EFFECT OF POSTPARTUM NUTRITION ON THE ONSET OF OVARIAN ACTIVITY IN BEEF COWS

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DEDICATION

In Loving Memory of My Parents

With all my Love, Respect and Admiration

Dr. Horacio Rubio Palacios

and

Dra. Velia Gutiérrez de Rubio

Who Taught Me the Value of Work and Education

Commit Thy Works Unto the Lord and Your Plans will Succeed

Proverbs 16:3

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CHAPTER I

INTRODUCTION

The beef cattle industry in the United States of America is supplied largely by a cow-calf production system dominated by many medium and small producers. In order to make a profit those producers must have very efficient cows on their ranches. An efficient cow is one that calves and weans a calf every 12 months. Reproduction is a major factor that influences the profitability of a ranch. In addition, breeding and genetic programs should be focus on improving the quality of weaned calves to meet the expectations of modern beef industry standards.

Low reproduction efficiency causes major economic losses to the livestock industry because of a reduction in the calf crop. The major case of inefficiency is a prolonged postpartum anestrus in beef cows. Suckling and nutrition are the two major factors that influence the length of postpartum anestrus. Other minor factors such as presence of bull, breed and age at calving are also associated with postpartum anestrus.

The effect of nutrition on postpartum reproduction is dependant on whether nutritional deficiencies occur before or after calving. Restricted nutrient

intake before calving results in thin cows at parturition, a prolonged postpartum anestrous period, and fewer cows in estrus during the breeding season. In contrast, greater energy intake before calving decreases the interval from calving to estrus, ovulation, and pregnancy. Greater nutrient intake prepartum also increases the percentage of cows exhibiting estrus during the breeding season.

Body condition is a useful indicator of energy status and potential rebreeding performance. The amount of body fat is positively correlated with the BCS in beef cows and heifers. When the amount of energy and protein is less than required, body fat is mobilized and BCS of cows and heifers decreases. Changes in body condition and body weight affect rebreeding performance. Nutrient intake and body fat stores have a decisive role in the secretion of hormones that regulate reproduction. If sufficient body stores of fat are not presented, pituitary hormones are not secreted after calving and estrous cycles are not established during the breeding season. Metabolic compounds and hormones, such as insulin and insulin-like growth factor-I, may indicate to the hypothalamus and/or pituitary as to the energy status of the cow. Insulin may potentiate the steroidogenic response to gonadotropins on the ovary and it may act on the pituitary to increase sensitivity of gonadotropes to GnRH. Inadequate nutrient intake affects the growth of the dominant follicle in cattle, and insulin may mediate the effects of acute changes in nutrient intake on follicular dynamics.

Suckling influences the secretion of gonadotropins and may delay ovulation. This contributes to an extended postpartum anestrus period, resulting in a decrease reproductive efficiency. Secretion of LH is reduced in suckled beef

cows and cyclic ovarian activity is suppressed during the early postpartum period. Nutrient intake and body energy reserves also influences the ovarian response to alteration of the suckling stimulus. The effect of short term calf separation (48 h) on ovarian function of cows is influenced by BCS and thin cows may not respond and body energy reserves influence the onset of ovarian activity after early weaning.

A better understanding of the hypothalamus–pituitary- ovary axis, is necessary to improve reproduction efficiency in beef cows, and how it is controlled by the energy status of the cow. In addition, knowing the role of metabolic compounds and hormones, as insulin and insulin-like growth factor-I, in the reproduction process in beef cows is also needed.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Prolonged postpartum anestrus in beef cows reduces the calf crop and causes major economic losses to the livestock industry. It is well established that nutrition has a profound influence on reproductive performance of domestic ruminants (Hammond, 1949; Robinson, 1996; Wettemann et al., 2003) and the effects have been reviewed extensively in dairy and beef cows (Short et al., 1990; Beam and Butler, 1999, Wettemann et al., 2003) with special emphasis on the period from calving to the first postpartum estrus. The major factors that control the length of postpartum anestrus are suckling and nutrition, with minor effects attributed to presence of a bull, breed, and age at calving (reviewed by Short et al., 1990; Wettemann et al., 2003).

Postpartum Reproduction of Beef Cows

Nutrition and reproduction

Nutritional effects on postpartum reproduction in beef cows have been well documented by the classical work of Wiltbank et al. (1962) and Dunn et al. (1969). Several detailed reviews have assessed the importance of nutrition on reproduction (Dunn and Kaltenbach, 1980; Short and Adams, 1988; Randel, 1990, Butler, 2000; Wettemann et al., 2003; Hess et al., 2005). The influence of nutrition on postpartum reproduction is dependant on whether the nutritional deficiency occurs before or after calving, and thus nutritional management during gestation and after calving are of major concern.

Follicular growth after calving

Secretion of FSH occurs within five days after parturition in beef cows (Schallenberger, 1985; Crowe et al., 1998). Follicular waves are established in beef cows within 10 to 20 d after parturition, and multiple follicular waves of growth can occur before the first postpartum ovulation (Murphy et al., 1990). The diameter of dominant follicles increases with successive waves until ovulation (Murphy et al., 1990, Stagg et al., 1995). In dairy cows, Beam and Butler (1999) indicated that the first postpartum dominant follicle has three possible fates: ovulation, atresia and turnover (followed by new wave emergence) or formation of a follicular cyst. A principal component of folliculogenesis is the secretion of LH during the early postpartum period. Inadequate pulsatile release of LH is

associated with follicular turnover and anestrus; moderate LH pulsatility is associated with ovulation; and extreme LH pulsatility, and lack of an LH surge, is associated with the development of cystic ovaries (Silvia et al., 2002).

Factors influencing postpartum reproduction

Prepartum nutrition

Restricted nutrient intake before calving results in thin cows at parturition, a prolonged postpartum anestrous period, and less cows in estrus during the breeding season (Wiltbank et al., 1962; Bellows and Short, 1978; Dunn and Kaltenbach, 1980; Wright et al., 1987). In contrast, greater protein and energy intake before calving, decreases the interval from calving to estrus and ovulation (Perry et al., 1991) and to pregnancy (Dunn et al., 1969). Greater nutrient intake prepartum also increases the percentage of cows exhibiting estrus during the breeding season (Corah et al., 1975; Spitzer et al., 1995) and increases pregnancy rates (Selk et al., 1988; Marston et al., 1995).

Body condition is a useful indicator of energy status and potential rebreeding performance (Dunn and Kaltenbach, 1980; Dziuk and Bellows, 1983; Randel, 1990). Multiparous cows with greater body condition before or at calving have greater pregnancy rates than thin cows (Warnick et al., 1981; Rutter and Randal, 1984; Rakestraw et al., 1986; Richards et al., 1986; Selk et al., 1988; Osoro and Wright, 1992). Nutrient requirements during the prepartum period may differ depending on body condition and body weight of cows entering the last third of gestation (DeRouen et al., 1994). There is a negative correlation

between body condition score (BCS) at calving and duration of postpartum anestrus (Richards et al., 1986; Wright et al., 1987, 1992), and cows calving with moderate BCS (5), had greater pregnancy rates regardless of BCS at six months of gestation (Morrison et al., 1999). First service conception rates were not effected, but overall pregnancy rates were less for thin cows compared with cows in moderate BCS at calving (Lake et al., 2004).

Inadequate nutrient intake has an adverse effect on ovarian function (Rasby et al., 1986) and alters follicular growth (Webb et al., 2004). Thin Hereford cows (BCS ≤4) had reduced ovarian, corpora lutea and follicular fluid weights compared with cows that had moderate to good body condition (Rasby et al., 1986). Perry et al. (1991) found that cows which consumed greater energy before calving had a greater number of large follicles after calving. Dietary intake may influence oocyte quality (Krisher, 2004). The magnitude and duration of a negative energy balance deficit in dairy cows during lactation is a major factor controlling follicular growth (Beam and Butler, 1999; Butler, 2000).

