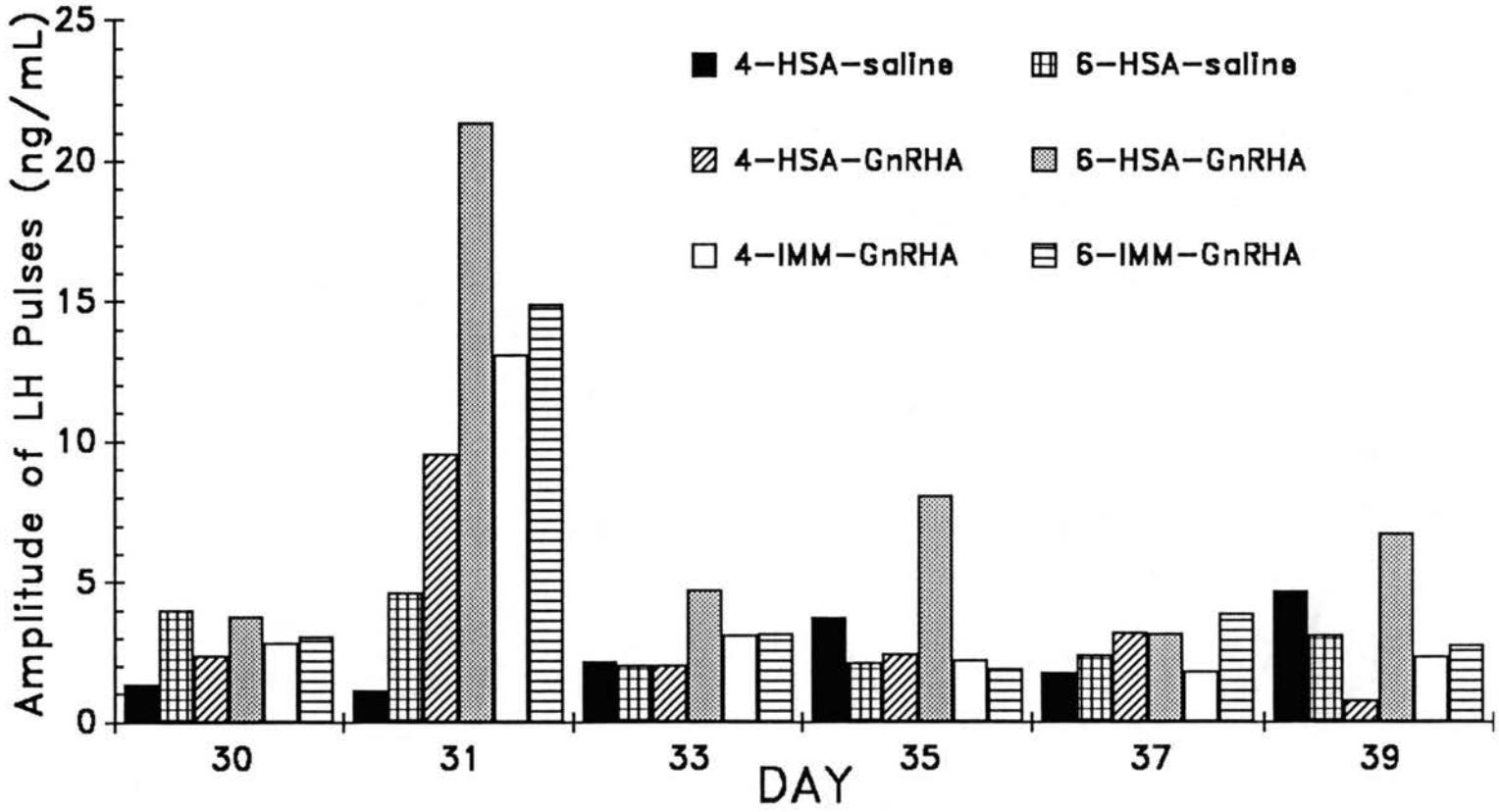

Figure 5. Least squares mean amplitudes of LH pulses in serum of postpartum beef cows infused with an analog of GnRH or saline (year 1)

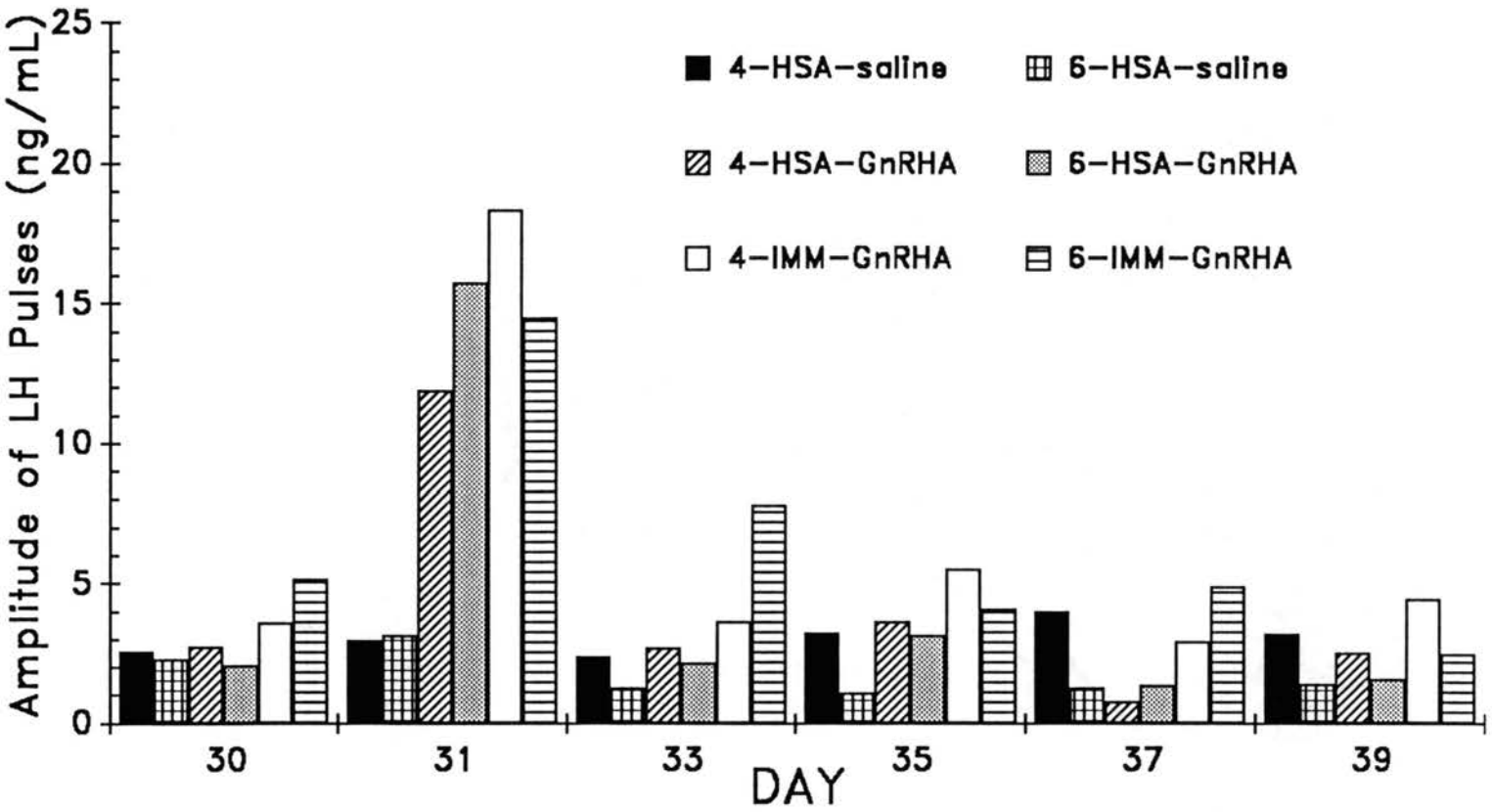

Figure 6. Least squares mean amplitudes of LH pulses in serum of postpartum beef cows infused with an analog of GnRH or saline (year 2)

LH pulses were detected in response to treatment with GnRH-A on d 31 PP (d 1 of infusions). Cows infused with GnRH-A had greater ($P < .001$) amplitudes of LH (HSA immunized, 16.6 ± 1.9 ng/mL; GnRH immunized, 17.6 ± 1.8 ng/mL) compared with cows infused with saline (4.5 ± 2.0 ng/mL). There was a tendency ($P < .12$) for BCS to influence the amplitudes of LH on d 31. The amplitudes of LH pulses were less ($P < .01$) in serum collected on d 33 through d 39 of infusion compared with d 31 and were not influenced by BCS or treatment.

Concentrations of FSH in serum (Table 1) from 32 cows (16 per year) were determined on d 30 PP, prior to the start of infusions of GnRH-A or saline. Concentrations of FSH in cows at 30 d PP averaged .44 ± .03 ng/mL and were not influenced by immunization against GnRH or BCS ($P >.17$). Neither the frequency of pulses of FSH (2.71 ± .29 pulses/6 h; $P >.31$) nor the amplitude of FSH pulses (.36 ± .08 ng/mL) at 30 d PP were influenced by body energy reserves of cows at calving. Immunization against GnRH did not affect concentrations of FSH, frequency of FSH pulses or the amplitude of FSH pulses.

Body condition of cows did not influence luteal function during treatment. Concentrations of progesterone in the plasma of cows with BCS of 4 and 6 were similar during pulsatile treatment with GnRH-A or saline. However, treatment influenced luteal activity (Table 2). More cows (with a BCS of 4 or 6) pulsed with GnRH-A had luteal

Table 1. Influence of BCS and immunization against GnRH on secretion of FSH in beef cows at 30 days post partum.

	Treatment				Standard Error
	4-HSA	4-GnRH	6-HSA	6-GnRH	
Cows, no	8	8	8	8	
Mean, ng/mL	.44	.41	.38	.53	.04
Pulses, no/6 h	2.50	2.75	2.88	2.75	.41
Amplitude					
Average, ng/mL	.51	.32	.30	.32	.12
Maximum, ng/mL	.71	.36	.44	.40	.18

BCS = 4 or 6; HSA = Immunized against human serum albumin; GnRH = Immunized against GnRH.

Table 2. Influence of BCS, immunization against GnRH and infusion of an analog to GnRH on luteal activity (LA) and reproductive function of postpartum beef cows.

	Treatment						Significant Effect
	4-S	4-G	4-IG	6-S	6-G	6-IG	
Cows, no	12	11	12	10	12	13	
Pregnant, %	92	91	64	100	88	85	TRT ($P < .1$)
C-C, d	100	93	95	87	80	91	BCS ($P < .05$)
LA for 3 d, %	17	58	46	20	90	42	TRT ($P < .03$)
LA PT, %	0	0	0	11	33	9	BCS ($P < .01$)
Prog > 2ng/ml, %	9	58	54	20	90	33	TRT ($P < .001$)
Days of LA, d	.4	3.2	2.4	1.3	4.1	2.1	TRT ($P < .001$)

BCS = 4 or 6; S = Saline; G = Pulsatile GnRH-A; IG = Immunized against GnRH and pulsatile GnRH-A. C-C = Calving to conception. PT = Post treatment.

activity for at least 3 days during treatment compared with cows pulsed with saline. When cows with a BCS of 6 were immunized against GnRH and pulsed with GnRH-A, a lesser percentage ($P < 0.03$) had luteal activity for 3 days during treatment compared with cows immunized against HSA (control) and pulsed with GnRH-A. The percentage of cows with greater than 2 ng/ml of progesterone, and the days of luteal activity during treatment, were influenced by treatment in a similar way as the percentage of cows with luteal activity was effected.

Luteal activity after treatment was influenced by BCS ($P < 0.01$). None of the cows with a BCS of 4 had luteal activity for 8 d in succession during the first 3 wk after treatment. Only one of the cows with a BCS of 6 in either the group of cows treated with saline or the group immunized against GnRH and pulsed with GnRH-A had luteal activity after treatment. However, 33% of the HSA immunized cows with BCS=6 that were infused with GnRH-A ($P < 0.08$, compared with saline) had luteal activity for 8 days during the first 3 wk after treatment. Pregnancy rate tended ($P < .10$) to be influenced by treatment especially in cows with a BCS of 4. When cows were immunized against GnRH and pulsed with GnRH-A, only 64% became pregnant compared with 92% of the saline pulsed cows and 91% of the cows pulsed with GnRH-A ($P < .06$). Days from calving to conception were influenced by BCS ($P < .05$) but not by

treatment. Cows with a BCS of 4 became pregnant an average of 10 days later than cows with a BCS of 6.

Discussion

Some variability in concentrations of LH and FSH was expected in cows at 30 d post partum. By the third week post partum, the pituitaries of cows on adequate diets have maximized their ability to synthesize and release gonadotropins (Moss et al., 1985; Leung et al., 1986) but after early weaning (Bishop, 1991) or nutritional restriction (Richards et al., 1989; Bishop and Wettemann, 1993) of beef cows, LH secretion may be decreased in cows with decreased body energy reserves. The lack of an influence of BCS on concentrations of FSH in cows at 30 d PP agrees with Walters et al., 1982 and Leung et al. (1986) and further emphasises the permissive role of FSH in the onset of estrous cycles following parturition (Carruthers et al., 1980; Williams et al., 1983). The mechanisms by which energy availability influences postpartum fertility may include an impaired ovarian response to gonadotropins, reduced pituitary responsiveness to GnRH and/or reduced pulsatile release of GnRH (Schillo, 1992). Increased mean concentrations of LH or FSH represent increased frequency and/or magnitude of pulses of these gonadotropins indicating the onset of ovarian activity in cows with adequate body energy reserves.

In this experiment, endogenous GnRH was replaced with pulsatile infusion of an analog of GnRH (GnRH-A) to study the direct effects of body energy reserves on the pituitary. The dosage was determined in a preliminary trial with postpartum cows. In that trial, injection (i.v.) of 675 ng of GnRH-A resulted in increased concentrations of LH that were similar to the amount of LH in serum in response to injection (i.v.) of 1 μg of GnRH (Wettemann, unpublished data). Intravenous infusion of GnRH-A resulted in greater than 3-fold increases in the amplitude of LH pulses compared with cows given saline on d 31 PP. Failure of pulses of GnRH-A on d 33 to d 39 to maintain increased concentrations of LH in serum may indicate a decrease in the releasable stores of LH in the pituitary, desensitization of receptors in the pituitary to the analog of GnRH, or an inability of the pituitary to maintain synthesis of LH at a rate necessary to maintain serum concentrations.