Postpartum nutrition

Inadequate nutrient intake after calving has detrimental effects on postpartum reproduction. The benefits of increased energy intake after calving are most apparent when cows calve with thin BCS (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980; Spitzer et al., 1995). Wiltbank et al. (1964) found that the interval from calving to the first estrus may be shortened when cows calve in thin BCS and are fed greater amounts of energy after calving. Increasing dietary

intake increased weight and BCS and decreased the interval to the first normal luteal phase (Lalman et al., 2000). Primiparous beef cows that calved with a BCS of 4 or 5 and were fed to gain 0.90 kg/d for the first 71 d postpartum, had shorter intervals to first postpartum estrus and ovulation, and a larger dominant follicle at the first estrus, compared with cows fed to gain 0.45 kg/d (Ciccioli et al., 2003). If cows calved in a very good body condition in the fall of the year, and nutritient intake was inadequate after calving, fewer cows exhibited estrus during the first 70 d after calving (Raskestraw et al., 1986). In a review by Randel (1990), conception rates at the first service ranged from 38 to 62% for energy restricted cows and from 66 to 84 % for well-fed cows. Pregnancy rates for cows fed diets with restricted energy after calving were from 50 to 76 % compared with 87 to 92 % for well-fed cows. Dietary energy intake after calving may not affect the length of the postpartum interval if cows calve with adequate body energy reserves and maintain adequate BCS during lactation (Richards et al., 1986; Marston et al., 1995; Spitzer et al., 1995; Stagg et al., 1998).

Follicular development after calving can be effected by postpartum nutrition. Inadequate energy after calving decreased rate of appearance of small (5.0 to 7.9 mm) and large (≥10mm) follicles, and a greater percentage of cows that received greater amounts of energy ovulated by 150 d post partum (Perry et al., 1991). Postpartum energy intake did not influence the interval to detection of the first dominant follicle, but the number of dominant follicles undergoing atresia before the first ovulation was greater for cows that received a low energy diet (Stagg et al., 1995). Restricted nutrient intake during the early postpartum period

depresses LH pulsatility and decreased the size of the largest follicle, indicating a delay in the establishment of functional dominance in underfed cows (Grimard et al., 1995). A negative energy balance in postpartum dairy cows impacts the population of ovarian follicles and the functional competence of the dominant follicle (Beam and Butler, 1999). Growth rate of the dominant follicle and concentrations of insulin and IGF-I in plasma were greater in cows fed a high energy compared with cows fed a low energy diet (Armstrong et al., 2001). Undernutrition during the early postpartum period may alter gene expression in preantral follicles, which will result in abnormal mature follicles that produce low quality oocytes or form corpora lutea with abnormal function. These altered functions may cause decreased fertility (Webb et al., 2003). Lack of follicular waves after calving is not the limiting factor for the onset of estrus and ovulation (Wettemann et al., 2003).

Suckling

Cyclic ovarian activity is suppressed during the early postpartum period in suckled beef cows (reviewed by Edgerton, 1980; Williams, 1990). Secretion of LH is reduced in suckled anovulatory beef cows compared with cows exhibiting normal estrous cycles (Humphrey et al., 1983) and the maximum diameter of anovulatory dominant follicles is smaller than during ovulatory estrous cycles (Perry et al., 1991). Continuous suckling delays ovulation and contributes to a long postpartum anestrous period, resulting in decreased reproductive efficiency (Wettemann, 1994).

The primary mechanism by which suckling, and the presence of a calf, delays ovarian function is through reduced secretory pulses of LH (Short et al., 1972; Short et al., 1990; Williams, 1990). Suckling delayed the onset of LH secretion in cows (Williams et al., 1982), whereas suppression of the suckling stimulus after 20 to 30 d post partum, increased LH secretion after calving (Stagg et al., 1998). The inhibition of LH secretion by suckling is controlled by GnRH-secreting neurons (Williams et al., 1983).

The effect of altering the suckling stimulus on LH secretion has been evaluated (Griffith and Williams, 1996; Mackey et al., 2001). If suckling occurs two or three times a day, the duration of ovarian acyclicity is longer, plasma LH concentrations are decreased, and sensitivity of the hypothalamic-hypophyseal axis to the inhibitory effects of estradiol on LH are increased compared with cows with weaned calves (Acosta et al., 1983; Short et al., 1990; Williams, 1990). Secretion of GnRH is inhibited in continuously suckled cows, compared with cows suckled once a day (Zalesky et al., 1990). Acute weaning of suckled anestrous beef cows is characterized by a rapid increase in pulsatile LH secretion within 48 to 96 h (Shively and Williams, 1989). The increase in LH secretion is initially accompanied with a rapid decrease in content of GnRH within the hypothalamus, followed by an increase (Malven et al., 1986). Short-term calf removal (Smith et al., 1979) and early weaning (Bellows et al., 1974) increase serum concentrations of LH and decrease the postpartum anestrous interval. The increase in LH in serum at calf separation can be markedly attenuated by the premature return of calves; an interval of 144 h of separation maybe required

for cows to respond to temporary weaning (Cutshaw et al., 1991). A greater suckling intensity, induced by twins (Sinclair et al., 1994) or by adoption of a foster calf (Wettemann et al., 1978), and suckling more than once daily extends the postpartum anovulatory period (Lamb et al., 1999).

Nutrient intake and body energy reserves influence the ovarian response to alteration of the suckling stimulus. Early weaning or once-daily suckling after 65 d post partum did not shorten the length of the anovulatory interval of primiparous beef cows that calved with moderate BCS (≥5) and were fed to maintain BCS until breeding (Bell et al., 1998). The effect of short term calf separation (48 h) on ovarian function of cows is influenced by BCS and thin cows may not respond (Wettemann et al., 1986). Body energy reserves influence the onset of ovarian activity after early weaning (Bishop et al., 1994).

Maternal identification of a cow's own calf influences the response to suckling (Williams and Griffith, 1995). This indicates that maternal recognition of a calf (maternal-offspring bond) is required to inhibit LH release and ovulation (Silveira et al., 1993). Removal of a cow's natural calve resulted in the expected increase in serum LH concentrations and pulse frequency within 48 h, and suckling by foster calves every 6 h for 4 d did not prevent the increase in LH. However, suckling by a cow's own calf at 6 h intervals maintained the suppressed secretion of LH which is typical of suckled, anovulatory cows (Williams and Griffith 1995). Postpartum intervals to onset of luteal activity for cows with weaned calves and for cows suckling foster calves were similar, and both were markedly shorted compared with cows suckling their own calves.

Vision and olfaction meditate the suckling-inhibition of LH secretion in cattle (Griffith and William, 1994). Repression of a cow's visual and olfactory senses blocked recognition of her calf and released the cow from the suckling-mediated inhibition of LH secretion (Griffith and Williams 1996). Consequently, the maternal-offspring bond is essential for the suckling-induced anovulation, and cows can use both olfactory and visual cues to identify their calves (Williams and Griffith, 1995).

Endogenous opioid peptides may mediate the suckling-inhibition of LH secretion in postpartum beef cows (Myers et al., 1989). Suckled anestrous cows had greater concentrations of opioid receptors in the preoptic-basal forebrain area than suckled cyclic cows (Trout and Malven, 1988). Administration of an opioid antagonist (naloxone) increased LH secretion in suckled cows (Whisnant et al., 1986), and the ability of opioids to inhibit LH secretion may decrease with days after parturition (Whisnant et al., 1986b).

Estrous cycles

Endocrine regulation

The duration of bovine estrous cycles averages 21 d, with a normal range of 18-26 d (Asdell et al., 1949; Woody et al., 1965; Swanson et al., 1972). Estimates of the duration of estrus range from 3 to 28 h in dairy (Allrich, 1994; Xu et al., 1997; Dransfield et al., 1998) and beef cows (White et al., 2002). Duration of estrous expression could be due to hypothalamic sensitivity to threshold concentrations of estradiol, which may differ among cows (Darwash et al., 2001). Concentrations of estrogens in plasma increase during proestrus (Wettemann et al., 1972) and progesterone concentrations are greatest during the 15 d of the luteal phase (Henricks et al., 1970; Swanson et al., 1972).

Waves of follicular growth occur at regular intervals during the estrous cycle, with two to four distinct cohorts of follicles emerging during the cycle. Each follicular wave has an inherent lifespan of 7 to 10 d as it progresses through emergence, selection, dominance and atresia or ovulation (Fortune et al., 1991). The emergence of each new wave is stimulated by a transient (1 to 2 d) increase in plasma FSH (Adams et al., 1992; Sunderland et al., 1994; Stagg et al., 1998) with selection of a follicle occurring during decreasing concentrations of FSH in plasma. The dominant follicle suppresses FSH secretion until the follicle either ovulates or becomes atretic. The first or subsequent dominant follicle of the cycle is capable of producing sufficient estrogen to induce estrus and subsequently ovulation. Ovulatory surges of LH and FSH occur at behavioral estrus in most domestic farm animals. Estrogen and progesterone govern gonadotropin release through positive and negative

feedback on the hypothalamus and anterior pituitary. The preovulatory surge of LH is initiated by the positive feedback of increased concentrations of estradiol on the hypothalamus and anterior pituitary (Beck and Convey, 1977; Kesner et al., 1981). Progesterone from the corpus luteum during the luteal phase of the estrous cycle suppresses the ovulatory surge of LH (Rahe et al., 1980; Walters et al., 1982).

Estradiol-17β in the absence of progesterone is the primary signal to the hypothalamus that induces estrus in cattle (Blache et al., 1991). Administration estradiol benzoate induces behavioral estrus in ovariectomized cows (Asdell et al., 1945; Carrick and Shelton, 1969), and immunization against estradiol prevents expression of estrus in beef heifers (Martin et al., 1978). When progesterone reaches a threshold concentration early in the luteal phase, it inhibits the occurrence of estrus (Vailes et al., 1992). Intensity of estrus expression may be not related to either dose or blood concentrations of estradiol-17β (Coe and Allrich, 1989). However dose of estradiol may influence the duration of estrus (Reames et al., 2005).

Estradiol is secreted by preovulatory follicles in the ovary (Ireland and Roche, 1982; Ireland and Roche, 1983). Measurement of plasma estradiol in the utero-ovarian vein verified that a single large antral follicle was responsible for increased concentration of estradiol during proestrus and estrus in cows (Ireland et al., 1984). Felck (1959) first proposed the “two-cell theory” where both thecal and granulosal cells of rat ovarian follicles were involved in the production of estradiol. Under the influence of gonadotropins, steroidogenesis occurs in two

cell types: the LH-responsive theca interna and the FSH-responsive granulosa cells (Fortune and Armstrong, 1978; Fortune and Armstrong, 1997). Luteinizing hormone acts via LH-receptors on the thecal cells to increase production of cAMP which activates genes that encode for cholesterol side-chain cleavage, 17α-hydroxylase, and C17,20 lyase which are required for androgen synthesis (Erickson et al., 1985). Increased enzyme activity (17α-hydroxylase) occurs as bovine follicles mature (Rodgers et al., 1986). Androstenedione is the principal aromatizable steroid produced through the Δ^5 pathway by bovine theca cells (Lacroix et al., 1974; Fortune, 1986). Androgens produced by theca cells diffuse across the follicular basement membrane to be utilized as substrate in estrogen biosynthesis by granulosa cells (Baird, 1977). Bovine granulosa cells supply progesterone to the thecal cells for androgens synthesis (Fortune, 1986). Androgen aromatization is regulated by FSH in granulosa cells (Dorrington et al., 1975). Aromatase activity is absent in immature, hypophysectomized rats but can be induced by FSH (Armstrong and Papkoff, 1976). Dieleman and Blankenstein (1984) found aromatization decreases approximately 14 h after the preovulatory LH surge in cows.

Frequent low amplitude pulses of LH occur during the follicular phase of the bovine estrous cycle (Rahe et al., 1980). Frequency of LH pulses is less, but amplitude is greater during the progesterone dominated luteal phase (Rahe et al., 1980). Estradiol increases synthesis and secretion of LH from the anterior pituitary gland at estrus. Increased serum concentrations of estradiol and luteinizing hormone occur concurrently during proestrus (Wettemann et al., 1972;

Echternkamp and Hansel, 1973; Chenault et al., 1975; Lemon et al., 1975). Basal concentration of LH begins to increase 5-6 d prior to estrus, with a preovulatory surge of LH occurring near the onset of estrus (±3 h; Henricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). Anterior pituitary content of LH and FSH reach maximal concentrations between d 18 to 20 of the estrous cycle, when concentrations of estradiol are increasing (Hackett and Hafs, 1969). Pituitary content of LH and FSH decreased 89 and 73%, respectively, from d 18 to 2 of the subsequent estrous cycle. In the absence of steroid feedback, pituitary and plasma concentrations of LH are negatively correlated (- 0.88) in ovariectomized heifers (Swanson et al., 1971).

The preovulatory surge of LH is maximal for 6 to 10.6 h (Henricks et al., 1970; Swanson and Hafs, 1971; Chenault et al., 1975). Kesner et al. (1981) suggested that estradiol induces the LH surge in cows by increasing the sensitivity of the bovine anterior pituitary to GnRH and by increasing secretion of GnRH through an ultra positive feedback loop. Estradiol initially reduces the ability of the anterior pituitary to release LH either by decreasing responsiveness of the gonadotropes to GnRH or by reducing GnRH release in cows. Concentrations of LH are reduced several hours (10 to 15 h) prior to the LH surge, although the anterior pituitary is capable of responding to exogenous GnRH. This indicates that estradiol reduces GnRH release below the threshold concentration that normally induces LH release. Secretion of GnRH resumes about 12 h after estradiol stimulation and induces a LH surge (Kesner et al., 1981). Maximum LH concentrations occurred when the diameter of the dominant

follicle is the greatest in dairy heifers, prior to first estrus (Swanson et al., 1972). Exogenous estradiol treatment of ovariectomized cows and heifers (Short et al., 1973; Beck and Convey, 1977; Imakawa et al., 1986), and ovariectomized ewes (Moss et al., 1981; Kasa-Vubu et al., 1992) induced LH release similar to an endogenous preovulatory surge.

Estrous behavior

The first postpartum ovulation in beef cows frequently is not preceded by estrous behavior (Wettemann, 1980; Short et al., 1990; Ciccioli et al., 2003). Estrus is usually expressed prior to the second ovulation in the majority of cows (King et al., 1976; Perry et al., 1991; Ciccioli et al., 2003; Looper et al., 2003). A transitory increase in concentrations of progesterone commonly preceded the first pubertal (Rutter and Randel, 1986) and postpartum estrus in beef cows (Perry et al., 1991b; Werth et al., 1996; Looper et al., 2003). Treatment with estradiol benzoate, after short-term progesterone treatment, increases the estrous response of anestrous cows (McDougall et al., 1992; Fike et al., 1997). It is likely that short-term transient luteal activity must precede the first postpartum estrus. Progesterone, acting on neural centers, may enhance the effect of estradiol on estrous behavior. Maximal concentrations of estradiol produced during late pregnancy may induce a refractory state to estradiol in the brain, which is reversed by progesterone exposure (Carrick and Shelton, 1969). Treatment of anestrous cows with progesterone, increased synthesis of LHβ mRNA in the anterior pituitary (Looper, 1999), LH secretion (Anderson et al.,

1996), and number of LH receptors and concentrations of estradiol within the largest follicle (Inskeep et al., 1988). This sequence of events could stimulate estrus and ovulation.

Detection of estrus

Expression of estrous behavior can be altered by numerous factors such as the number of cows expressing estrus (Helmer and Britt, 1985; Floyd, 2001), age of the cow (Mathew et al., 1999), environmental temperature (Gwazdauskas, 1985; White et al., 2002), or days after calving (Pennington et al., 1986). Detection of estrus by human observation has been the method of choice to identify cows in estrus and time to inseminate (Foote, 1975; Lehrer et al., 1992). Numerous factors such as housing arrangement, milk yield, floor surface, feet and leg problems and estrus status of herd mates effect the expression of estrus (Senger, 1994).

Low to moderate estrous detection efficiencies achieved on most farms reflect inadequate methods, but short duration of estrus with few mounts received could also be a problem. Estrous detection aids have been developed to assist observation; some of these include mount detectors, tail chalk, teaser bulls or androgenized cows with chinball markers, and video recordings (Macmillan and Curnow, 1977; Sawyer et al., 1986; Senger, 1994). Recent advances in detection methods include automated methods such as pedometry and rump mounted, pressure-sensitive electronic mount detection devices

(Pennington et al., 1986; Senger, 1994; Stevenson et al., 1996; White et al., 2002).

Marking the tail heads of cows with paint or a livestock marker has been used as an effective indicator of estrus (Foote, 1975; Macmillan and Curnow., 1977). When an estrous cow is mounted by other cows or bulls, the paint is either partially or totally removed. Efficiencies of estrous detection using tail paint vary from 44 to 96% (Macmillan and Curnow, 1977, Sawyer et al., 1986).