The influence of BCS on the amount of LH in serum in response to the analog of GnRH may represent increased synthesis of gonadotropins (Hamernick et al., 1986; Dalkin et al., 1989) or a greater number of GnRH receptors (Wise et al., 1984; Katt et al., 1985) in response to increased endogenous GnRH released in cows with greater BCS (Rasby et al., 1992). The increased LH released after treatment with a GnRH analog is probably not due to greater releasable stores of LH in the pituitary of cows with BCS=6, as

pituitary weights and content of LH in the pituitary of nonlactating cows were not influenced by nutrient intake or BCS (Rasby et al, 1991). Thin (BCS=3), nonlactating cows had greater concentrations of LH in serum in response to an injection of GnRH, but cows with BCS of 5, 6 or 7, fed to maintain BW, had similar concentrations of LH after injection of GnRH (Rasby et al., 1992). In ovariectomized ewes, concentrations of LH and messenger RNA for the α- and ß-subunits of LH are decreased when a critical amount of BW is lost as a result of severe nutrient restriction (Kile et al., 1991) and intravenous infusion of GnRH for 21 d restores gonadotropins. Messenger RNA for LH and FSH are differentially regulated by GnRH in castrated rats (Dalkin et al., 1989) and ovariectomized ewes (Hamernik and Nett, 1988). Hourly infusion of GnRH for 7 d increased the amount of messenger RNA for LH in the pituitary of ewes after hypothalamic-pituitary disconnection. It is possible that the frequency and/or amplitude of endogenous pulses of GnRH, including concentrations of GnRH not bound to antibodies in cows immunized against GnRH (Wettemann and Castree, 1993), were greater in cows with BCS=6 at 31 d PP compared with thin (BCS=4) cows, thus resulting in greater concentrations of LH in serum during treatment with GnRH-A.

BCS of cows influenced the onset of luteal activity in beef cows after, but not during, treatment with GnRH-A. Luteal activity during treatment with GnRH-A could be due to ovulation or luteinization of follicles that are on the

ovary. Luteal activity after treatment indicates the onset of normal cycles is under the control of endogenous GnRH. The influence of BCS on luteal activity and days from calving to conception are in agreement with Dunn et al. (1980) and Selk et al. (1988). When cows in thin (BCS=4) to very good condition (BCS=7) were fed 100% of requirements and exposed to bulls until at least 100 d post partum (Selk et al., 1988), the single most important factors influencing pregnancy rate was the body condition of cows at calving.

The decreased luteal activity, during and after treatment, in cows immunized against GnRH and pulsed with GnRH-A, compared with HSA immunized cows infused with GnRH-A, and decreased pregnancy rates in cows immunized against GnRH compared with control (HSA) immunized cows, suggest, in agreement with Bishop et al. (1992), that growth of follicles is blocked by immunization against GnRH. Immunization of cows during the postpartum interval delays the onset of luteal activity and reduces the percentage of cows pregnant after a limited breeding season (O'Connell and Wettemann, 1990). The effects of GnRH immunization on the secretion of gonadotropins during the postpartum interval have not been evaluated. Immunization of heifers against GnRH (Wettemann and Castree, 1993; O'Connell, 1990) blocks normal reproductive cycles but does not suppress basal LH secretion in heifers (O'Connell, 1990). Pituitary stores of LH and FSH are reduced by immunization of ewes

against GnRH (Adams and Adams, 1986) but basal secretion of LH and FSH are unchanged. In this study, immunization of cows against GnRH during the postpartum interval did not influence secretion of LH or FSH, prior to treatment with GnRH-A (d 30 PP) or in response to GnRH-A (d31 - d39).

Pregnancy rate tended ($P<.10$) to be influenced by treatment. Within cows with thin BCS, only 64% of cows immunized against GnRH and pulsed with GnRH-A were pregnant compared ($P <.07$) with 92% of control cows and 91% of cows pulsed with GnRH-A. The percentage of cows in good body condition score that were pregnant at the end of the breeding season was not influenced by treatment.

We conclude that body energy reserves (BCS = 4 or 6) do not have a direct effect on the pituitary to influence gonadotropin secretion. BCS influences LH secretion in response to an analog of GnRH only on the first day of treatment. BCS influences luteal activity after treatment, but BCS does not influence the ovarian response to an analog of GnRH. Immunization of postpartum anestrous cows against GnRH did not influence basal secretion of LH or FSH, or GnRH-A stimulated LH release. However, immunization against GnRH tended to reduce the percentage of cows with luteal activity after treatment and the percentage of cows pregnant at the end of the breeding season. Body energy reserves appear to have a permissive role in controlling LH secretion. If body condition is adequate no effect is evident on LH, but as condition

decreases below a minimum BCS, LH concentrations in serum decrease and this decrease is associated with anestrus.

Implications

Cows in this condition have decreased reproductive performance and may fail to be pregnant at the end of the breeding season. Concentrations of LH from the pituitary are decreased in cows with thin BCS probably due to decreased release of GnRH from the hypothalamus. Determination of the location of effects of body energy reserves will aid in attempts to increase reproductive performance in beef cows.

CHAPTER IV

OVARIAN RESPONSE AFTER GONADOTROPIN TREATMENT OF HEIFERS IMMUNIZED AGAINST GONADOTROPIN-RELEASING HORMONE (GnRH)

ABSTRACT: Angus x Hereford heifers were used to determine if the ovaries of heifers immunized against GnRH would respond to exogenous gonadotropins. The ovarian response of prepuberal heifers to the same gonadotropins was also evaluated. Postpuberal heifers (POST; n = 23; 491 ± 23 kg) were assigned to a 2 x 2 factorial design: immunization against GnRH conjugated to human serum albumin (HSA) or HSA, and control (C; saline) or treatment with gonadotropins (GTH). The GTH treatment consisted of 2000 IU PMSG + 1000 IU hCG and C heifers were given saline (s.c.). Prepuberal (PRE; n = 11; 221 ± 20 kg) heifers were given one of three doses of gonadotropins: C, GTH or one half GTH (LGTH). Immunization against GnRH (GnRH-IMM) caused cessation of estrous cycles (progesterone < 1 ng/ml plasma for 3 wk) and GTH treatment began within 2 wk after anestrus. Control (HSA-IMM) heifers were treated on d 9 to 11 of the estrous cycle. All heifers were given PGF (i.m.)

on d 2 (d 0 = start of GTH treatment) and 2000 IU of hCG (i.m.) on d 4. Blood samples were collected daily between d 0 and d 8 and every second day through d 14 and progesterone and estradiol were quantified. Ovaries of POST heifers were evaluated by ultrasound: size and number of follicles ≥ 6 mm and the number of corpora lutea (CL) were determined. Concentrations of estradiol and progesterone were increased by GTH treatment of POST heifers but the response was reduced by GnRH-IMM (IMM x GTH x day, $P < .01$). Number of follicles ≥ 6 mm (d 0-14) was increased after GTH (GTH x day, $P < .001$) but was decreased ($P < .03$) by GnRH-IMM. Immunization against GnRH reduced total follicles on d 4 in heifers on both C and GTH treatments. Gonadotropin treatment increased the number of follicles ≥ 16 mm on d 14 from 0.0 ± 0 in C to 3.4 ± .4 in GTH treated heifers. The number of CL was increased by GTH (GTH x day, $P < .001$) but the response was reduced ($P < .01$) in GnRH-IMM heifers compared with HSA-IMM heifers. Concentrations of estradiol in PRE heifers increased between d 0 and d 14 in GTH and LGTH heifers, but not in C heifers (trt x day, $P < .001$). Concentrations of progesterone in PRE heifers on d 10 were greater ($P < .01$) in GTH and LGTH heifers compared with C heifers. Treatment with gonadotropins, at a dose sufficient to increase estradiol and progesterone concentrations in PRE heifers, stimulated folliculogenesis in heifers immunized against GnRH. We conclude that gonadotropin treatment will induce

follicular growth and ovulation in heifers that are anestrus due to immunization against GnRH.

Introduction

Immunoneutralization of GnRH effectively isolates the anterior pituitary from hypothalamic stimulation and results in cessation of estrous cycles in rats (Fraser, 1975), gilts (Esbenshade and Britt, 1985), ewes (Clarke et al., 1978), mares (Garza et al., 1986) and heifers (Johnson et al., 1988; Wettemann and Castree, 1994). Ovaries from females immunized against GnRH weighed less and had decreased numbers of corpora lutea compared with control animals (Fraser, 1975; Clarke et al., 1978). After immunization against GnRH and the onset of anestrus, fewer follicles > 10 mm were detected in mares (Garza et al., 1986) and heifers (Johnson et al., 1988) and morphologically distinct follicles were not present on the ovaries of gilts (Traywick and Esbenshade, 1988). Pituitary stores of LH and FSH are reduced by immunization of ewes against GnRH (Adams and Adams, 1986), but basal secretion of gonadotropins are unchanged. Similarly, basal secretion of LH is maintained in heifers that become anestrus (O'Connell, 1990) after immunization against GnRH. As antibody titers against GnRH decrease with time after immunization, estrous cycles are reinitiated in heifers (O'Connell, 1990).

Attempts to restore cyclic ovarian activity in gilts and heifers, while titers against GnRH were adequate to cause anestrus, were not successful. Intravenous infusion of an analog of GnRH stimulated release of LH and FSH in anestrous gilts (Traywick and Esbenshade, 1988), and pulsatile infusion of an analog of GnRH increased concentrations of LH and the amplitude of LH pulses in anestrous heifers (O'Connell, 1990) but neither treatment caused reinitiation of estrous cycles. Neither injection of PMSG nor extracts from porcine pituitaries increased estradiol concentrations or caused follicular development in gilts that were anestrus after immunization against GnRH (Esbenshade, 1987).

The objective of this experiment was to determine if exogenous gonadotropins would stimulate follicular growth in heifers that were anestrus as a result of active immunization against GnRH.

Material and Methods

Heifers were maintained in a drylot with ad libitum access to prairie grass hay and water. A protein supplement and minerals were fed to meet NRC (1984) requirements for maintenance. Heifers were weighed and a body condition score (BCS; Wagner et al., 1988) was assigned at the booster immunization (experiment 1), at

treatment (d 0), and on d 14 to verify that diets maintained BW and BCS.

Experiment 1. Postpuberal (POST) Angus x Hereford heifers (n = 23) that were 24 mo of age and exibiting normal estrous cycles were used. Twelve of the heifers had been immunized against GnRH conjugated to human serum albumin (HSA). A primary immunization was given 15 mo previously and booster immunizations were given 12 and 14 mo previously. Eleven of the heifers (HSA-IMM; controls) had been immunized against HSA. GnRH immunized heifers were given a booster immunization (GnRH-IMM) to enhance antibody production and to cause cessation of estrous cycles. Control heifers received a booster immunization against HSA. GnRH (Sigma Chemical Co., St. Louis, MO) was conjugated to HSA (Sigma Chemical) by the carbodiimide reaction (Fraser, 1975; O'Connell, 1990). For primary immunizations, GnRH conjugated to HSA or HSA was emulsified in Freund's complete adjuvant (Sigma Chemical). For booster immunizations, GnRH conjugated to HSA or HSA was emulsified in Freund's incomplete adjuvant (Sigma Chemical).

Blood samples were collected weekly commencing on the day that the booster immunization was given and continuing until the heifers immunized against GnRH became anestrus (concentrations of progesterone < 1 ng/mL for 3 wk). Antibody titers were quantified each week in serum using a procedure similar to that described by Esbenshade and Britt

(1985). Anestrous heifers (n = 12) at 3 to 5 wk after the cessation of estrous cycles were alloted by titers and assigned to treatment with gonadotropins (PG 600, Intervet America Inc., Millboro, DE). Estrous cycles of heifers immunized with HSA (n = 11; HSA-IMM) were synchronized with prostaglandin $F_{2\alpha}$ (PGF; Lutalyse, The Upjohn Co., Kalamazoo, MI) and heifers were treated between d 9 and 11 (d 0 = estrus) of the subsequent cycle.

Treatments were arranged in a 2 x 2 factorial design. Half of the anestrous (GnRH-IMM; n = 6) heifers and six of the control (HSA-IMM) immunized heifers received gonadotropins (2000 IU PMSG + 1000 IU hCG; GTH). The remaining heifers were given saline (s.c.; C). Treatments (10 mL) were administered (s.c.; 40, 30 and 30% of the total dose) at 6 h intervals on d 0. All heifers were given PGF on d 2 and 2000 IU hCG (i.m.; Rugby Laboratories, Inc., Rockville Centre, N.Y.) on d 4.

Blood samples (15 mL) were collected prior to treatment (d 0), daily through d 8 and every second day through d 14. Oxalic acid (1.25 mg) was added to each sample and samples were cooled to 4° C. Samples were centrifuged (3000 x g for 20 min) within 4 h and plasma was decanted and stored at -20° C. Concentrations of progesterone (Bishop and Wettemann, 1993a) and estradiol (Hallford et al., 1979) were quantified in plasma by RIA. Additional blood samples were collected for serum on d 0 and d 14. Samples were allowed to clot for 16 h at 4° C,

then centrifuged (3000 x g for 20 min), and serum was decanted and stored at -20° C. Antibody titers against GnRH were quantified as the amount of ^{125}I-GnRH that was bound to antibodies in a 1:1000 dilution of serum.

Ovaries of POST heifers were examined with an Aloka 210 portable ultrasound scanner equipped with a 5 MHz transducer designed for intrarectal examinations (Pierson and Ginther, 1987). Both ovaries of each heifer were evaluated just prior to treatment on d 0 and on d 2, 4, 6, 8 and 14. Ovarian ultrasound images were recorded on video tape and projected on a screen. The size of the ovary, and the size, location, and numbers of follicles and corpora lutea (CL) were determined. Numbers of follicles ≥ 6 mm were categorized as medium (6 - 10 mm), large (11 - 15 mm) and X-large (> 16 mm).

Split plot analyses of variance were used to determine the effects of reproductive state and gonadotropin treatment on concentrations of estradiol and progesterone in plasma and numbers and sizes of ovarian follicles and number of corpora lutea. The main effects were treatment combinations and interactions. The subplot was time after administration with GTH and interactions. Concentrations of estradiol and progesterone in plasma, and numbers and sizes of follicles and number of corpora lutea on the ovaries of GnRH-IMM heifers were compared to HSA-IMM heifers on the same day after GTH treatment using Bonferroni t-statistics (Gill,1973). Pearson's correlation

coefficients were used to relate concentrations of estradiol in plasma with sizes and numbers of follicles and progesterone concentrations with number of corpora lutea.

Experiment 2. Prepuberal Angus x Hereford heifers (n=12) were randomly assigned to one of three doses of gonadotropins. Heifers were given C or GTH as described for experiment 1 or one half GTH (1000 IU PMSG + 500 hCG; LGTH). Treatments with PGF and hCG were given, and blood plasma was collected as described in experiment 1.

Split plot analyses of variance were used to determine the effects of gonadotropin treatment on concentrations of estradiol and progesterone in plasma of PRE heifers. The main effects were GTH treatments and the subplot was time after administration of treatments and interactions. Orthogonal contrasts were used to compare the effects of the three doses of gonadotropins on concentrations of estradiol and progesterone in plasma on a given day.