The use of pedometers to monitor activity of cows was first reported by Kiddy (1977). When cows are in estrus, their physical activity increases (Farris, 1954, Reimers et al., 1985). Lehrer et al. (1992) reviewed the effectiveness of pedometry-aided detection of estrus (when comparing with visual observation) and found that the accuracy of detection of estrus using pedometry varied from 22 to 100%, and that efficiency of visual observation varied from 60 to 100% .

Electronic estrous detection systems are important tools for researchers to investigate duration of estrous, onset of estrus expression, mounting frequency, breed effects on behavior, synchronized estrous expression, and time of AI relative to esxpression of estrus (Rae et al., 1999). The HeatWatch system (DDx, Inc., Denver CO), is an automated mount monitoring system that consists of individual rump-mounted mount detectors that transmit the occurrence of each mount (time and duration of mount) via radio signal to a receiver. A buffer then stores the mount data, until accessed with a computer, using HeatWatch software (Nebel et al., 1995; Xu et al., 1997; White et al., 2002).

Stevenson et al. (1996) compared the effectiveness of the HeatWatch system to twice daily visual observation for estrous detection in beef heifers. The HeatWatch system increased the efficiency of estrous detection by 37% (100% vs 73%) over visual observation. In a similar study, Borger et al. (1996) compared the efficiency and accuracy of twice daily visual observation to HeatWatch in 74 mature beef cows. The use of the HeatWatch system improved the efficiency of estrous detection compared with visual observation (91.1% vs 65.8%, respectively). The accuracy of estrous detection by HeatWatch and visual observation were 87.5% and 91.5%, respectively.

Role of the Insulin-Like Growth Factor System in the Ovary

The insulin-like growth factor-I system

The insulin-like growth factor (IGF) system is composed of the IGF-I and IGF-II peptides, six structurally homologous high-affinity IGF binding protein (IGFBPs), IGFBP-specific proteases, a family of IGFBP-related (low affinity) proteins, and two IGF receptors (Hwa et al., 1999). The IGF system is the most extensively studied growth factor systems in the ovary (Poretsky et al., 1999). Insulin-like growth factor-I and IGF-II, are mitogenic, and antiapoptotic peptides that promote differentiation and also have insulin-like metabolic effects mediated by binding to specific high-affinity membrane receptors. The type I IGF receptor mediates the metabolic and growth promoting actions of IGF-I and IGF-II at target cells through the tyrosine kinase pathway. The type II IGF receptor is important in IGF-II turnover and may mediate signals involved in angiogenesis or other processes. Insulin-like growth factors circulate bound primarily to IGFBP-3, as well as other IGFBPs, that prolong their half lives and also facilitate transcapillary transport to tissues. Locally produced IGFBPs modulate (mostly inhibit) IGF actions at target cells and some have IGF independent actions. Insulin-like growth binding proteins have up to two orders of magnitude greater affinities for the IGFs than do the IGF receptors. Insulin-like growth factor binding proteins-specific proteases, that have been identified in a variety of cell types and in body fluids, decrease the affinities of specific IGFBPs for IGF peptides. A

group of IGBP proteases are members of matrix metalloproteinase (MMP), metzincin, and serine protease superfamilies (Hwa et al., 1999).

Expression of IGF and IGF Receptor

During folliculogenesis in the human, IGF expression is follicle stage-specific and is compartmentalized. Insulin-like growth factor-II mRNA is expressed in theca and perifollicular vessels of all follicles (El-Roeiy et al., 1993). In small antral follicles of normal ovaries, IGF-II mRNA and protein are expressed in granulosa and thecal cells. Although IGF-II is in atretic antral follicles, expression by thecal cells is minimal (Poretsky et al., 1999).

Actions of IGF-I and -II in the ovary

A number of functions in the ovary are either modulated by IGF-I alone or in concert with gonadotropins. For the most part in the rat, IGF-I acts on granulosa cells to amplify actions of gonadotropins (Grimes et al., 1994; Jia et al., 1986), and IGF-I may also stimulate interstitial cells (Cara et al., 1988; Magoffin et al., 1990). In humans, the in vitro effects of IGF-I on granulosa and theca cells have been investigated, eventhough the endogenous ligand in human ovaries is IGF-II. Estradiol stimulates synthesis of IGF-I and IGF-II by human granulosa and granulosa-luteal cells (Poretsky et al., 1999). Maturation of immature human oocytes in vitro is augmented by IGF-I (Gomez et al., 1993) and IGF-I is antiapoptotic in follicles. Apoptosis of granulosa and luteal cells within the follicle is enhanced by IGFBPs (Chun et al., 1994). The percentages of

ovine, porcine, rabbit and rat granulosa cells that express P450 side chain cleavage enzyme are increased by IGF-I in synergy with FSH (Urban et al., 1994). Insulin-like growth factor-I stimulates estradiol and progesterone secretion by porcine granulosa cells in vitro (Balwant et al., 1997). Furthermore, IGF-I increases estradiol and progesterone secretion alone or in combination with FSH in murine, bovine, ovine and caprine follicles (Campbell et al., 1995; Gong et al., 1994), and IGF-I can enhance the expression of FSH receptor in granulosal cells (Minegishi et al., 2000).

Insulin-Like Growth Factors in Follicular Fluid and Serum

Constituents within follicular fluid (FF) of the human Graffian follicle originate from both the circulation and local intraovarian production. (Van Dessel et al., 1996). Concentrations of IGF-I are similar in follicular fluid from estrogen- vs androgen-dominant follicles and concentrations are not correlated with follicular size. Follicular fluid IGF-II is primarily from local intraovarian production of granulosa and perhaps theca cells, in addition to some contribution from the circulation.

Insulin-Like Growth Factor Binding Proteins: Expression and Action within the Ovary

Six high-affinity IGFBPs have been identified, and expression of mRNA for five IGFBPs have been detected in the human ovary (El-Roeiy et al., 1994). Expression of IGFBP-1 mRNA occurs in granulosa cells of dominant follicles as well as in the corpora lutea. Follicular expression of mRNA for IGFBP-2, -3, -4 and -5 was detected in human thecal cells from small antral follicles and dominant follicles (Poretsky et al., 1999). During the human menstrual cycle expression of IGFBP is dependent on the functional status of the follicle as androgen-dominant follicles (high A:E ratio) have greater concentrations of IGFBP-2 and IGFBP-4 compared with healthy estrogen-dominant follicles (Cataldo and Giudice, 1992). Insulin-like growth factor binding protein-4 is a potent inhibitor of FSH and IGF-II stimulated granulosa cell steroidogenesis (Mason et al., 1998). Greater concentrations of IGBPs decrease intrafollicular levels of bioavailable IGFs, which contribute to atresia in androgen-dominant follicles (Poretsky et al., 1999).

Regulation of Ovarian IGF Binding Proteins

Granulosa cell secretion of IGFBP is inhibited by gonadotropins and insulin-like peptides, which enhances IGF availability and gonadotropin action within the follicle (Poretsky et al., 1999). Chandrasekher et al. (1995) found that modulation of IGF action is also influenced by IGFBP proteases that decrease the binding of IGFBPs to IGFs. An IGFBP-4 protease is present in estrogen-

dominant follicular fluid of humans, but not in androgen-dominant follicular fluid. Protease activity for IGFBP-4 also occurs in dominant follicles of bovine (Rivera et al., 2001), porcine (Besnard et al., 1997), and ovine (Mazerbourg et al., 1999) ovaries. Insulin-like growth factor-4 is highly conserved in bovine, ovine, human, mouse, and rat ovarian follicles (Poretsky et al., 1999). Conover et al. (1999) found that the IGFBP-4 protease in human ovarian follicular fluid is pregnancy-associated plasma protein-A (PAPP-A), which is a large dimeric glycoprotein with a molecular weight of 44 kD (Oxvig et al., 1993). Pregnancy-associated plasma protein-A is an active enzyme (Lawrence et al., 1999) and IGFBP-4 is its only substrate. Granulosa cells from small follicles (≤ 8mm) secrete less PAPP-A, whereas granulosa cells from dominant follicles (≥ 9mm) secrete greater amounts of PAPP-A (Chandrasekher et al., 1995).

Metabolic hormones and postpartum reproduction

Insulin

Insulin is a regulator of carbohydrate, fat and protein metabolism (Poretsky et al., 1999) and is important in the regulation of thermogenesis (Rothwell and Stock, 1988). However, the role of insulin on reproduction is not fully understood. Insulin is secreted by the pancreas and may cross the blood-brain barrier. The most direct route for peripheral circulatory insulin to enter the cerebrospinal fluid is via passage across the "blood-cerebrospinal fluid barrier" (Schwartz et al., 1992). Insulin receptors have been identified in the hypothalamus (Adamo et al., 1989), however fasting, diabetes and obesity do not influence the content of insulin receptors in the brain as observed in peripheral tissues such as the liver (Havrankova et al., 1979).

Infusions of insulin directly into the ventral hypothalamus of rats reduce food intake and body weight (McGowan et al., 1990). The ability of centrally admininstered insulin to reduce food intake is attenuated when animals are metabolizing more fat relative to carbohydrates (lipolytic as opposed to lipogenic, Arase et al., 1988). Food deprivation suppresses concentrations of insulin in blood of rats (Schwartz et al., 1992), and feed restriction results in reduced plasma concentrations of insulin in cattle (Richards et al., 1989; Bossis et al., 1999).

Insulin influences secretion of gonadotropins by effects on the hypothalamus and pituitary through modulation of GnRH neuronal activity in response to metabolic status (Van Houten et al., 1979). Release of GnRH is

increased eight-fold by low concentrations of insulin, but this only occurs when glucose is available (Arias et al., 1992; Hileman et al., 1993; Dhuyvetter and Caton, 1996). Intraceroventricular infusion of insulin to ovariectomized diet-restricted ewes, increases concentrations of LH, indicating that insulin is a component of hypothalamic mechanisms regulating secretion of LH (Daniel et al., 2000). Beef heifers on a greater nutrient intake, had greater concentrations of insulin and LH in serum, and reach puberty at a younger age, compared with animals on restricted nutrient intake (Yelich et al., 1996).

Insulin may potentiate the steroidogenic response to gonadotropins on the ovary (Davoren and Hsueh, 1984; Willis et al., 1996) and may act on the anterior pituitary to increase sensitivity of gonadotropes to GnRH (Soldani et al., 1994). Insulin is an important signal mediating nutritional effects on follicular dynamics in cattle (Webb et al., 2004). McCann and Hansel (1986) found that abnormal pituitary and luteal functions in fasted heifers were associated with concurrent fasting-induced changes in insulin and glucose metabolism. In addition, ovulatory increases in plasma insulin and IGF-I concentrations were more pronounced during the preovulatory period in cattle offered a high energy diet (Armstrong et al., 2001). Insulin receptors are present in granulosa, thecal and stromal cells in humans and other animals (Poretsky and Kalin, 1987; El-Roeiy et al., 1993; Samoto et al., 1993) and insulin enhances luteal cell steroidogenesis in vitro (Spicer et al., 1993; Moniaux et al., 1994). Bovine granulosa in culture cells are critically dependent on the presence of physiological concentrations of insulin (Glister et al., 2001). Insulin stimulates proliferation and steroidogenesis of

bovine granulosa cells in vitro (McArdle et al., 1991; Spicer and Echternkamp, 1995; Gutierrez et al., 1997b) and increases progesterone production (Staples et al., 1998). Insulin is a potent stimulator of FSH-induced estradiol secetion by bovine granulosa cells (Spicer et al., 1994), and insulin infusion during a superovulatory regime in cattle increased intrafollicular concentrations of estradiol in large graafian follicles by five-fold and increased the diameter of large follicles (Simpson et al., 1994). There is correlation between diet-induced increases in circulating concentrations of insulin with increased estradiol production by cultured granulosa cells from small antral (1 to 4 mm) follicles (Armstrong et al., 2002b). Restricted nutrient intake decreases circulating concentrations of insulin in cows (Richards et al., 1989b; Armstrong et al., 1993; Bossis et al., 1999; Armstrong et al., 2001) and granulosa cells from nutritionally induced anoestrous cows have the capacity to respond to insulin in vitro (Hamilton et al., 1999).

Inadequate nutrient intake affects the growth of dominant follicle (Murphy et al., 1991) in cattle, and insulin may mediate the effects of acute changes in nutrient intake on follicular dynamics (Webb et al., 2004). Decreased concentrations of insulin in plasma after calving are associated with a negative energy balance and decreased fertility of dairy cows (Beam and Butler, 1999; Butler, 2000). Administration of insulin increases follicular growth by increasing the number of small follicles and reducing the number of atretic follicles in swine (Matamoros et al., 1991). Treatment of primiparous sows with insulin increases the percentage in estrus (Whitley et al., 2002). Infusion of insulin into beef

heifers increased the diameter of the dominant follicle (Simpson et al., 1994) and ovulation rate in energy-deprived beef heifers (Harrison and Randel, 1986). Follicular recruitment can be enhanced by insulin. Insulin concentrations are greater in heifers fed twice maintenance, with no carryover after the diet was changed, and the increase in number of small follicles was positively associated with circulating insulin (Gutierrez et al., 1997). Insulin concentrations can be affected by BCS at calving. Ciccioli et al. (2003) found that concentrations of insulin in plasma during 7 wk before the first estrus were greater for cows with a BCS 5 at calving compared with cows with BCS 4, however postpartum nutrition did not affect concentrations of insulin before estrus for cows with a greater nutrient intake.

The initiation of the first ovulation is delayed in dairy cows selected for high genetic merit for milk yield and is associated with reduced concentration of insulin in plasma (Butler, 2000). Gong et al. (2002) found that feeding a diet that increased plasma concentrations of insulin in dairy cows increased the proportion of cows that ovulated within 50 days of calving, reduced the interval from calving to first ovulation, and tended to reduce the intervals from calving to first service and to conception. Concentrations of insulin in follicular fluid of dairy cows fed a corn grain diet are 26 % greater than in their counterparts fed corn gluten meal (Landau et al., 2000). The content of insulin in follicles was significantly affected by follicular status; preovulatory follicles had greater insulin concentrations than subordinate follicles. Therefore, nutrient intake can effect intrafollicular insulin contents and might influence reproductive status of the animals.

Insulin may facilitate production of IGF-I by the liver (Keisler and Lucy, 1996). Increased insulin and a concominant decrease in growth hormone (GH) is an important relationship to consider when evaluating nutritional impacts on reproduction (Hawkins et al, 2000). The functional relationship between insulin and GH with respect to reproduction appears to be anabolic in nature (Hess et al., 2005).

Insulin-like growth factor –I

The somatotropic axis has been implicated as a mediator of metabolic status to the central nervous system (Keisler and Lucy, 1996). Insulin-like growth factor-I, is a mitogenic GH dependent serum peptide with structure and functions closely related to insulin and IGF-II. IGF-I increases granulosal cell proliferation and steroidogenesis in cattle, sheep and pigs (Spicer and Echternkamp, 1995). Insulin-like growth factor-I may act via autocrine, paracrine, and/or endocrine mechanisms (Armstrong and Benoit, 1996). The liver is the main source of systemic IGF-I and GH is the primary regulator of hepatic IGF-I gene expression and secretion (Etherton and Bauman, 1998). Expression of the IGF-I gene occurs in granulosal cells (Hernandez et al., 1989; Wang et al., 1997). Murphy et al. (1987) subjected the total RNA from rat ovaries to a liquid hybridization/RNAase protection assay to establish the ovary as a site of IGF-I production. Porcine ovarian follicles and corpora lutea also express mRNA for IGF-I (Gadsby et al., 1996). Cellular localization studies have established that granulosal cells are the major ovarian cell type that express the IGF-I mRNA

(Wathes et al., 1995; Leeunberg et al., 1996; Bao and Garverick, 1998; Ge et al., 2000). Treatment of ewes with recombinant GH significantly increased secretion of IGF-I by ovarian follicles *in vitro*, indicating that IGF-I gene expression in ovaries may be modulated by GH (Gong et al., 1996).

Insulin-like growth binding proteins (IGFBPs) constitute a heterogenous group of at least six distinct proteins capable of binding IGFs, with affinities in range of 10^{-10} to 10^{-9} M. Functions of the IGFBPs are to transport IGFs from the circulation to the peripheral tissues, to maintain a reservoir of IGFs in the circulation, to potentiate or to inhibit IGFs, and to maintain IGF-independent biological effect (Stewart et al., 1996). Concentrations of plasma or follicular IGFBPs change during folliculogenesis. Concentrations of IGFBP-3 are similar in dominant follicles when compared with healthy subordinate follicles (Nicholas et al., 2002), however, concentrations of IGFBP-2, -4 and -5 are significantly less in dominant follicles than in subordinate follicles (Monget et al., 1993; Cwyfan-Hughes et al., 1997; Armstrong et al., 1998; Mihm et al., 2000; Spicer et al., 2001). Changes in the steady-state concentration of IGFBPs in follicular fluid result from a combination of changes in gene expression (Armstrong et al., 1998) and proteolysis (Rivera et al., 2001; Spicer et al., 2001).