Results and Discussion

Experiment 1. POST heifers immunized against GnRH were anestrus (1st of 3 consecutive samples with progesterone < 1 ng/mL plasma) by 3.3 ± .1 wk after the booster was given. GTH treatment was initiated within 5 wk after the onset of anestrus. At treatment, heifers weighed 491 ± 23 kg, had a BCS of 6.2 ± .2, and the antibody titers against GnRH (^{125}I-GnRH bound at 1:1000 dilution) were 69.5 ± 3.2 % in

GnRH-IMM heifers and non-detectable in HSA-IMM heifers. Similar to a previous report (Wettemann and Castree, 1994), concentrations of progesterone in plasma indicated that the CL present at the time of the booster immunizations had normal lifespans but subsequent ovulations were inhibited.

Concentrations of estradiol were 6.6 ± .7 pg/mL in cyclic (HSA-IMM) heifers (on d 9 to 11 of the estrous cycle) and 5.4 ± .7 pg/mL ($P > .2$) in anestrous (GnRH-IMM) heifers prior to GTH treatment. Concentrations of estradiol in POST heifers (Figure 7) increased after GTH but the response was reduced by immunization against GnRH (IMM x GTH x day; $P<.01$). Concentrations of estradiol in GnRH-IMM heifers given C injections averaged 5.0 ± 1.0 pg/mL and did not differ throughout the sampling period. HSA-IMM heifers given C had maximum concentration of estradiol (12.5 ± 3.0 pg/mL) on d 4, indicating follicular growth after luteal regression induced by PGF treatment (Fortune and Quirk, 1988). GnRH-IMM heifers given GTH had increased concentrations of estradiol in plasma on d 4 and concentrations tended ($P < .1$) to be greater than in GnRH-IMM + C and HSA-IMM + C during d 5 through 8. Concentrations of estradiol on d 4 were greater ($P < .05$) in HSA-IMM heifers treated with GTH compared with all other treatments and concentrations of estradiol in plasma were elevated in GTH treated HSA-IMM heifers on d 7 and 8, indicating a second wave of follicular growth. Concentrations of estradiol in heifers immunized against

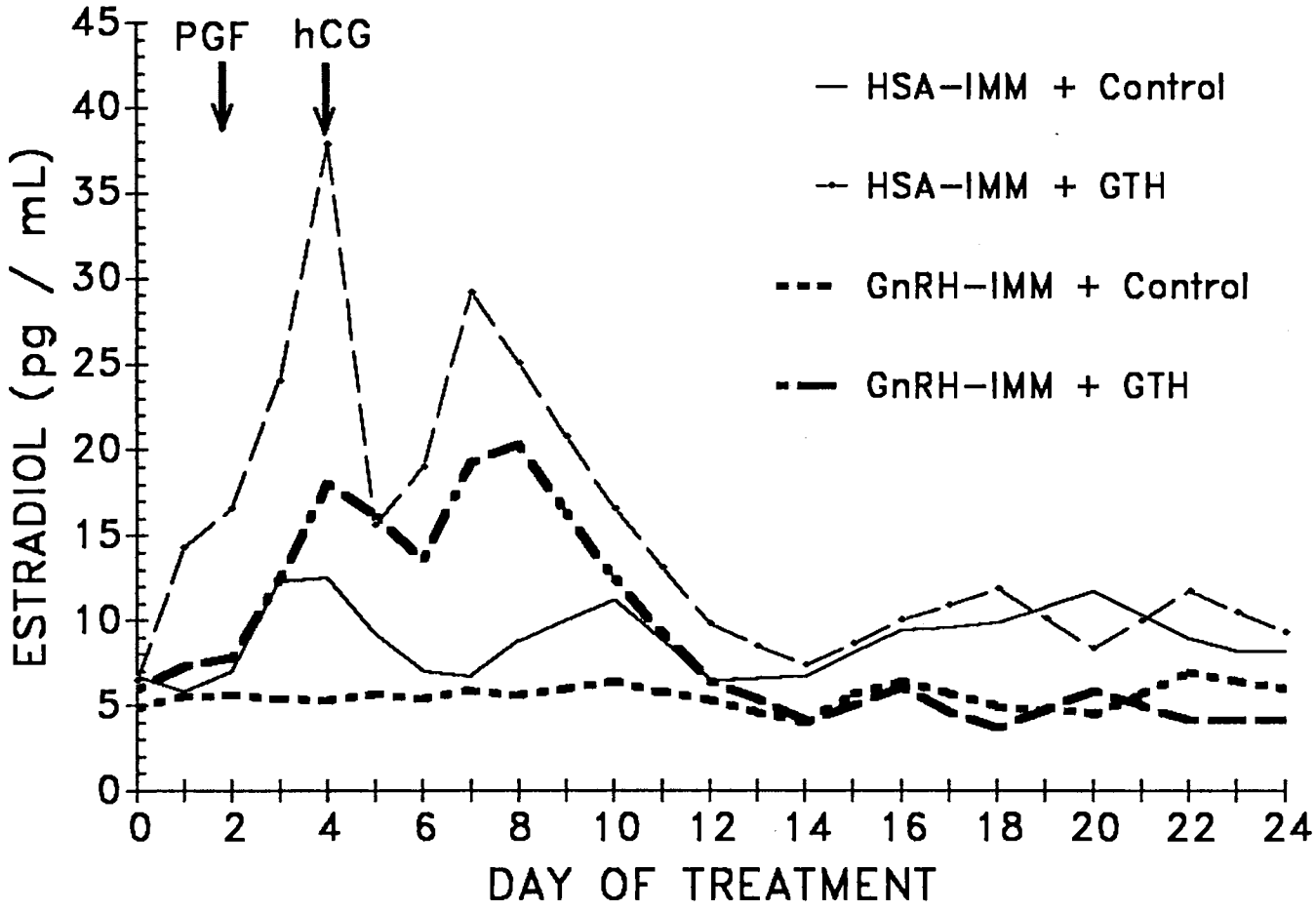

Figure 7. Least squares mean concentrations of estradiol in plasma of heifers immunized against GnRH or human serum albumin (HSA) and treated with gonadotropins (GTH) or saline

GnRH averaged 5.8 ± 6 pg/mL on d 14 through d 24 and were not influenced by GTH treatments. Concentrations of estradiol in control (HSA-IMM) heifers between d 14 and 24 were indicative of normal follicular growth (Fortune and Quirk, 1988), but estradiol concentrations of d 14 through the end of the trial were decreased ($P < .001$) by immunization against GnRH.

Concentrations of progesterone (Figure 8) at treatment (d 0) were 6.2 ± .3 and 0.5 ± .3 ng/mL for HSA-IMM and GnRH-IMM heifers, respectively. On d 7 concentrations of progesterone were similar in HSA-IMM heifers 2.9 ± 2.7 ng/mL and in GnRH-IMM heifers (1.4 ± 2.6 ng/mL) treated with GTH. Maximum concentrations of progesterone were detected in GTH treated heifers and in cyclic (HSA-IMM) heifers on d 14. Treatment with GTH increased (GTH x day; $P < .001$) concentrations of progesterone between d 7 and d 14. Concentrations of progesterone on d 14, were greater ($P < .01$) in GTH heifers (31.8 ± 2.6) compared with C treated heifers (5.4 ± 2.7). Immunization against GnRH did not influence ($P > .3$) the increase in progesterone in response to GTH.

Concentrations of progesterone on d 24 averaged 2.18 ± .87 ng/mL in POST heifers and were not effected by treatments. the decline in progesterone concentrations occuring between d 14 and d 24 was influenced by treatment with GTH and GnRH-IMM (GTH x IMM x day, $P < .03$).

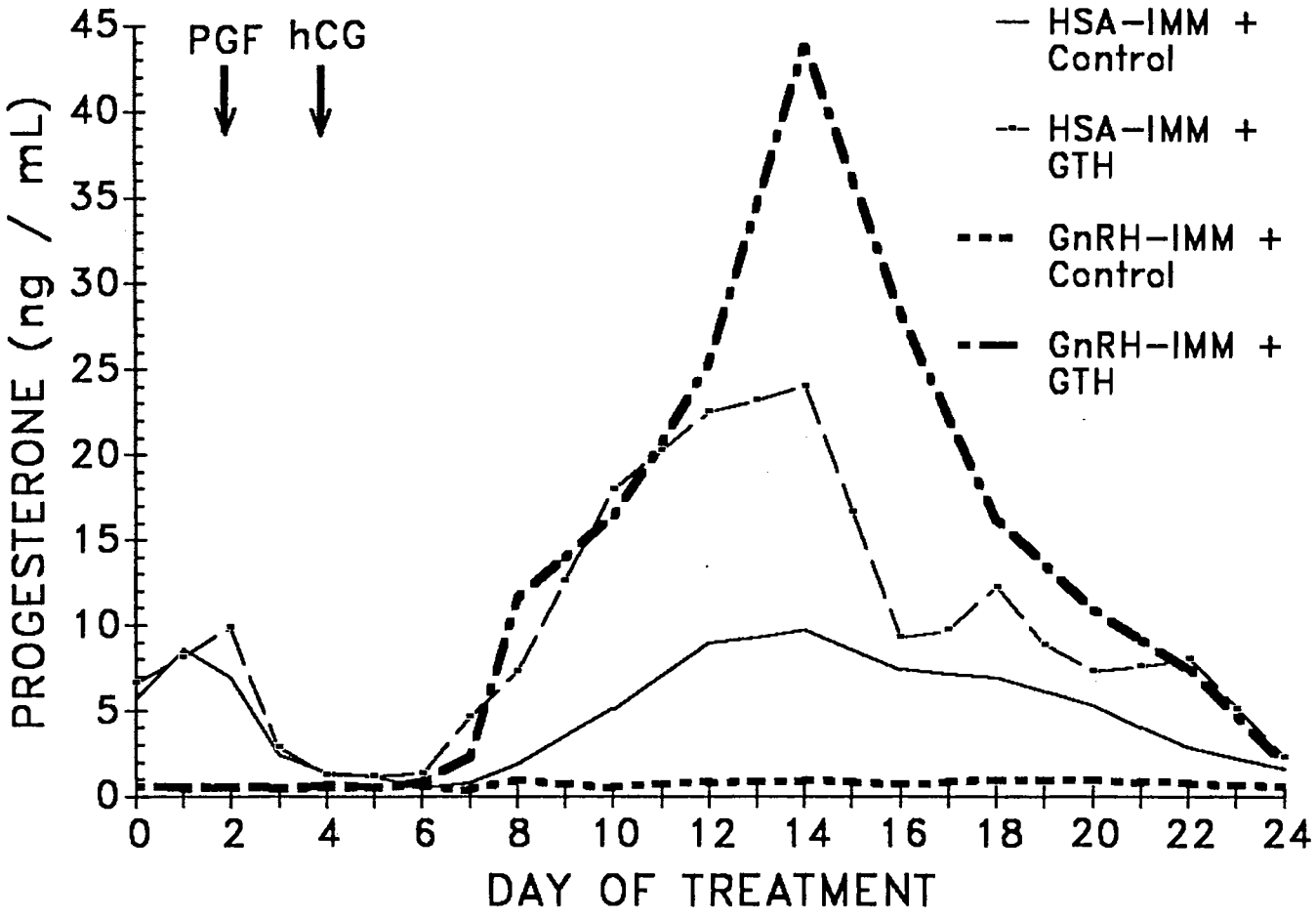

Figure 8. Least squares mean concentrations of progesterone in plasma of heifers immunized against GnRH or human serum albumin (HSA) and treated with gonadotropins (GTH) or saline

Hallford et al. (1979) also found increased concentrations of estradiol and progesterone within 2 days after treatment of cows with PMSG on day 5 or 17 of the estrous cycle and PMSG treatment altered secretion of estradiol, progesterone and endogenous gonadotropins in heifers (Bevers et al., 1989). The two-cell theory for regulation of estradiol production (Fortune and Quirk, 1988) indicates that both LH and FSH are important during the early follicular phase, however, only a surge of LH is essential for ovulation. Immunization against GnRH decreased secretion of endogenous gonadotropins in pigs (Esbenshade and Britt, 1985) and ewes (Adams and Adams, 1986), but did not decrease basal concentrations of LH in serum of heifers (O'Connell, 1990). Estradiol concentrations in GnRH-IMM heifers indicate that follicular maturation was reduced compared with HSA-IMM heifers.

The total number of follicles on ovaries (Figure 9) is the sum of the medium (6-10 mm), large (11-15 mm) and X-large (≥ 16 mm) follicles. Injection of GTH increased the total number of follicles (GTH x day; $P < .01$) and GnRH-IMM decreased (IMM x day, $P < .03$) the the total number of follicles. Estradiol concentrations in plasma, adjusted for day of treatment, were related to total follicles > 6mm ($r = .54$, $P < .01$).

The number of medium and large follicles (Figure 10) on ovaries of heifers increased with day after GTH treatment (GTH x day, $P < .001$) and GnRH-IMM decreased the

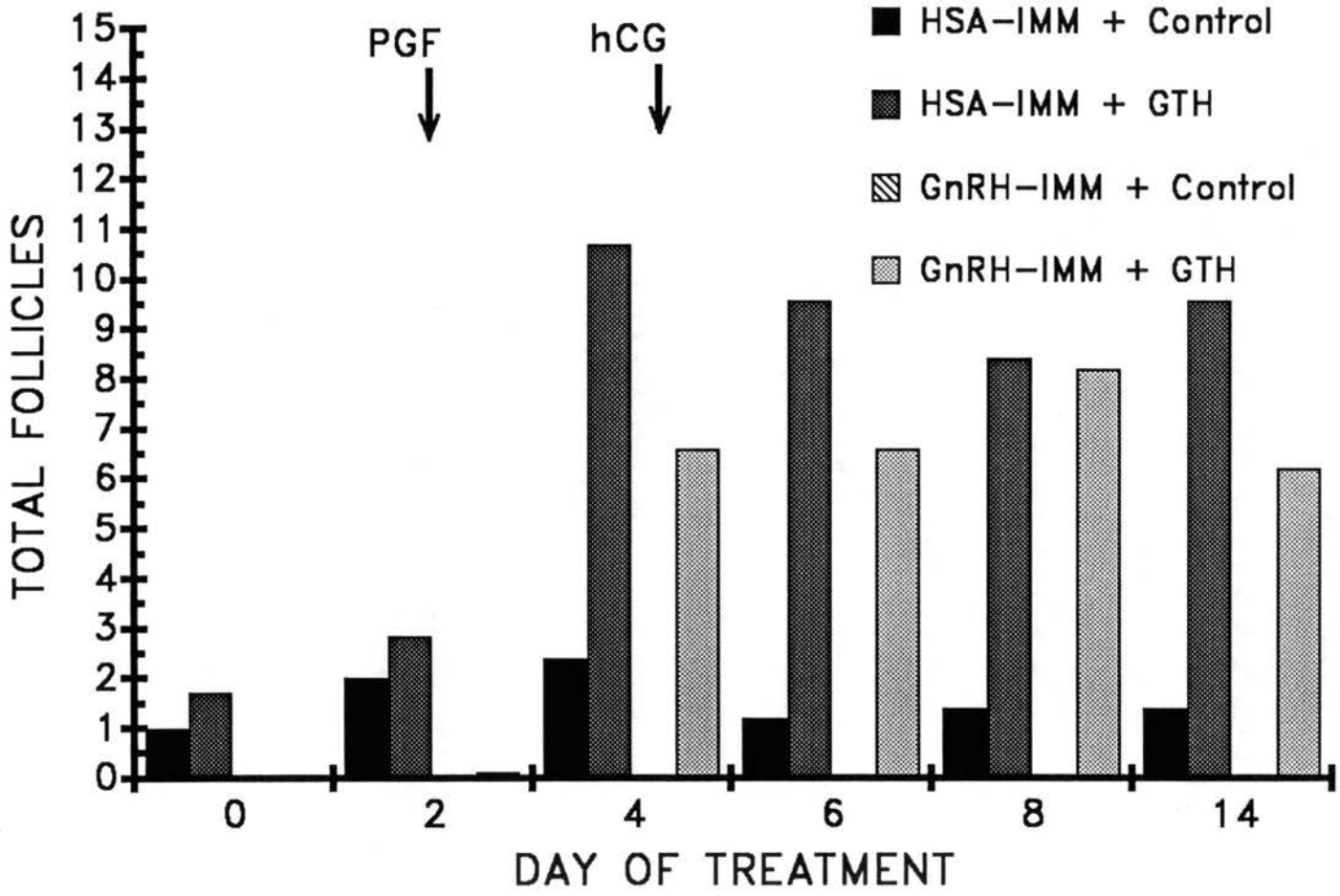

Figure 9. Total number of follicles on ovaries of heifers immunized against GnRH or human serum albumin (HSA) and treated with gonadotropins (GTH) or saline

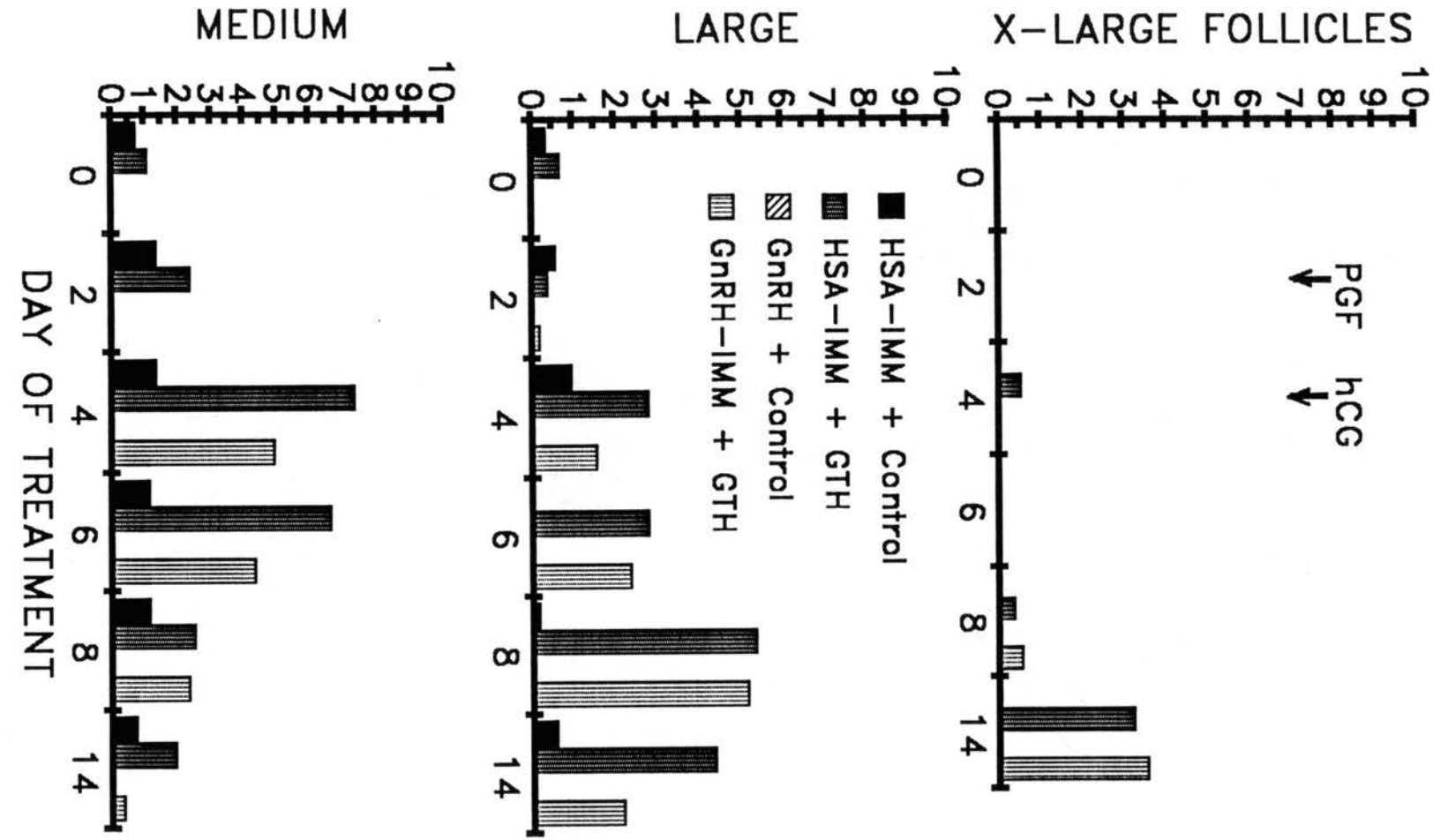

Figure 10. Numbers of medium (6-10 mm), large (11-15 mm) and x-large (≥ 16 mm) follicles on ovaries of heifers immunized against GnRH or human serum albumin (HSA) and treated with gonadotropins (GTH) or saline

number of medium ($P < .02$) and large ($P < .07$) follicles on all days. No follicles ≥ 6 mm were detected on ovaries of GnRH-IMM + C heifers. On d 14, only GTH treated heifers had X-large follicles (0.0 ± 0 for C versus 3.4 ± .4 for GTH heifers; $P < .001$). Estradiol concentrations in plasma on d 6 were correlated with the number of medium follicles ($r = .95$, $P < .001$), and on d 8 with the number of large follicles ($r = .94$, $P < .001$). Hallford et al. (1979) observed a relationship between maximum estradiol concentrations and the number of CL and follicles > 10 mm in diameter at 7 to 10 d after estrus ($r = .58$) in PMSG treated cows.

Only HSA-IMM heifers had CL at treatment and the number of CL (0.93 ± .14) did not change ($P > .3$) through d 4. Treatment with GTH increased (GTH x day; $P < .001$) the number of CL (Figure 11), but the number of CL was decreased by GnRH-IMM (IMM x day; $P < .01$). The maximum number of CL were present on d 14, but GnRH-IMM + GTH heifers had fewer ($P < .01$) CL compared with HSA-IMM +GTH heifers (2.8 ± .2 versus 4.4 ± .2, respectively). Concentrations of progesterone were related to the numbers of CL during d 0 through d 14 ($r = .82$; $P < .001$) and on d 14 ($r = .79$, $P < .001$). In cows and heifers given PMSG (Hallford et al., 1979), concentrations of progesterone were related ($r = .75$) to the number of CL at 7 to 10 days after estrus.

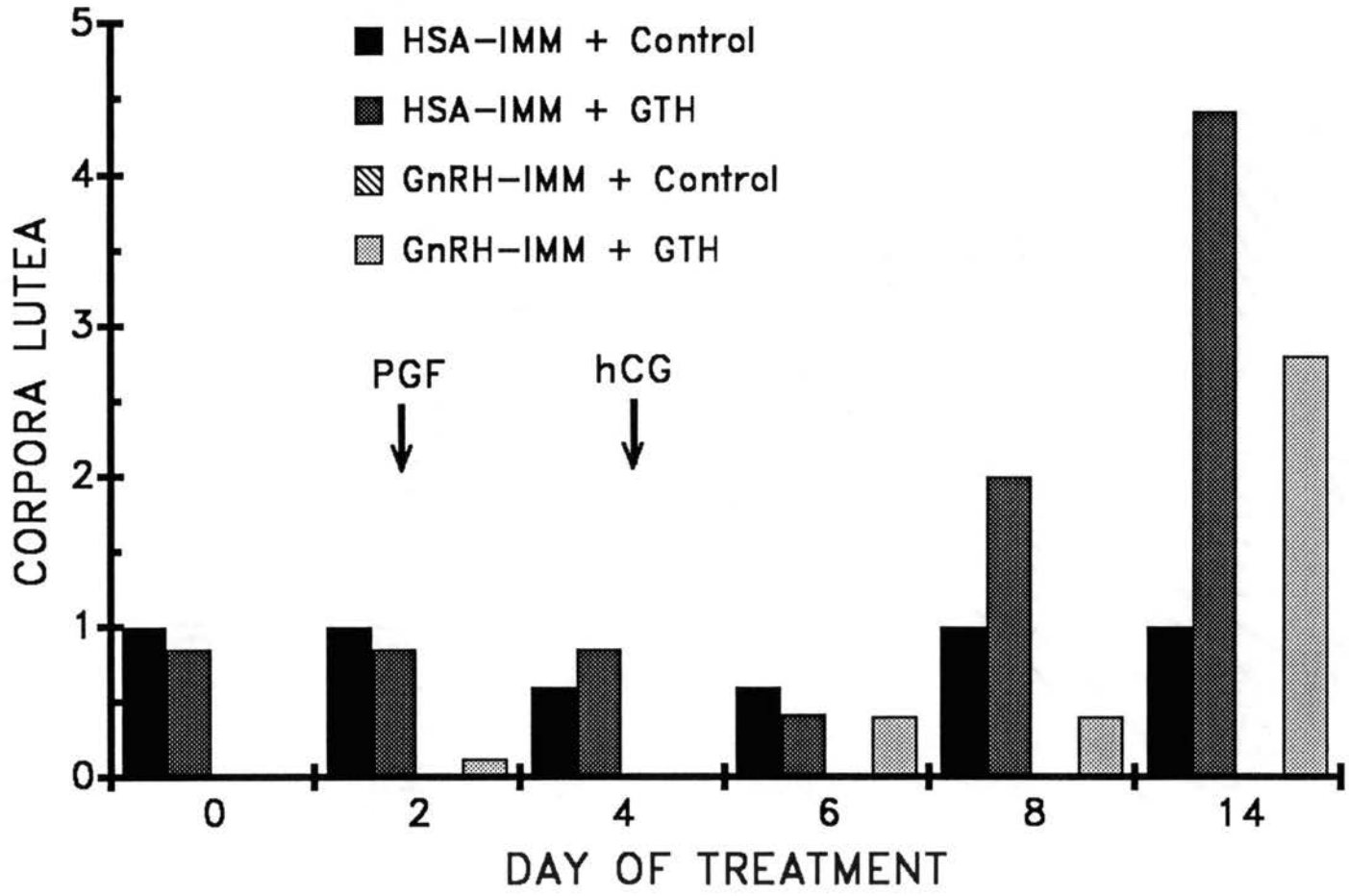

Figure 11. Number of corpora lutea on ovaries of heifers immunized against GnRH or human serum albumin (HSA) and treated with gonadotropins (GTH) or saline

Experiment 2. Average BW of heifers at treatment was 221 ± 20 kg. The largest of the heifers (270.9 kg; assigned to C treatment) had concentrations of progesterone > 5 ng/mL prior to treatment and was excluded from the analyses. Concentrations of progesterone and estradiol in plasma of heifers on d 0 averaged 0.8 ± 0.1 ng/mL and 5.1 ± 2.7 pg/mL, respectively, and were similar for all treatments. Concentrations of estradiol during d 0 through d 14 (Figure 12) were increased (GTH x day; $P < .001$) by GTH. Maximum concentrations of estradiol after GTH were on d 5, and were greater in GTH heifers (51.0 ± 4.4 pg/mL) compared with LGTH heifers (31.5 ± 4.4 pg/mL).

Concentrations of progesterone in plasma of prepuberal heifers on d 8, 10 and 14 (Figure 13) were increased (GTH x day; $P < .05$) by GTH. On d 10 (6 d after hCG) concentrations of progesterone were greater ($P < .01$) in both groups of GTH treated heifers compared with controls and GTH treated heifers had greater concentrations of progesterone ($P < .05$) compared with LGTH heifers. Maximum concentrations of progesterone after GTH (d 14) were greater ($P < .03$) in GTH heifers compared with C heifers, but did not differ significantly due to dose of GTH. Concentrations of progesterone averaged .88, 5.3 and 20.6 ng/mL on d 24 for control, LGTH and GTH heifers, respectively. On that day, concentrations of progesterone tended ($P < .09$) to be greater in prepuberal heifers treated with gonadotropins compared to controls, and