Insulin-like growth factor-I is associated with physiological processes such as onset of puberty (Jones, et al., 1991; Yelich et al., 1996), postpartum anestrus (Roberts et al., 1997; Beam and Butler, 1997, 1998; Stagg et al., 1998) and first postpartum estrus (Ciccioli et al., 2003). Concentrations of IGF in plasma have been associated with the onset of lactation (Taylor et al., 2004). Concentrations

of IGF-I in plasma during the prepuberal period were significantly related to IGF-I plasma during the start of the first lactation, and heifers that had lower IGF-I concentrations had delayed ovulation and altered reproductive function during the first lactation (Taylor et al., 2004). Decreased concentrations of IGF-I are associated with delayed puberty in cattle (Granger et al., 1989) and increased postpartum anestrous intervals (Rutter et al.; 1989; Nugent et al., 1993). Concentrations of IGF-I are reduced in nutritionally anestrous cows (Richards et al., 1991) and in short-term (48 h) fasted heifers (Spicer et al., 1992). Serum IGF-I in humans is reduced in patients with protein-calorie malnutrition (Soliman et al., 1986) and minimal serum IGF-I in chronically malnourished individuals can be normalized by nutritional rehabilitation (Thissen et al., 1994). Concentrations of IGF-I in adolescents with anorexia nervosa are reduced and the amount of weight deficit is negatively correlated with plasma IGF-I (Counts et al., 1992). Decreased concentrations of IGF-I in serum of fasted obese men are correlated with the decrease in excretion of urinary urea, suggesting that concentrations of IGF-I in serum may be an indicator of nitrogen loss (Clemmons et al., 1981).

Changes in systemic concentrations of IGF-I are associated with ovarian activity in dairy cows (Webb et al., 1999). Concentrations of IGF-I in plasma are positively correlated with body energy reserves and amount of feed intake (Rutter et al., 1989; Bishop et al., 1994; Vandehaar et al., 1995; Yelich et al., 1996; Bossis et al., 2000; Armstrong et al., 2001; Rausch et al., 2002). Concentrations of IGF-I were greater in heifers with greater nutrient intake during the 10 weeks before puberty (Yelich et al., 1995). Radcliff et al. (2004), found that greater

nutrient intake by Holstein heifers increased serum concentrations of IGF-I and decreased serum GH. Negative energy balance during early lactation (Spicer et al., 1990; Vicini et al., 1991; Sharma et el., 1994; Kobayashi et al., 1999; 2002), chronic (Richards et al., 1991, 1995; Bossis et al., 1999) or acute nutritional restriction (Armstrong et al., 1993; Armstrong et al., 2001; White et al., 2001; Kobayashi et al., 2002), and 48-h fasting (Spicer et al., 1992; Amstalden et al., 2000) reduce plasma concentrations of IGF-I in cattle. Decreases in plasma IGF-I were associated with acute nutrient restriction during the periparturient period in dairy cows (Kobayashi et al., 2002). Concentration of IGF-I 7 wk before the first estrus were greater in postpartum cows with greater nutrient intake (Ciccioli et al., 2003).

The majority of IGF-I in follicular fluid is derived from the systemic circulation in ruminants, (Leeuwenberg et al., 1996), therefore the availability of IGF-I to follicles is reduced when plasma concentrations are reduced (Schoppee et al., 1996). In consequence, this may result in a failure of the dominant follicle to ovulate in the early postpartum period (Beam and Butler, 1999). Most reports indicate that follicular concentrations of IGF-I are not influenced by nutrient intake (Spicer et al., 1991; 1992). However, if nutritionally induced anestrous cows are infused with 2 μg of GnRH every hour, concentrations of IGF-I in follicular fluid increase (Hamilton et al., 1999) and cows resumed ovarian activity (Vizcarra et al., 1997).

Overall, undernutrition increases GH secretion in cattle (Armstrong et al., 1993; Bossis et al., 199) and concentrations of IGF-I in plasma and hepatic IGF-I

mRNA are decreased (VanderHaar et al., 1995). This is probably due to an insulin-dependent down-regulation of the GH receptor (Thissen et al., 1994; Kobayashi et al., 1999; Butler et al., 2003).

The biovailability of IGF-I in plasma and its clearance from serum is controlled by IGFBP (Thissen et al., 1994). Peripheral concentrations of IGFBPs are regulated by feed intake in cattle, and IGFBP-3 in plasma is positively correlated with dietary intake (Rausch et al., 2002) and increased growth rate (Vestergaard et al., 1995). Insulin-like growth factor binding protein-2, is associated with inadequate nutritional status (Armstrong and Benoit, 1996). In dairy cows, 2 d of feed restriction increased IGFBP-2, and IGFBP-3 was not altered (McGuire et al., 1995). Restricting heifers to 54% of maintenance for 84 d increased plasma IGFBP-2 by 79%, but plasma IGFBP-3 was not altered (Vanderharr et al., 1995). Roberts et al. (1997) found that concentrations of IGFBP-2 in serum of beef cows at 2 wk post partum was diminished, and concentrations of IGFBP-3 increased in cows that resumed estrus by 20 wk post partum compared with anestrous cows. However, ewes fed a lower plane of nutrition had greater amounts of IGFBP-2, and ewes in thin body condition (≤ 3) had decreased plasma concentrations of IGFBP-3 and -4 compared with ewes in good (>3) body condition (Snyder et al., 1999). Increased dietary energy decreases the steady-state concentration of mRNA encoding IGFBP-2 and -4 in small antral follicles, which in turn increases the bioavailability of locally produced IGF-II and systemically derived IGF-I in follicles (Webb et al., 2003; Armstrong et al., 2003). Expression of mRNA for IGFBPs occurs in the ovary (Bao and

Garverich, 1998) and IGFBP-2, -3, -4 and -5 have been detected in the ovarian follicular fluid of beef cows (Funston et al., 1996). Fasting increases mRNA for IGFBP-1 and -2 in the liver of rats (Tseng et al., 1992) and also increases peripheral concentrations of IGFBP-1 and -2 (Orlowski et al., 1990; Murphy et al., 1991). Fasting increased plasma concentrations of IGFBP-I (Busby et al., 1988; Baxter et al., 1993) and IGFBP-2 (Clemmons et al., 1991; Smith et al., 1995).

Other metabolic signals that regulate reproduction

Leptin

Leptin is derived from the Greek term ‘leptos’ which means thin (Zeiba et al., 2005). In 1994, the gene for the protein was cloned and sequenced from both mice and humans. Leptin is a 16- kDa protein produced and secreted from adipocytes (Zhang et al., 1994). Leptin has a central role in the regulation of body energy homeostasis (appetite, energy expenditure, nutrient partitioning between tissues and body composition), cell differentiation and proliferation (Kershaw et al., 2004), regulation of metabolism (Baile et al., 2000), reproduction (Chehab et al., 1996), immune and renal functions (Cioffi et al., 1996), angiogenesis (Sierra-Honigmann et al., 1998), blood pressure control (Frühbeck, 1999), and bone formation (Ducy et al., 2000).

Concentrations of leptin and expression of adipocyte ob mRNA are strongly correlated with estimates of obesity, total fat mass, percent body fat, and body mass index (Ahima and Flier, 2000). Leptin gene expression has been detected in adipose tissue (Chilliard et al., 2001), pituitary glands (Yonekura et al., 2003), mammary glands (Bonnet et al., 2002), fetal tissues (Muhlhausler et al., 2003), rumen, abomasum and/or duodenum (Yonekura et al., 2002), and muscle (Wang et al., 1998). In ruminants, leptin may be involved in stress responses, as it modulates the hypothalamic-pituitary-adrenal axis (Heiman et al., 1997), and leptin receptors have been identified in the adrenal medulla and cortex (Cao et al., 1997).

Frisch (1980) suggested that the amount of body fat could in some way trigger initiation of reproductive function in female rats and humans. Leptin regulates reproductive function (Cunningham et al., 1999; Keisler et al., 1999) and signals the adequacy of energy stores for reproduction by interacting with different target organs in the hypothalamic-pituitary-gonadal axis (Frühbeck et al., 1998).