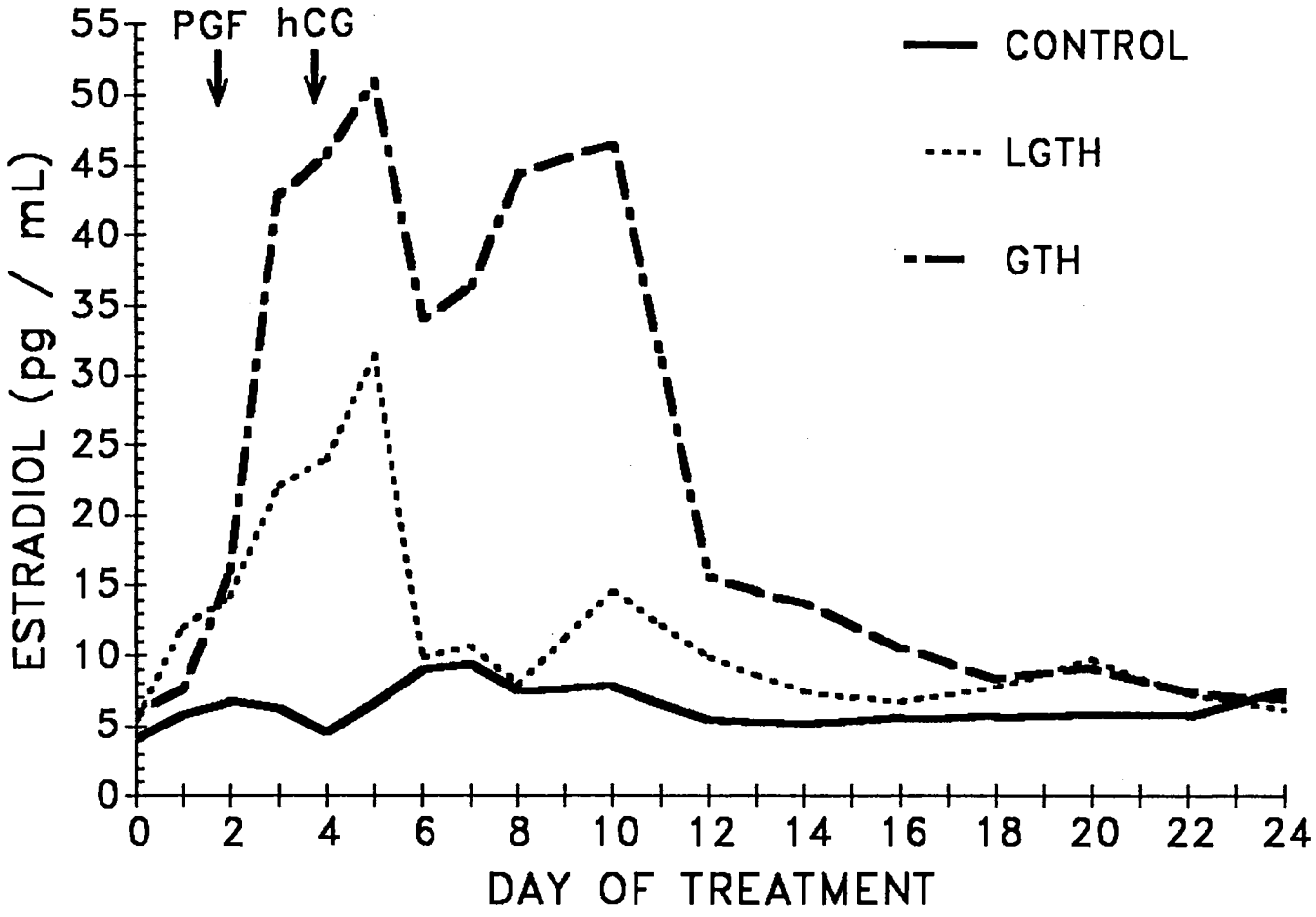

Figure 12. Least squares mean concentrations of estradiol in plasma of prepuberal heifers treated with gonadotropins (GTH), half of GTH (LGTH) or saline

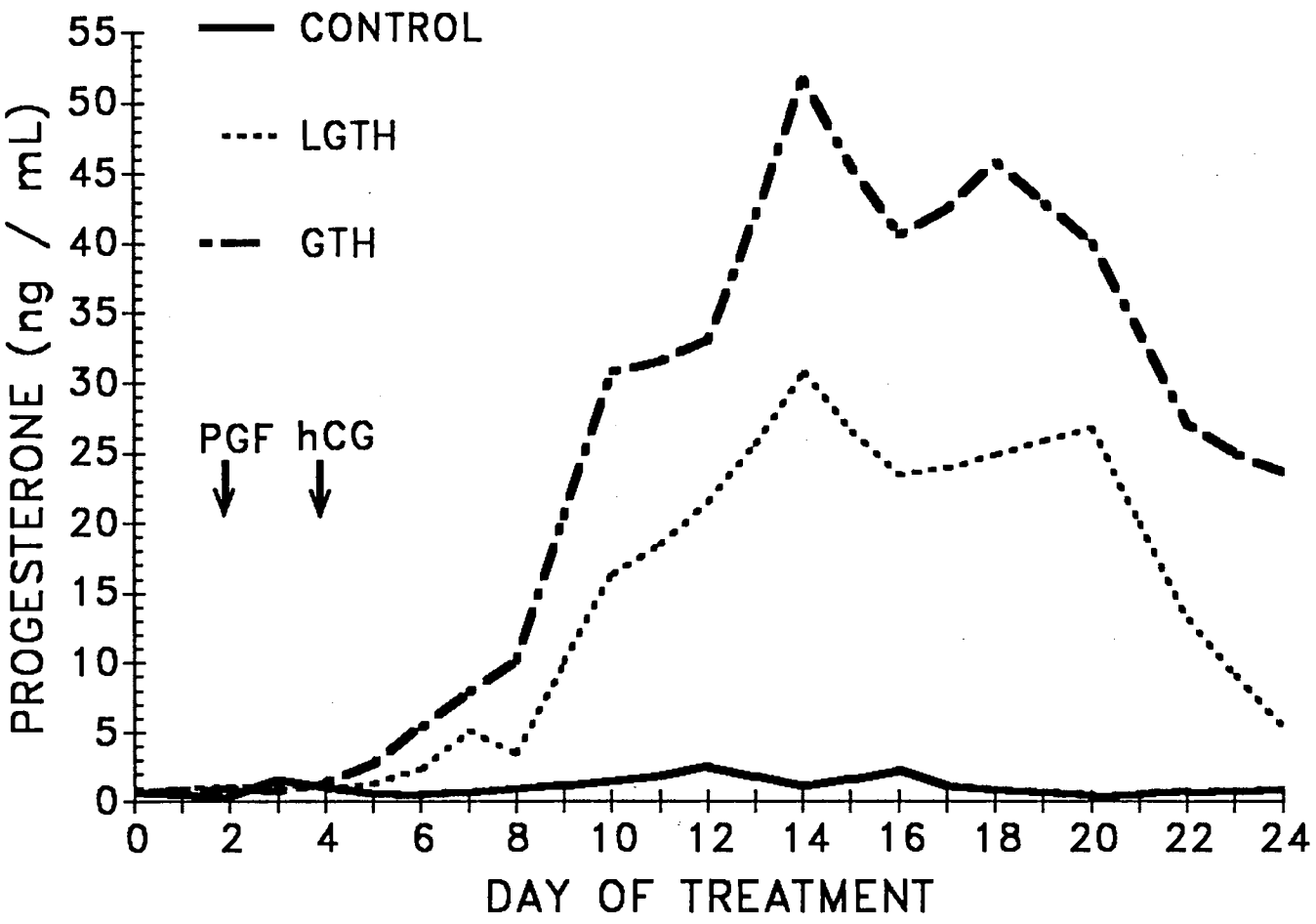

Figure 13. Least squares mean concentrations of progesterone in plasma of prepuberal heifers treated with gonadotropins (GTH), half of GTH (LGTH) or saline

heifers receiving the total GTH treatment had greater ($P < .06$) concentrations of progesterone in plasma than those receiving a dose based on BW (LGTH). Induction of ovulation and superovulation by administration of PMSG, with or without other gonadotropins, has been documented in prepuberal calves, lambs and gilts (see review by Foote, 1972). The maximum number of follicles occurred on d 5, and ovulation probably occurred on d 6, about 40 h post hCG treatment (Graves and Dzuik 1968). Greater concentrations of progesterone in GTH than LGTH heifers on d 10 indicate that more follicles were capable of responding to hCG.

In agreement with other reports (Fraser 1975; Garcia et al., 1986; Johnson et al., 1988) fewer follicles and CL were detected in heifers immunized against GnRH. The ultrasound equipment used in this study could not be used to quantify follicles < 6 mm in diameter. However, similar estradiol concentrations in heifers before administration of GTH indicate that, unlike the gilt (Traywick and Esbenshade, 1988) total cessation of follicular growth does not occur after immunization against GnRH in cattle. Other studies with heifers (O'Connell, 1990; Wettemann and Castree, 1994) indicate that immunization against GnRH blocks the surge of LH necessary to cause ovulation. In gilts immunized against GnRH, PMSG or exogenous LH and FSH did not cause synthesis of estradiol or follicular growth (Esbenshade, 1987). In addition, the possibility exists that immunization against GnRH may have a direct effect at

the ovary in gilts because infusion of antibodies to GnRH into ovaries of prepuberal gilts decreased the number of viable follicles present on the ovary (Patton et al., 1991). GnRH-like peptides have been identified in bovine ovaries (Aten et al., 1987; Ireland et al., 1988) but a direct effect of GnRH on the ovaries of cows has not been determined.

In conclusion, treatment with exogenous gonadotropins, at a dose sufficient to increase estradiol and progesterone concentration in prepuberal heifers, caused increased concentration of estradiol, follicular growth and ovulation in anestrous heifers immunized against GnRH. Antibody titers against GnRH that are sufficient to prevent ovulation and/or development of corpora lutea, do not prevent the ovary from responding to exogenous gonadotropins.

Implications

Immunization against GnRH is an effective tool to study the hypothalamic-pituitary-ovarian axis in livestock. The administration of exogenous hormones in the absence of natural hormones is the classic approach to study endocrine mechanisms. An increased understanding of the regulation of gonadotropins by the hypothalamus, will aid in the improvement of reproductive efficiency at puberty and after parturition.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine the mechanisms through which nutrition, body energy reserves and GnRH influence pulsatile secretion of gonadotropins in cattle. Two specific objectives of this research were: 1) to determine if body energy reserves have a direct effect on the pituitary to influence secretion of gonadotropins, and 2) to determine if exogenous gonadotropins will stimulate follicular growth in heifers that are anestrus as a result of immunoneutralization of endogenous GnRH. Seventy beef cows were used in experiment 1 which was conducted over two years. Cows in good body condition score (BCS; Wagner et al., 1988) were randomly assigned to diets during gestation so as to calf with a BCS (1 = emaciated, 9 = obese) of 4 or 6. Treatments were arranged in a 2 (BCS=4 or 6) by 3 (control immunization infused with saline or an analog of GnRH, or immunization against GnRH and infusion of an analog) factorial design. Primary immunizations were administered to cows at approximately 265 days of gestation and a booster immunization was given 14 days post partum.

Lactating cows and their calves were maintained in individual pens in a barn between days 25 and 39 post partum. Pulsatile (2 mL/1.25 min) infusions of saline or an analog of GnRH (GnRH-A; des-Gly^{10}, (D-Ala^{6})-LHRH; 675 ng) were randomly assigned at treatment. Pulses were given once per h for 198 h commencing on day 31 post partum.

Concentrations of LH and FSH on day 30 post partum averaged 4.3 ± .2 ng/mL and .4 ± .1 ng/mL, respectively, and were not influenced by immunization against GnRH or BCS. Prior to treatment with GnRH-A, the frequency of LH pulses was greater ($P < .06$) in cows with BCS = 6 compared with thin (BCS = 4) cows. Pulsatile infusion of GnRH-A increased LH concentrations and the amplitude of pulses of LH, but the response was dependent on the BCS of the cows (BCS * TRT * Day; $P < .01$). Maximum concentrations of LH in serum and the amplitude of LH pulses on day 31 (day 1 of treatment) were greater ($P < .05$) in cows treated with GnRH-A compared with those infused with saline. During year 2, cows in good condition immunized against HSA and treated with GnRH-A had greater concentrations of LH compared with thin (BCS=4; HSA immunized and GnRH-A) cows. There was a tendency for BCS to influence the amplitude of LH pulses on day 31 post partum. Mean concentrations of LH and the amplitude of LH pulses were decreased ($P < .01$) in serum collected on day 33 through day 39 of infusion compared with day 31 post partum and were not influenced by treatment or BCS. Immunization of cows against GnRH did

not influence the response of LH to GnRH-A during the infusion period.

Cows with a BCS of 6 (good) at calving conceived in 86 day ($P < 0.05$) post partum compared with 96 day for cows with a BCS of 4 (thin). Pregnancy rate tended ($P < .10$) to be influenced by treatment. Within cows with BCS=4, only 64% of cows immunized against GnRH and pulsed with GnRH-A were pregnant compared ($P < .07$) with 92% of control cows and 91% of cows pulsed with GnRH-A. Treatment did not influence the percentage of cows in good condition (BCS=6) that were pregnant.

Body energy reserves influence the number of pulses of LH, but not FSH, in cows at 30 day post partum. In all cows infused with an analog of GnRH, the amount of LH released on the first day of infusion was greater than concentrations on subsequent days of treatment indicating that the analog caused maximal release of LH early in the treatment period. Decreased concentrations of LH on day 33 through 39 compared with day 31 indicate that either the releasable pool of LH was exhausted and subsequent synthesis under the influence of GnRH-A was not adequate to maintain concentrations of LH in serum or that the pituitary became less sensitive to stimulation by the analog. Greater releasable stores of LH in the pituitary of postpartum cows may be due to increased GnRH stimulation indicating the onset of reproductive cycles in cows with adequate body energy reserves. Although BCS did not

influence the ovarian response to an analog of GnRH, luteal activity after treatment was influenced by body energy reserves. The effects of BCS on ovarian function are thus mediated through hypothalamic secretion of GnRH. The effects of BCS on the pituitary appear to be manifested as decreased stores of LH.

Immunization of postpartum anestrous cows against GnRH did not influence basal secretion of LH or FSH or GnRH-A stimulated LH release, but tended to reduce the percentage of cows with luteal activity during and after treatment and the percentage of cows pregnant at the end of the breeding season compared with non-immunized cows pulsed with GnRH-A. Immunization against GnRH may decrease the ovulatory surge of gonadotropins in postpartum anestrous cows in a similar manner as evident in heifers (Wettemann and Castree, 1994).

In the second study, Hereford and Angus x Hereford heifers were used to determine if the ovaries of heifers immunized against GnRH would respond to exogenous gonadotropins. The ovarian response of prepuberal heifers to the same gonadotropins was also evaluated. Postpuberal heifers previously immunized against GnRH conjugated to HSA or HSA were given booster immunizations to control reproductive cycles. Gonadotropin treatments were arranged in a 2 by 2 factorial design. Half of the anestrous (GnRH-IMM; n = 6) heifers and six of the control (HSA-IMM) immunized heifers received gonadotropins (2000 IU PMSG + 1000 IU hCG; GTH). Prepuberal heifers were given one of

three doses of gonadotropins: C, GTH or one half G (LGTH). The remaining heifers were given saline (s.c.; C). All heifers were given prostaglandin $F_{2\hat{A}}$ (PGF) on day 2 to cause regression of corpora lutea and 2000 IU of human chorionic gonadotropin (hCG) on day 4 to cause ovulation of large follicles.

Immunization against GnRH (GnRH-IMM) caused cessation of estrous cycles. Concentrations of estradiol and progesterone were increased by GTH treatment of POST heifers but the response was reduced by GnRH-IMM. Number of follicles · 6 mm was increased after GTH but the response was decreased by immunization against GnRH. Immunization against GnRH reduced total follicles on day 4 in heifers on both C and GTH treatments. Gonadotropin treatment increased the number of follicles · 16 mm on day 14. The number of CL was increased by GTH but the response was reduced ($P < .01$) in GnRH-IMM heifers compared with HSA-IMM heifers.