Leptin receptors are localized in several reproductive tissues (reviewed by Spicer et al., 2001), including testis of mice (El-Hefnawy et al., 2000), ovine anterior pituitary and hypothalamic regions (Dyer et al., 1997), and the neuroendocrine reproductive axis in monkeys (Finn et al., 1998). Leptin treatment accelerated onset of puberty and behavioral estrus in lean mice (Chehab et al., 1997) and prevents the delay in puberty induced by food restriction (Cheung et al., 1997). Leptin inhibited weight gain in fed rats but prevented the delay in puberty that occurs with nutrient restriction (Gruaz et al., 1998). Serum concentrations of leptin and IGF-I, and gene expression for leptin, increased as heifers approached puberty (Garcia et al., 2002). Serum concentrations of leptin change with stage of the menstrual cycle in women (Teirmaa et al., 1998); concentrations are greater in mid-luteal plasma compared with during the follicular phase (Hardie et al., 1997), and concentrations of leptin decrease after menopause (Rosenbaum et al., 1996).

Leptin receptors have been found on neuropeptide Y (NPY) neurons in the hypothalamus (Finn et al., 1998). Neuropeptide Y is a 36-amino acid neuropeptide that is involved in food intake and neuroendocrine control

(Houseknecht and Portocarrero, 1998). NPY is a potent stimulator of feed intake and inhibitor of gonadotropin secretion (McShane et al., 1992; Kalra and Kalra, 1996). Leptin receptors have been found on NPY neurons in the hypothalamus (Finn et al., 1998). Leptin has been proposed as a metabolic signal to the central nervous system that control pulsatile LH release (Barash et al., 1996), and intraventricular administration of leptin decreases NPY and can restore LH secretion (Shwartz et al., 1996; Ahima et al., 1999).

Plasma concentrations of leptin are positively related with nutrient intake in mature gestating cows (Lents et al., 2005) and increased postpartum nutrient intake increased BCS and concentrations of leptin in lactating beef cows (Ciccioli et al., 2003).

Nonesterified Fatty Acids

Degree of negative energy balance is positively correlated with non-esterified fatty acids (NEFA) in plasma of dairy cows (Canfield and Butler, 1990; Staples et al., 1990) and beef cows after calving (Richards et al., 1989b) and in beef heifers (Bossis et al., 1999). Plasma NEFA were similar during the first 2 to 3 weeks post partum in dairy cows with ovulatory or anovulatory first-wave dominant follicles (Beam and Butler, 1997, 1998). Nonesterified fatty acids are indicators of energy status in pregnant beef cows (Russel and Wright, 1983). Plasma nonesterified fatty acids were greater in cows with greater fat deposition during the last 4 wk of pregnancy (Guedon et al., 1999). Concentrations of

NEFA during 7 wk before estrus were greater in cows with greater BCS comarted with thinner cows (Ciccioli et al., 2003).

Glucose

Concentrations of glucose in plasma of dairy cows during the first 3 to 4 weeks postpartum (Beam and Butler, 1997, 1998) are usually minimal compared with later weeks of lactation. The ovary uses glucose as a source of energy (Rabiee et al., 1999). Inadequate concentrations of glucose in plasma due to feed restriction or fasting is associated with decreased LH pulsatility in sheep (Clarke et al., 1980), monkeys (Chen et al., 1992) and cows (Yelich et al., 1996). Minimal LH pulse frequency during negative energy balance may result from inhibition of the hypothalamic GnRH pulse generator by inadequate energy. Plasma concentrations of glucose were positively correlated with frequency of LH pulses in prepuberal heifers fed two different levels of nutrition (Yelich et al., 1996). Glucose may also have a role during the breeding period as greater glucose concentrations before insemination are associated with a greater conception rate (Forshell et al., 1991; Pehrson et al., 1992). Nutritional restriction in beef cows and loss of weight and BCS are associated with reduced concentrations of glucose in plasma (Richards et al., 1989a; Rutter and Mann, 1991; Grimard et al., 1995). Concentrations of glucose in plasma are reduced during restriction of nutrient intake prior to cessation of ovulation (Richards et al., 1989b; Bossis et al., 1999). Vizcarra et al. (1996) found that cows with a BCS of

6 at calving had greater concentrations of glucose during the subsequent breeding season than cows that calved with a BCS of 4 or 5.

Summary

An understanding of the endocrine mechanisms that control postpartum anestrus is essential to decrease the interval from calving to conception. It is well established that nutrition has a profound influence on reproductive performance of domestic ruminants with special emphasis on the period from calving to the first postpartum estrus. Many factors influence the length of postpartum anestrus; suckling and nutrition are major factors while minor factors are presence of bull, breed and age at calving. The effect of nutrition on postpartum reproduction is dependant on whether the nutritional deficiencies occur before or after calving and thus nutritional management during gestation and after calving are of major concern.

The functions of metabolic hormones during the reestablishment of ovarian activity such as the insulin-like growth factor system, insulin, nonsterified fatty acids and leptin have been studied. Inadequate nutrient intake affects the growth of the dominant follicle in cattle. Insulin may as well mediate the effects of acute changes in nutrient intake on follicular dynamics. Concentrations of steroids should reflect steroidogenic capacity in cows with different nutrient intake. Thus, metabolic hormones may exert a direct effect on the ovary and could mediate the effects of nutrient intake on reproductive function (Wettemann and Bossis, 2000).

The first postpartum estrus in beef cows is usually preceded by a transient increase in plasma progesterone and is followed by a normal luteal phase. The

ability of the dominant follicles to produce estradiol is limited during the postpartum anovulatory period.

Therefore, the objectives of this research are: 1) to determine the effect of post partum nutrition on: concentrations of insulin, IGF-I, progesterone, androstenedione, estradiol, IGF-I binding proteins (IGFBP) in follicular fluid (FF) of dominant follicles (DF) and abundance of mRNA for IGFBP -4, -5, aromatase and pregnancy-associated plasma protein-A in granulosal cells of DF, and 2) to determine if treatment with GnRH or estradiol influences the onset of first estrus and luteal activity of postpartum anestrous beef cows.

CHAPTER III

Influence of postpartum nutrition of primiparous beef cows on insulin-like growth factor-I , insulin and insulin-like growth factor binding proteins in plasma and follicular fluid, and mRNA for aromatase, insulin-like growth factor binding proteins -4 and -5 and pregnancy-associated plasma protein-A

ABSTRACT

Effects of nutrition on insulin-like growth factor-I (IGF-I) and insulin in plasma and dominant follicles (DF) were evaluated at 72 ± 2d and at 56 ± 9 d (experiment 1 and experiment 2 respectively) after calving in anovulatory primiparous Angus x Hereford cows (Exp 1 n= 12; Exp 2 n= 28). Body condition score (BCS = 1 emaciated, 9= obese) at calving was 4.5 ± 0.1 in experiment 1 and 4.8 ± 0.2 in experiment 2. Cows were stratified based on BCS at calving and randomly assigned to one of two postpartum nutritional treatments: maintain (M), 2.27 kg of a 40% CP supplement per day and ad libitum hay; or gain (G), ad libitum access to a 50 % concentrate diet and hay. Estrus was monitored with electronic mount detectors (HeatWatch) and blood samples were collected twice a week starting at 30 d postpartum. Ovarian follicles were evaluated daily by ultrasonography commencing at 42 d (Exp. 1) or 30 d (Exp. 2) after calving. Body condition score at aspiration of the DF was greater for H (5.1 ±0.3 and 4.8 ± 0.2) than M (4.5 ±