Exogenous gonadotropins increased concentrations of estradiol in plasma of prepuberal heifers indicating the ability of the PMSG + hCG to cause follicular growth in prepuberal heifers. Concentrations of estradiol in PRE heifers increased between day 0 and day 14 in GTH and LGTH heifers, but not in prepuberal heifers given saline Concentrations of progesterone in prepuberal heifers on day 10 were greater in GTH and LGTH heifers compared with C heifers. Treatment with gonadotropins, at a dose

sufficient to increase estradiol and progesterone concentrations in prepuberal heifers, stimulated folliculogenesis in heifers immunized against GnRH.

Treatment of anestrous heifers with exogenous gonadotropins caused increased concentration of estradiol, follicular growth and ovulation. Antibody titers against GnRH that are sufficient to prevent ovulation and/or development of corpora lutea, do not prevent the ovary from responding to exogenous gonadotropins. These results suggest that GnRH, or GnRH-like peptides, may not have direct effects on the bovine ovary.

Evidence presented here demonstrates that body energy reserves influence the secretion of LH in postpartum beef cows before and on the first day of a pulsatile infusion of a constant amount of an agonist of GnRH. Body energy reserves do not influence the ovarian response to treatment with an analog of GnRH, but BCS does influence luteal activity after treatment. GnRH has little if any direct role in regulation of ovarian function in the bovine. Reduced secretion of LH in nutritionally anestrous beef cows (Richards et al., 1989a) can be compensated for by pulsatile infusion of GnRH (Bishop and Wettemann, 1993a) and thin cows have decreased release of GnRH from infundibular stalk-median eminence (Rasby et al., 1992). Collectively these results suggest that the effects of nutrition and body energy reserves on reproduction are mediated through the hypothalamic release of GnRH and not

due to direct effects at the level of the pituitary or the ovary.

The proposed pulse generator for GnRH in the hypothalamus of cows has not been identified. Additional research is being conducted to determine the optimum pattern of infusion of GnRH into nutritionally anestrous cows. Studies are under way to perfect techniques of immunization of cows against GnRH, thus separating the pituitary from hypothalamic stimulation. Collectively, these techniques will aid in understanding the mechanisms by which nutrition and body energy reserves influence release of GnRH from the hypothalamus.

Literature Cited

Adams, T.E. and B.M. Adams. 1986. Gonadotroph function in ovariectomized ewes actively immunized against gonadotropin-releasing hormone (GnRH). Biol. Reprod. 35:360.

Adams, T.E. and B.M. Adams. 1990. Reproductive function and feedlot performance of beef heifers actively immunized against GnRH. J. Anim. Sci., 68:2793-2802.

Allrich, R.D., D.L. Cook and T.A. Winters. 1984. Influence of dexamethasone, progesterone, GnRH and testosterone on estrous behavior of estradiol- treated ovariectomized Holstein heifers. J. Anim. Sci. 59 (Suppl.1):316.

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

Arimura, A., L. Debeljuk and A.V. Schally. 1974. Blockade of the preovulatory surge of LH and FSH and of ovulation by anti-LH-RH serum in rats. Endocrinol. 95:323.

Armstrong, E.G., C.A. Villee and J.R. Ville. 1977. Characterization and comparison of estrogen and androgen receptors of calf anterior pituitary. J. Steroid Biochem. 8:285.

Armstrong, J.D. and J.H. Britt. 1985. Pulsatile administration of gonadotropin-releasing hormone in anestrous sows: Endocrine changes associated with GnRH-induced and spontaneous estrus. Biol. Reprod. 33:375.

Armstrong, J.D., and J.H. Britt. 1987. Nutritionally-induced anestrus in gilts: metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. 65:508.

Aten, R.F., A.T. Williams and H.R. Behrman. 1986. Ovarian Gonadotropin-Releasing hormone-like protein(s): Demonstrations and characterization. Endocrinol. 118:961.

Aten, R.F., J.J. Ireland, C.W. Weems and H.R. Behrman. 1987. Presence of gonadotropin-releasing hormone-like proteins on bovine and ovine ovaries. Endocrinol. 120:1727.

Baba, Y., H. Matsuo and A.V. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem. Biophys. Res. Commun. 44:459.

Becker, S.E. and A.L. Johnson. 1992. Effects of gonadotropin-releasing hormone infused in a pulsatile or continuous fashion on serum gonadotropin concentrations and ovulation in the mare. J. Anim. Sci. 70:1208.

Bellows, R.A., R.E. Short and R.B. Stagmiller. 1979. Research areas in beef cattle reproduction. Beltsville Symposia in Agric. Research 3. Animal Reproduction. John Wiley and Sons. pp. 3.

Bennett, H.P.J. and C. McMartin. 1979. Peptide hormones and their analogues: Distribution, clearance from circulation, and inactivation of in vivo. Pharmacol. Rev. 30:247.

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

Bergendahl, M, A. Perheentupa and I. Huhtaniemi. 1991. Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. Biol. Reprod. 44:413.

Bevers, M.M., S.J. Dieleman, H.T.M. van Tol, D.M. Blankenstein and J. van den Broek. 1989. Changes in pulsatile secretion patterns of LH, FSH, progesterone, androstenedione and oestradiol in cows after superovulation with PMSG. J. Reprod. Fert. 87:745.

Bex, F.J. and A. Corbin. 1981. Lutenizing hormone-releasing hormone (LHRH) and LHRH agonist termination of pregnancy in hypophysectomized rats: extrapituitary site of action. Endocrinol. 108:273.

Bishop, D.K. 1991. Influence of body energy reserves, early weaning and treatment with gonadotropin releasing hormone on luteinizing hormone and ovarian activity in beef cows. MS Thesis. Oklahoma State Univ., Stillwater.

Bishop, D.K. and R. Wettemann. 1992. Luteinizing hormone (LH) after treatment of nutritionally anestrous cows with naloxone. Biol. Reprod. 46 (Suppl. 1):158.

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

Bishop, D.K., R.P. Wettemann, J.V. Yelich and L.J. Spicer. 1993b. Effect of gonadotropin treatment on the ovarian response of heifers immunized against GnRH. J. Anim. Sci. (Suppl. 1) 71:2.

Braden, T.D. and P.M. Conn. 1992. Gonadotropin releasing hormone and its actions. In; W.F. Crowley, Jr. and P. M. Conn (Ed.) Modes of Action of GnRH and GnRH Analogs. pp. 26.

Breire, B.H., J.J. Bass, J.H. Butler and P.D. Gluckman. 1986. The somatrotrophic 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.

Brinkley, H.J. 1981. Endocrine signaling and female reproduction. Biol. Reprod. 24:22.

Britt, J.H., R.J. Kittock and D.S. Harrison. 1974. Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J. Anim. Sci. 39:915.

Bronson, F.H. and J.M. Manning. 1991. The energetic regulation of ovulation: A realistic role for body fat. Biol. Reprod. 44:945.

Brown, J.L. and J.J. Reeves. 1983. Absence of specific luteinizing hormone releasing hormone receptors in ovine, bovine and porcine ovaries. Biol. Reprod. 29:1179.

Button, E.J. and A. Whitehouse. 1981. Subclinical anorexia nervosa. Phycol. Med. 11:509.

Carlberg, K.A., M.T. Buckman, G.T. Peake and M.L. Riedesel. 1983. Body composition of oligo/amenorrheic athletes. Med. Sci. Sports Exerc. 15:215.

Carmel, P.W., S. Araki and M. Ferin. 1976. Pituitary stalk portal blood collection in Rhesus monkeys: Evidence for pulsatile release of Gonadotropin-Releasing hormone (GnRH). Endocrinol. 99:243.

Carruthers, T.D., E.M. Convey, J.S. Kesner, H.D. Hafs and K.W. Cheng. 1980. The hypothalamo-pituitary gonodotrophic axis of suckled and nonsuckled dairy cows postpartum. J. Anim. Sci. 51:949.

Chang, C.F., A.J. Roberts and J.J. Reeves. 1987. Increased luteinizing hormone secretion and ovarian function in heifer actively immunized against estrogen and progesterone. J. Anim. Sci. 65:771.

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

Clarke, I.J. 1989. The GnRH/gonadotropin axis in the ewe, cow and sow. Dom. Anim. Endocrinol. 6:1.

Clarke, I.J. 1992. Exactitude in the relationship between GnRH and LH secretion. In: W. F. Crowley, Jr. andP. M. Conn (Ed.) Modes of Action of GnRH and GnRH Analogs. pp 179.

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

Clarke, I.J. and J.T. Cummins. 1985a. GnRH pulse frequency determines LH Pulse amplitude by altering the amount of releasable LH in the pituitary glands of ewes. J. Reprod. Fert. 73:425.

Clarke, I.J. and J.T. Cummins. 1985b. Increased GnRH pulse frequency associated with estrogen-induced LH surges in ovariectomized ewes. Endocrinol. 116:2376.

Clarke, I.J. and J.T. Cummins. 1987. The significance of small pulses of gonadotrophin-releasing hormone. J. Endocrinol. 113:413.

Clarke, I.J., G.B. Thomas, B. Yao and J.T. Cummins. 1987a. GnRH secretion thoughout the ovine estrous cycle. Neuroendocrinol. 46:82.

Clarke, I.J., H.M. Fraser and A.S. McNeilly. 1978. Active immunization of ewes against luteinizing hormone releasing hormone, and its effects on ovulation and gonadotrophin, prolactin and ovarian steroid secretions. J. Endocrinol. 78:39.

Clarke, I.J., J.T. Cummins and D.M. DeKretser. 1983. Pituitary gland function after disconnection from direct hypothalamic influences in the sheep. Neuroendocrinol. 36:376.

Clarke, I.J., J.T. Cummins, J.K. Findlay, K.J. Burman and B.W. Doughton. 1984. Effects on plasma luteinizing hormone and follicle stimulating hormone of varying the frequency and amplitude of gonadotropin-releasing hormone pulses in ovariectomized ewes with hypothalamo-pituitary disconnection. Neuroendocrinol. 36:214.

Clarke, I.J., R.J.E. Horton and B.W. Doughton. 1990. Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes. Endocrinol. 127:1470.

Clarke,I. J.,J. T. Cummings, M. E. Crowder and T. E. Nett. 1987b. Pituitary receptors for gonadotropin-releasing hormone in relation to changes in pituitary and plasma luteinizing hormone in ovariectomized-hypothalamo pituitary disconnected ewes. I. Effect of changing frequency of gonadotropin-releasing hormone pulses. Biol. Reprod. 37:749.

Clayton, R.N., J.P. Harwood and K.J. Catt. 1979. Gonadotropin-relasing hormone analogue binds to luteal cells and inhibits progesterone production. Nature 282:90.

Conn, P.M. (ed.), 1986. Endocrine Reviews: GnRH vol. 7(1):1-125. Baltimore, MD: The Endocrine Society.

Conn, P.M., C.A. McArdle, W.A. Andrews and W.R. Huckle. 1987. The molecular basis of gonadotropin-releasing hormone (GnRH) in action in the pituitary gonadotrope. Biol. Reprod. 36:35.

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.

Cosgrove, J.R., J.E. Tilton, M.G. Hunter and G.R. Foxcroft. 1992. Gonadotropin-independent mechanisms participate in ovarian responses to realimentation in feed-restricted prepubertal gilts. Biol. Reprod. 47:736.

Cox, N.M. and J.H. Britt. 1982. Pulsatile administration of gonadotropin releasing hormone to lactating sows: Endocrine changes associated with induction of fertile estrus. Biol. Reprod. 27:1126.

Currie, W.D., K.A. Ellis, S.A. Gourley, I.B.J.K. Joseph, S.J. Cook and N.C. Rawlings. 1993. Luteinizing and follicle-stimulating hormone secretion during median eminence perfusion or intravenous infusion with gonadotropin-releasing hormone antisera in ovariectomized ewes. Anim. Reprod. Sci. 31:277.

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. Endocrinol. 125:917.

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.

Day, M.L., K. Imakawa, P.L. Wolfe, R.J.Kittok and J.E. Kinder. 1987. Endrocrine mechanisms of puberty in heifers: Role of hypothalamo-pituitary estradiol receptors in negative feedback of estradiol on lutenizing hormone secretion. Biol. of Reprod. 37:1054.

De Kretser, D.M. and D.M. Robertson. 1989. The isolation and physiology of inhibin and related proteins. Biol. Reprod. 40:33.

Dierschke, D.J., A.N. Bhattacharya, L.E. Adkinson and E. Knobil. 1970. Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinol. 87:850.

Dobson, H. and P.D.G. Dean. 1974. Radioimmunoassay of oestrone, oestradiol-17α and -17ß in bovine plasma during the oestrous cycle and last stages of pregnancy. J. Endocrinol. 61:479.

Drost, M. and W.W. Thatcher. 1992. Application of gonadotropin releasing hormone as theraputic agent in animal reproduction. Anim. Reprod. Sci. 28:11.

Duggan, S.L., R.P. Wettemann, K.S. Lusby, J.V. Yelich and D.K. Bishop. 1992. Immunization of heifers against gonadotropin releasing hormone: Effectiveness of adjuvants. Okla. Agr. Exp. Sta. Res. Rep. MP-136:389.

Dunn, T.G. and C.C. Kaltenbach. 1980. Nutrition and the postpartum interval of the ewe, sow and cows. J. Anim. Sci. 51 (Suppl. 2):29.

Dziuk, P.J. and R.A. Bellows. 1983. Management of reproduction of beef cattle, sheep and pigs. J. Anim. Sci. 57(Suppl.2):355.

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.

Echternkamp, S.E., C.L. Ferrel and J.D. Rone. 1982. Influence of pre- and post-partum nutrition on LH secretion in suckled postpartum beef heifers. Theriogenol. 18:283.

Esbenshade, K.L. 1987. Ovarian response to pregnant mare serum gonadotropin and porcine pituitary extract in gilts actively immunized against gonadotropin releasing hormone. J. Anim. Sci. 65:1768.

Esbenshade, K.L. 1991. Passive immunization of the pig against gonadotropin releasing hormone during the follicular phase of the estrus cycle. Theriogenol. 35:869.

Esbenshade, K.L. and J.H. Britt. 1985. Active immunization of gilts against gonadotropin-releasing hormone: Effects on secretion of gonadotropins, reproductive function and responses to agonists of gonadotropin-releasing hormone. Biol. Reprod. 33:569.

Esbenshade, K.L., A.J. Zieck and J.H. Britt. 1990. Fegulation and action of gonadotropins in pigs. J. Reprod. Fertil., Suppl. 40:19-32.

Findlay, J.K. 1993. An update on the roles of inhibin, activin and follistatin as local regulators of folliculogenesis. Biol. Reprod. 48:15.

Foote, R.H. 1972. Induced ovulation and fertility in prepuberal cattle, sheep and swine. J. Anim. Sci. 34 (Suppl. 1):49.

Fortune, J.E. and S.M. Quirk. 1988. Regulation of steroidogenesis in bovine preovulatory follicles. J. Anim. Sci. 66 (Suppl.2):1.

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 system. Endocrinol. 116:375.

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. Endocrinol. 125:342.

Foxcroft, G.R. and M.G. Hunter. 1985. Basic physiology of follicular maturation in the pig. J. Reprod. Fertil. Suppl. 33:1.

Fraser, H.M. 1975. Effects of antibodies to luteinizing hormone releasing hormone on reproductive functions in rodents. In: E. Nieschlag (Ed.) Immunization with Hormones in Reproductive Research. pp 107-117.

Fraser, H.M. 1976. Physiological effects of antigody to luteinising hormone releasing hormone releasing hormone. Physiological Effects of Immunity Against Reproductive Hormones. pp 137.

Fraser, H.M. 1980. Inhibition of reproductive function by antibodies to luteinizing hormone releasing hormone. In: J.P. Hearn (Ed.) Immunological Aspects of Reproduction and Fertility Control. pp. 143.

Fraser, H.M. and J. Sandow. 1977. Gonadotrophin release by a highly active analogue of luteinizing hormone releasing hormone in rats immunized against luteinizing hormone releasing hormone releasing hormone. J. Endocrinol. 74:291.

Fraser, H.M. and T.G. Baker. 1978. Changes in the ovaries of rats after immunization against luteinizing hormone releasing hormone. J. Endocr. 77:85.

Fraser, H.M., I.J. Clarke and A.S. McNeilly. 1977. Inhibition of ovulation and absence of postive feedback in ewes immunized against luteinizing hormone releasing hormone. J. Endocrinol. 74:45.

Fraser, H.M. 1977. Reversal of the inhibitory action of an antiserum of luteinizing hormone releasing homrone (LH-RH) by an inactive fragment of LH-RH. J. Endocrinol. 73:393.

Fraser, H. M. and A. S. McNeilly. 1982. Effect of immunoneutralization of luteinizing hormone releasing hormone on the estrogen-induced leutinizing hormone and follicle-stimulating hormone surges in the ewe. Biol. Reprod. 27:548.

Frisch, R.E. and R. Revelle. 1970. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 169:397.

Frisch, R.E. 1987. Body fat, menarche, fitness and fertility. Human Reprod. 2:521.

Frisch, R.E. 1978. Population, food intake and fertility. Science 199:22.

Frisch, R.E. and J.W. McArthur. 1974. Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance and onset. Science 185:949.

Frisch, R.E. 1982. Malnutrition and fertility. Science 215:1272.

Frisch, R.E. 1988. Loss of fat from dieting or exercise can lead to infertility that is reversible with fat gain. It is possible that fat tissue exerts a regulatory effect on the reproductive ability of human females. Scientific American 258 (3):88.

Garza, F. Jr., D.L. Thompson, Jr., D.D. French, J.J. Wiest, R.L. St. George, K.B. Ashley, L.S. Jones, P.S. Mitchell and D.R. McNeill. 1986. Active immunization of intact mares against gonadotropin-releasing hormone: Differential effects on secretion of luteinizing hormone and follicle-stimulating hormone. Biol. Reprod. 35:347.

Gill, J.L. 1973. Current status of multiple comparisons of means in designed experiments. J. Dairy Sci. 56:973.

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

Goodman, R.L. and E. Knobil. 1981. The sites of action of ovarian steriods in the regulation of LH secretion. Neuroendocrinol. 32:57.

Gordon, K. and G.D. Hodgen. 1991. GnRH analogues in ovarian stimulation. Ann. N.Y. Acad. Sci. 626:238.

Goubau, S., D.W. Silversides, A. Gonzalez, B. Laarveld, R.J. Mapletoft and B.D. Murphy. 1989. Immunization of sheep against modified peptides of gonadtropin releasing hormone conjugated to carriers. Dom. Anim. Endo. 6:339.

Graves, C.N. and P.J. Dziuk. 1968. Control of ovulation in dairy cattle with human chorionic gonadotropin after treatment with 6α-methyl-17α-acetoxy-progesterone. J. Reprod. Fert. 17:169.

Grieger, D.M. and J.J. Reeves. 1990. Active immunization of beef heifers against luteinizing hormone: II. Evaluation of conjugation technique on antigenicity of LH. J. Anim. Sci. 68:3747.

Grieger, D.M., R. Scarborough, D.M. deAvila, H.E. Johnson and J.J. Reeves. 1990. Active immunization of beef heifers against luteinizing hormone: III. Evaluation of dose and longevity. J. Anim. Sci. 68:3755.

Hage-van Noort, M., W.C. Puijk, W.M.M. Shaaper, D. Kuperus, N.J.C.M. Beekman, H.H. Plasman, H. Lankhof, C.J.G. Wensig and R. H. Meloen. 1992. Immunomodulation of reproductive systems. Anim. Reprod. Sci. 28:187.

Haisenleder, D.J., A.C. Dalkin, G.A. Ortolano, J.C. Marshall and M.A. Shupnick. 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. Endocrinol. 128:509.

Hallford, D.M., E.J. Turman, R.P. Wettemann and C.E. Pope. 1979. Endocrine and reproductive response of beef cows to PMSG. J. Anim. Sci. 49:1030.

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

Hamernik, D.L. and T.M. Nett. 1988. Gonadotropin-releasing increases the amount of messenger ribonucleic acid for gonadotropins in ovariectomized ewes after hypothalamic-pituitary disconnection. Endocrinol. 122:959-966.

Handelsman, D.J. and R.S. Swerdloff. 1986. Parmacokinetics of gonadotropin-releasing hormone and its analogs. Endocrinol. Rev. 7:95.

Hawes, B.E. and P.M. Conn. 1992. Development of gonadotrope desensitization to gonadotropin-releasing hormone (GnRH) and recovery are not coupled to inositol phosphate production of GnRH receptor number. Endocrinol. 131:2681.

Hawes, B.E., S. Barnes and P.M. Conn. 1993. Cholera toxin and pertussis toxin provoke differential effects on luteinzing hormone release, inositol phosphate production, and gonadotropin-releasing hormone (GnRH) receptor binding in the gonadotrope: Evidence for multiple guanyl nucleotide binding proteins in GnRH action. Endocrinol. 132:2124.

Herman, M.E. and T.E. Adams. 1990. Gondotrophin secretion ofovariectomised ewes: Effect of passive immunization against gonadotrophin-releasing hormone (GnRH) and infusion of a GnRH agonist and estradiol. Biol. Reprod. 42:273.

Houseknecht, K.L., D.L. Boggs, D.R. Campion, J.L. Sartin, T.E. Kiser, G.B. Rampasek and H.E. Amos. 1988. Effect of dietary energy source and level of serum growth hormone, insulin-like growth factor-I, growth and body composition in beef heifers. J. Anim. Sci. 66:2916.

Humphrey, W.D., D.R. Koritnik, C.C. Kaltenbach, T.G. Dunn and G.D.Niswender. 1976. Progesterone and LH in postpartum suckled beef cows. J. Anim. Sci. 43:290.

Hyland, J.H., P.J. Wright, I.J. Clarke, R.S. Carson, D.A. Langsford and L.B. Jeffcott. 1987. Infusion of gonadotropin-releasing hormone (GnRH) induces ovulation and fertile oestrus in mares during seasonal anoestrus. J. Reprod. Fert. Suppl. 35 pp 211.

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

Imakawa, K., M.L. Day, D.D. Zalesky, M. Garcia-Winder, R.J. Kittock and J.E. Kinder. 1986b. Influence of dietary-induced weight changes on serum luteinizing hormone, estrogen and progesterone in the bovine female. Biol. Reprod. 35:377.

Imakawa, K., M.L. Day, M. Garcia-Winder, D.D. Zalesky, R.J. Kittock, B.D. Schanbacher and J.E. Kinder. 1986c. Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. J. Anim. Sci. 63:565.

Ireland, J.J., R.F. Aten and H.R. Behrman. 1988. GnRH-like proteins in cows: Concentrations during corpora lutea development and selective localization in granulosa cells. Biol. Reprod. 38:544.

Jeffcoate, S.L., D.T. Holland and H.M. Fraser. 1976. Anti-LH-RH sera in the investigation of reproduction. Physiological Effects of Immunity Against Reproductive Hormones. pp 121.

Johnson, A.L. 1986. Induction of ovulation in anestrous mares with pulsatile administration of gonadotropin-releasing hormone. Am. J. vet. REs. 47:983.

Johnson, A.L. and S.E. Becker. 1988. Use of gonadotropin-releasing hormone (GnRH) treatment to induce multiple ovulations in the anestrous mare. J. Equine Vet. Sci. 8:130.

Johnson, H.E., D.M. DeAvila, C.F. Chang and J.J. Reeves. 1988. Active immunization of heifers against luteinizing hormone releasing hormone, human chorionic gonadotropin and bovine luteinizing hormone. J. Anim. Sci. 66:719.

Kanematsu, S., K. Kishi and S. Mikami. 1979. Rise of prolactin and changes in fine structure of the anterior hypophysis stalk section in rats. Endocrinol. 105:427.

Katt, J.A., J.A. Duncan, L. Herbon, A. Barkan and J.C. Marshall. 1985. The frequency of gonadotropin-releasing hormone stimulation determines the number of pituitary gonadotropin-releasing hormone receptors. Endocrinol. 116:2113.

Karsch, F.J., E.L. Bittman, D.L. Foster, R.L. Goodman, S.J. Legan and J.E. Robinson. 1984. Neuroendocrine basis of seasonal reproduction. Recent Prog. Horm. Res. 40:185.

Kaynard, A.H. and F.J. Karsch. 1988. Hypophyseal actions of pulsatile gonadotropin-releasing hormone in the ewe: Development and application of a new experimental model. Neuroendocrinol. 48:287.

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. Endocrinol. 108:1386.

Kesner, J.S., R.R. Kraeling, G.B. Rampacek and B. Johnson. 1987. Absence of an estradiol-induced surge of luteinizing hormone in pigs receiving unvarying pulsatile gonadotropin-releasing hormone stimulation. Endocrinol. 121:1862.

Khalid, M., W. Haresign and M.G. Hunter. 1991a. Regulation of pituitary GnRH receptors by continuous infusion of GnRH in the seasonally anestrous ewe: evidence of ovarian involvment. Anim. Reprod. Sci. 24:271.

Khalid, M., W. Haresign and M. G. Hunter. 1991b. Pituitary GnRh receptor dynsmics during continulus infusion of a low dose of GnRH in seasonally anoestrous ewes. Anim. Reprod. Sci. 25:41.

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. Endocrinol. 128:843.

Killen, J.H., D.W. Forrest, F.M. Byers, G.T. Schelling and C. R. Long. 1989. Gonadotropin-releasing hormone-induced luteinizing hormone release in heifers: Effect of nutrition during gestation. J. Anim. Sci. 67:496.

Kinder, J.E., M.L. Day and R.J. Kittock. 1987. Endocrine regulation of puberty in cows and ewes. J. Reprod. Fert., (Suppl.34):167.

Knobil, E. 1974. On the control of gonadotropin secretion in the rhesus monkey. Rec. Prog. Horm. Res. 30:1.

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

Knobil, E. 1981. Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. Biol. Reprod. 24:44.

Knobil, E. 1993. Editorial: Inhibition of luteinizing hormone secretion by fasting and exercise: "Stress" or specific metabolic signals? Endocrinol. 132:1879.

Kraeling, R.R., C.R. Barb and G.B. Rampacek. 1986. Ovarian response of the hypophyseal stalk-transected pig to pregnant mare serum gonadotropin. Domest. Anim. Endocrinol. 3:177.

Krey, L.C., W.R. Butler and E. Knobil. 1975. Surgical disconnection of the medical basal hypothalamus and pituitary function in the Rhesus monkey. I. Gonadotropin secretion. Endocrinol. 96:1073.

Lamming, G.E. and B.J. McLeod. 1988. Continuous infusion of GnRH reduces the LH response to an intravenous GnRH injection but does not inhibit endogenous LH secretion in cows. J. Reprod. Fert. 82:237.

Lamming, G.E., D.C. Wathes and A.R. Peters. 1981. Endocrine patterns of the post-partum cow. J. Reprod. Fertil. (Suppl.30):155.

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. Endocrinol. 125:351.

Lee, P.C. 1987. Nutrition, fertility and maternal investment in primates. J. Zool. 213:409.

Le Magnen, J. 1983. Body energy balance and food intake: A neuroendocrine regulatory mechanism. Physiol. Reviews. 63:314.

Leung, K., V. Padmanabhan, L.J. Spicer, H.A. Tucker, R.E. Short and E.M. Convey. 1986. Relationship between pituitary GnRH-binding sites and pituitary release of gonadotrophins in post-partum beef cows. J. Reprod. Fert. 76:53.

Little, D.E., C.H. Rahe, J.L. Fleeger and P.G. Harms. 1982. Episodic release of LH during gestation in the cow. J. Reprod. Fert. 66:687.

Lobb, D.K. and J. Dorrington. 1992. Intraovarian regulation of follicular development. Anim. Reprod. Sci. 28:343.

Loetz, E.R., R.P. Wettemann and R.D. Geisert. 1990. Active immunization of pregnant beef cows against GnRH. Okla. Agri. Exp. Sta. MP-129:330.

Mann, D.R., K.G. Gould and K. Wallen. 1992. Use of gonadotropin releasing hormone analogs to influence sexual and behavioral development. In; W.F. Crowley, Jr. and P.M. Conn (Ed.) Modes of Action of GnRH and GnRH Analogs. pp 347.

Marshall, J.C., A.C. Dalkin and D.J. Haisenleder. 1992. Regulation of gonadotropin gene expression by gonadotropin releasing hormone. In; W.F. Crowley, Jr. and P.M. Conn (Ed.) Modes of Action of GnRH and GnRH Analogs. pp 55.

Martin, R.J., B.D. White and M.G. Hulsey. 1991. Most adults maintain a remarkably stable body weight. The mechanisms that govern eating, obesity and the body's energy balance are more complex than once thought. Amer. Scient. 79:528.

Matsuo, H., Y. Baba, R.M.G. Nair, A. Arimura and A.V. Schlly. 1971. Structure of porcine LH and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43:1334.

Mattern, L.G., D.L. Helmreich, and J.L. Cameron. 1993. Diurnal pattern of pulsatile luteinizing hormone and testosterone secretion in adult male Rhesus monkeys (Macaca mulatta): Influence of the timing of daily meal intake. Endocrinol. 132:1044.

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

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.

McShane, T.M., T. May, J.L. Miner and D.H. Keisler. 1992. Central actions of neuropeptide-Y may provide a neuromodulatory link between nutrition and reproduction. Biol. Reprod. 46:1151.

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

Moss, G.E., J.R. Parfet, C.A. Marvin, R.D. Allrich and M.A. Diekman. 1985. Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. J. Anim. Sci. 60:285.

Moss, G.E., J.R. Parfet, M.A. Diekman, R.P. Lemenager and K.S. Hendrix. 1982. Pituitary luteinizing hormone (LH), follicle stimulating hormone (FSH), serum progesterone (P) and hypothalamic gonadotropin-releasing hormone (GnRH) in beef cows in varied body condition. J. Anim. Sci. 55 (Suppl.1):374.

Naor, Z. and G.V. Childs. 1986. Binding and activation of gonadotropin-releasing hormone receptors in pituitary and gonadal cells. Int. Rev. Cytology. 103:147.

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

Nett, T.M., A.M. Akbar and G.D. Niswender. 1974. Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anestrous ewes. Endocrinol. 94:713.

Nett, T.M., D. Cermak, T. Braden, J. Manns and G.D. 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.

Nett, T.M., D. Cermak, T. Braden, J. Manns and G.D. Niswender. 1988. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. II. Changes during the postpartum period. Dom. Anim. Endocrinol. 5:81.

Nett, T.M., M.E. Crowder, G.E. Moss and T.M. Duello. 1981. GnRH-receptor interaction. V. Down regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biol. Reprod. 24:1145.

NRC. 1984. Nutrient Requirements of Beef Cattle. 6th Edition. National Academy of Science. National Academy Press, Washington, D.C.

O'Connell, C.M. 1990. Immunization against gonadotropin releasing hormone alters puberty, secretion of luteinizing hormone and ovarian activity in beef heifers. Master of Science Thesis. Okla. State Univ.

O'Connell, C.M. and R.P. Wettemann. 1990. Immunization of postpartum cows against gonadotropin releasing hormone influences the onset of luteal activity and establishment of pregnancy. Okla. Agri. Exp. Sta. MP-129:334.

Patton, M.L., K.L. Esbenshade and W.L. Flowers. 1991. Impairment of follicular development by intra-ovarian infusion of gonadotropin-releasing hormone antiserum in prepubertal pigs. J. Reprod. Fert. 93:393.

Peters, A.R. 1984. Reproductive activity of the cow in the post-partum period. I. Factors affecting the length of the post-partum acyclic period. Brit. Vet. J. 140:76.

Pierson, R.A. and O.J. Ginther. 1987. Reliability of diagnostic ultrasonography for identification and measurement of follicles and detecting the corpus luteum in heifers. Theriogenol. 28:929.

Plant, T.M., L.C. Krey, J. Moossy, J.T. McCormack, D.L. Hess and E. Knobil. 1978. The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female Rhesus monkey (Macaca mulata). Endocrinol. 102:52.

Pugliese, M.T. 1990. Endocrine function adaptions in undernutrition. World Rev. Nutr. Diet. 62:186.

Pugliese, M.T., F. Lifshitz, G. Grad, P. Fort and M. Marks-Katz. 1983. Fear of obesity: A cause of short stature and delayed puberty. New Engl. J. Med. 309:508.

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. Endocrinol. 107:498.

Rasby, R.J., R.P. Wettemann, P.G. Harms, K.S. Lusby and J.J. Wagner. 1992. GnRH in the infundibular stalk-median eminence is related to percentage body fat in carcasses of beef cows. Dom. Anim. Endocr. 9:71.

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.

Reddy, V. 1981. Protein energy malnutrition: An overview. Prog. Clin. Biol. Res. 77:227.

Reeves, J.J., C.F. Chang, D.M. deAvila, D.M. Grieger, H.E. Johnson and A.J. Roberts. 1989. Vaccines against endogenous hormones: A possible future tool in animal production. J. Dairy Sci. 72:3363.

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.

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.

Richards, M.W., L.J. Spicer and R.P. Wettemann. 1989c. Insulin-like growth factor-I (IGF-I) in serum of beef cows fed maintenance or restricted diets: Relationships with non-esterified fatty acids (NEFA), glucose, insulin and LH in serum. J. Anim. Sci. 67 (Supple.1):319.

Richards, M.W., R.P. Wettemann, L.J. Spicer and G.L. Morgan. 1991. Nutritional anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biol. Reprod. 44:961.

Riley, G.M., A.R. Peters and G.E. Lamming. 1981. Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of Gn-RH. J. Reprod. Fert. 63:559.

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) ß-subunit messenger ribonucleic acids are uncoupled from pulsatility of LH secretion during sexual maturation of the heifer. Biol. Reprod. 46:435.

Roberts, A.J.,.D.M. de Avila, J.D. Gerber, and J.J. Reeves. 1990. Active immunization of beef heifers against luteinizing hormone: I. Evaluation of protein carriers and adjuvants on antigenicity of LH. J. Anim. Sci. 68:3742.

Robertson, D.M., L.M. Foulds, L. Leversha, F.J. Morgan, M.T.W. Hearn, H.G. Burger, R.E.H. Wettenhalland D.M. de Krester. 1985. Isolation of inhibin from bovine follicular fluid. Biochem. Biophys. Res. Commun. 126:220.

Roche, J.F., J. Ireland and S. Mawhinney. 1981. Control and induction of ovulation in cattle. J. Reprod. Fertil. Suppl. 30:211.

Rodriguez, R.E. and M.E. Wise. 1989. Ontogeny of pulsatile secretion of gonadotropin-releasing homrone in the bull calf duirng infantile and pubertal development. Endocrinol. 124:248.

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

Sakurai, H., B.M. Adams and T.E. Adams. 1992. Pattern of gonadotropin-releasing hormone (GnRH)-like stimuli sufficient to induce follicular growth and ovulation in ewes passively immunized against GnRH. Biol. Reprod. 47:177.

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

SAS. 1982. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Schally, A.V., A. Arimura and Y. Baba. 1971. Isolation and porperties of the FSH and LH-releasing hormone. Biochem. Biophys. Res. Commun. 43:393.

Schanbacher, B.D. 1984. Active immunization against LH-RH in the male. In: D.B. Crighton (Ed.) immunological aspects of repoduction in mammals. pp. 345. Butterworths, London.

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

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

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

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. of Reprod. 32:547.

Schwartz, N.B. 1968. A model for the regulation of ovulation in the rat. Rec. Prog. Horm. Res. 25:1.

Schwartz, M.W., D.P. Figlewicz, D.G. Baskin, S.C. Woods and D.Porte, Jr. 1992. Insulin in the brain: A Hormonal regulator of Energy Balance. Endocrine Reviews. Vol. 13:387.

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

Short, R.E., And D.C. Adams. 1988. Nutritional and hormonal interrelationships in beef cattle reproduction. Can. J. Anim. Sci. 68:29.

Short, R.E. and R.A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. 32:127.

Short, R.E., R.A. Bellows, R.B. Staigmiller, J.G. Berardinelli and E.E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci. 68:799.

Siebert, B.D. and J.B.F. Field. 1975. Reproductive activity in beef heifers following post-weaning feeding on spear grass hay alone or with supplements. Australian J. Exp. Agri. and Anim. Hus. 15:12.

Smith, N.J. 1980. Excessive weight loss and food aversion in athletes simulating aneorexia nervosa. Pediatrics 66:139.

Spicer, L.J., and S.E. Echternkamp. 1986. Ovarian follicular growth, function and turnover in cattle: A review. J. Anim. Sci. 62:428.

Spicer, L.J., P. Matton, S.E. Echternkamp, E.M. Convey and H.A. Tucker. 1987. Relationships between histological signs of atresia, steroids in follicular fluid, and gonadotropin binding in individual bovine antral follicles during postpartum anovulation. Biol. of Reprod. 36:890.

Spicer. L.J., W.B. Tucker and G.D. Adams. 1990. Insulin-like growth factor-I in dairy cows: Relationships among energy balance, body condition score, ovarian activity and estrous behavior. J. Dairy Sci. 73:929.

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.

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.

Staigmiller, R.B. and B.G. England. 1982. Folliculogenesis in the bovine. Theriogenol. 17:43.

Steiner, R.A., W.J. Bremner and D.K. Clifton. 1982. Regulation of leutinizing hormone pulse frequency and amplitude by testosterone in the adult male rat. Endocrinol. 111:2055.

Stumpf, T.T., M.W. Wolfe, M.L. Day, J.A. Stotts, P.L. Wolfe, R.J. Kittok and J.E. Kinder. 1991. Effect of 17ß-estradiol on the preovulatory surge of LH in the bovine female. Theriogenol. 36:201.

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 beta-subunits in pituitaries of cows against LH-releasing hormone. Biol. Reprod. 47:776.

Takahashi, M., J.J. Ford, K. Yoshinaga and R.O. Greep. 1978. Active immunization of female rats with luteinizing hormone releasing hormone (LHRH). Biol. Reprod. 17:754.

Tatman, W.R., M.B. Judkins, T.G. Dunn and G.E. Moss. 1990. Luteinizing hormone in nutrient-restricted ovariectomized ewes. J. Anim. Sci. 68:1097.

Terasawa, E. and A.C. Gore. 1992. Regulation of pulsatile LHRH release in primates. In; W.F. Crowley, Jr. and P.M. Conn (Ed.) Modes of action of GnRH and GnRH Analogs. pp 256.

Tortonese, D.J., P.E. Lewis, H. Papkoff and E.K. Inskeep. 1990. Roles of the dominant follicle and the pattern of oestradiol in induction of preovulatory surges of LH and FSH in prepuberal heifers by pulsatile low doses of LH. J. Reprod. Fertil. 90:127.

Traywick, G.B. and K.L. Esbenshade. 1988. Pulsatile administration of gonadotropin-releasing hormone agonist to gilts actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 66:2209.

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.

Vigersky, R.A., A.E. Anderson, R.H. Thompson and D.L. Loriaux. 1977. Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. N. Eng. J. Med. 297:1141.

Villa-Godoy, A.,T.L. Hughes, R.S. Emery, W.J. Enright, A.D. Ealy, S.A. Zinn and R.L. Fogwell. 1990. Energy balance and body condition influence luteal function in holstein heifers. Dom. Anim. Endocrinol. 7:135.

Vizcarra, J.A. 1991. Factors influencing reproduction of beef heifers: Age at puberty, nutritional anestrus, glucose in plasma and consumption of cobalt. Masters of Science Thesis. Okla. State Univ.

Vizcarra, J.A., R.P. Wettemann and G.L. Morgan. 1994. Influence of frequency of GnRH pulses on release of LH in nutritional anestrous beef cows. (in preparation).

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.

Wagner, W.C., R. Saatman and W. Hansel. 1979. Reproductive physiology of the postpartum cow. II. Pituitary, adrenal and thyroid function. J. Reprod. Fert. 18:501.

Walters, D.L., M.F. Smith, P.G. Harms and J.N. Wiltbank. 1982. Effect of steroids and/or 48 hr calf removal on serum luteinizing hormone concentrations in anestrous beef cows. Theriogenol. 18.349.

Warren, M.P. 1983. Effects of undernutrition on reproductive function in the human. Endocrine Rev. 4:363.

Waters, S.B., B.E. Hawes and P.M. Conn. 1990. Stimulation of luteinzing hormone releasing by sodium fluoride is independent of protein kinase-C activity and unaffected by desensitization to gonadotropin-releasing hormone. Endocrinol. 126:2583.

Weesner, G.D., P.G. Harms, N.H. McArthur, J.M. Wilson, D.W. Forrest, T.J. Wu, and D.W. Pfaff. 1993. Luteinizing hormone-releasing hormone gene expression in the bovine brain: Anatomical localization and regulation by ovarian state. Biol. of Reprod. 49:431.

Wettemann, R.P. 1980. Postpartum endocrine function of cattle, sheep and swine. J. Anim. Sci. 51 (Suppl.2):2.

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.

Wettemann, R.P. and J.W. Castree. 1994. Immunization of heifers against gonadotropin-releasing hormone delays puberty and causes the cessation of estrous cycles. J. Reprod. Fertil. (In press).

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. Theriogenol. 24:565.

Williams, G.L. 1990. Suckling as a regulator of postpartum rebreeding in cattle: A review. J. Anim. Sci. 68:831.

Williams, G.L., T. Talavera, B.J. Peterson, J.D. Kirsch and J.E. Tilton. 1983. Coincident secretion of follicle-stimulating hormone and luteinizing hormone in early postpartum beef cows: Effects of suckling and low-level increases in systemic progesterone. Biol. Reprod. 29:362.

Wiltbank, J.N. 1970. Research needs in beef cattle reproduction. J. Anim. Sci. 31:755.

Wise, M.E., D. Nieman, J. Stewart and T.M. Nett. 1984. Effect of number of receptors for gonadotropin-releasing hormone on the release of luteinizing hormone. Biol. Reprod. 31:1007.

Woller, M.J., J.K. McDonald, D.M. Reboussin and E.I. Terasawa. 1992. Neuropeptide Y is a neuromodulator of pulsatile luteinizing hormone-releasing hormone release in the gonadectomized Rhesus monkey. Endocrinol. 130:2333.

Wu, F.C.W., D.C. Irby, I.J. Clarke, J.T. Cummins and D.M. de Kretser. 1987. Effects of gonadotropin-releasing hormone pulse-frequency modulation on luteinizing hormone, follicle-stimulating hormone and testosterone secretion in hypothalamo/pituitary-disconnected rams. Biol. of Reprod. 37:501.

Yelich, J.V., R.P. Wettemann, K.S. Lusby, H.G. Dolezal and D.K. Bishop. 1991. Influence of growth rate on body composition of beef heifers at puberty. J. Anim. Sci. (Suppl. 1) 69:41.

Yelich, J.V., R.P. Wettemann, H.G. Dolezal, K.S. Lusby and D.K. Bishop. 1993. Carcass lipid content and growth hormone in heifers at puberty. J. Anim. Sci. (Suppl. 1) 71:19.

Zimbelman, R. 1966. Effects of progestagens on ovarian and pituitary activities in the bovine. J. Reprod. Fert. Suppl. 1:9.

VITA

David Keith Bishop

Candidate for the Degree of

Doctor of Philosophy

Thesis: REGULATION OF GONADOTROPIN SECRETION IN BEEF COWS BY GONADOTROPIN RELEASING HORMONE (GnRH) AND BODY ENERGY RESERVES

Major Field: Animal Breeding and Reproduction

Biographical:

Personal Data: Born in San Angelo, Texas; December 24, 1957, the son of L. W. Jr. and Lavell Bishop.

Education: Graduated from Comanche High School, Comanche, Oklahoma, May 1976; received Bachelor of Science in Agriculture Business Management at Cameron University, December 1987; received Master of Science Degree in Animal Science at Oklahoma State University, May 1991; completed the requirements for the Doctor of Philosophy Degree in December, 1993.

Professional experience: Halliburton Services 1978-1985; North Ridge Enterprises 1985-1986; Graduate research and teaching assistant at Oklahoma State University 1988-1993; Visiting Assistant Professor at Oklahoma State University 1993.

Professional Organizations: American Society of Animal Science, Animal Science Graduate Student Association.