0.1 and 4.3 ± 0.3 in Exp.1 and 2, respectively) cows and postpartum interval to estrus with luteal activity was longer for M cows (132 ± 2 and 95 ± 24) than for H (109.7 ± 15.2 and 80 ± 11d, in Exp. 1 and 2, respectively). Maximum size of DF was influenced by nutritional treatment in Exp. 1 (12.2 ± 0.4 and 11.1 ± 0.7 mm; G and H cows, respectively) but it was not influenced by nutritional treatment (13.2 ± 1.6 mm) in Exp. 2. Postpartum interval to luteal activity increased in cows with lower body condition score at calving. Concentrations of IGF-I in FF were greater for H (34.4 ± 7.0 and 34.0 ± 10.7 ng/ml) than M (24.0 ± 3.7 and 23.6 ± 8.5 ng/ml, for Exp. 1 and 2, respectively) cows and plasma concentrations of IGF-I prior to aspiration were also greater in G (36.6 ± 3.5 and 33.6 ± 11.7 and ng/ml) than in M (24.7 ± 4.6 and 18.6 ± 8.2 ng/ml, for Exp. 1 and 2) cows. Concentrations of insulin in FF and plasma were greater for G than M cows in Exp. 1 and Exp 2. In Exp. 2, concentrations of IGFBP-4 and -5 in plasma were 30% greater ($P < 0.01$) in G than M cows. Concentrations of IGFBP-4 and -5 in FF were 68 and 48%, respectively, greater ($P < 0.05$) for G than M cows. Concentration of IGFBP-2 and -5 in plasma at follicular aspiration were positively correlated with follicle size ($P < 0.05$). BCS at calving was positively correlated with IGFBP-2, -4 and -5 in plasma at aspiration of follicles. Concentration of IGF-I in plasma at aspiration and in FF was positively correlated with IGFBP-3 and -4 in FF. Abundance of mRNA for aromatase, IGFBP-4 and -5, and for pregnancy-associated plasma protein-A were not affected by treatment. These results indicate that concentrations of IGF-I and insulin in FF are influenced by nutritional intake and may be related to follicular function. Changes in follicular fluid IGFBP

concentrations, rather than local translational regulation, may have a role in dietary induced changes in postpartum follicular growth.

Key Words: Follicle, IGF-I, Ovary, Postpartum Beef Cows

INTRODUCTION

Nutrient intake and body energy reserves are major regulators of ovarian function in beef cows (Richards et al., 1989; Wettemann and Bossis, 2000). Prolonged restriction of dietary energy intake by cows results in loss of body weight and body condition, and cessation of estrous cycles (Richards et al., 1989). Body condition score (BCS) is an indicator of the nutritional status of cow and increased BCS is required for the resumption of estrous cycles in nutritionally induced anovulatory heifers (Bossis et al., 2000). Cows calving with thin BCS (≤ 4) have longer intervals to first estrus (Spitzer et al., 1995; Lents et al., 2000) compared with cows with a BCS ≥ 5. The interval from calving to first estrus is longer for heifers fed a low energy diet after calving compared with heifers fed a high energy diet (Spitzer et al., 1995; Ciccioli et al., 2003). Metabolic hormones may exert a direct effect on the ovary and could mediate the effects of nutrient Intake on reproductive function (Keisler and Lucy, 1996; Wettemann and Bossis, 2000). Feed restriction increases concentrations of GH, and greater nutrient intake increases plasma concentrations of insulin-like growth factor-I (IGF-I), insulin and leptin in cows (Ciccioli et al., 2003; Lents et al., 2005). Amount of insulin-like growth factor-I binding proteins in plasma may be related to the postpartum anovulatory period in beef cows (Roberts et al., 1997; White, 2004).

The objectives of this study were to evaluate the effects of nutrient intake of primiparous cows after calving on: 1) concentrations of insulin, IGF-I, progesterone, androstenedione, estradiol, IGF-I binding proteins (IGFBP) in

follicular fluid (FF) of dominant follicles (DF) and 2) abundance of mRNA for IGFBP-4 , -5, aromatase and pregnancy-associated plasma protein-A in granulosal cells of DF, and 3) relationship of IGF-I and insulin in plasma and follicular fluid.

MATERIALS AND METHODS

Animals and Experimental Protocol

The Institutional Animal Care and Use Committee of Oklahoma State University approved all animal-related procedures used in this study.

Angus x Hereford primiparous cows, were maintained on dormant native grass pasture during the last third of gestation and were supplemented with 1.6 kg/d (as-fed basis) of a 38 % CP soybean meal-based supplement (1.9 cm pellet) to maintain BW so they would calve with a (BCS; 1= emaciated, 9= obese; Wagner et al., 1988) of 4 or 5. Body weight and BCS were determined after cows were denied access to feed and water for 16 h each month, from 60 d before to 150 d after parturition. The BCS at calving was the last BCS recorded prior to calving and the first weight recorded after calving was used to determined BW changes during treatments. Two experiments were conducted and the only difference between experiments 1 and 2 was the days after calving that ovarian function was evaluated. In experiment 1, DF were aspirated an average of 70 ± 2 d after calving, and in experiment 2, aspiration was on 56 ± 9 d after calving. Cows in experiments 1 and 2 calved in February and March of two successive years (n = 12 and n = 28 respectively).

At calving, cows were stratified by calving date and BCS and randomly assigned to nutritional treatments. Cows were fed to maintain (M) body weight or to gain (G) 0.5 kg/d. Maintain cows were supplemented with 2.27 kg/d (as-fed basis) of a 38 % CP supplement and G cows had free access to a high-energy feed (1.61 Mcal NE_m/kg DM, 0.90 Mcal NE_g/kg DM, and 11.1% CP). The ration was composed (% DM) of rolled corn (39.7%), ground alfalfa pellets (35.5%), cottonseed hulls (22%), cane molasses (2.5%) and salt (0.3%). After (65 days) nutritional treatments, all cows were maintained in the same pasture and fed the M diet until the first postpartum, estrus.

Ovarian Function and Estrous Behavior

Experiment 1. Size of the ovarian follicles was evaluated daily by transrectal ultrasonography (7.5 MHz probe; Aloka 500V, Corometrics Medical Systems, Wallingford, CT) commencing at 42 d post partum. Ultrasonography images were recorded with a VHD recorder (Panasonic PV-V4520; Matusushita Electric Corp. of America, Secuucus, NJ) and viewed at a later time to confirm the size of DF. Size of follicles was calculated as the mean of the longest and shortest diameters (Pierson and Ginther, 1988). At 70 ± 2 d after calving, when growth of DF plateaued (< 0.8 mm increase in diameter in 24 h), follicular fluid (FF) was obtained by transvaginal ultrasound-guided follicular aspiration. Briefly, epidural anesthesia was induced with 5 mL of 2% lidocaine, then the ovary was hold against the vaginal wall, and a vaginal 5 –MHz probe; Aloka 500V, Corometrics Medical Systems, Wallingford, CT) was used to guide an 18 G, 55

cm needle (Cook Veterinary Products, Spencer, IN) to puncture the follicle and aspirate the FF to a 3 mL syringe. Follicular fluid was placed in 5 mL cryogenic polypropylene conical vials on ice for 10 min and then centrifuged at 2000 x g for 7 min to separate fluid and granulosa cells. Follicular fluid and granulosa cells and stored in 5 mL cryogenic polypropylene conical vials. TRIzol (500 µL; Invitrogen Corp., Carlsbad CA) was added to vials containing granulosa cells. Follicular fluid and granulosa cells were immediately frozen in liquid nitrogen, and FF was stored at -20 ^{o}C and granulosa cells were stored at -80 ^{o}C until analyzed.

Estrous behavior was monitored using a radiotelemetric pressure sensitive device (HeatWatch, DDx Inc., Denver, CO) attached to the rump of cows at 30 d post partum. Onset of estrus was defined as the first of two mounts received within 4 h. The end of estrus was defined as the last mount received with a mount received 4 h before and without receiving a mount during the next 12 h.

Concentrations of progesterone were used to determine luteal activity. Onset of luteal activity was determined when plasma samples had ≥ 0.5 ng/mL of progesterone in 2 consecutive samples after first behavioral estrus.

Experiment 2. Methods used to evaluate ovarian function and estrous behavior described for experiment 1 were used for experiment 2 with the exception that transvaginal ultrasonography was started at 30 d after calving and DF were aspirated at 56 ± 9 d after calving.

Blood Sampling

Blood samples were obtained on Monday and Thursday of each week from 4 wk after calving to 3 wk after first estrus. Cows had access to feed and water prior to sampling. Caudal vein blood was collected in vacutainers (10 mL) containing EDTA (0.1 ml of a 15% solution). Tubes were immediately placed on ice, centrifuged (2500 x g for 15 min) at 4 ^{o}C within 3 h after collection, and plasma was recovered and stored at -20 ^{o}C until hormones and IGFBP were quantified.

Radioimmunoassays (RIAs)

Concentrations of insulin, IGF-I and progesterone, were quantified in plasma samples. Insulin, IGF-I, androstenedione, progesterone and estrogen were quantified in FF.

Concentrations of insulin in plasma and FF were quantified with a solid phase RIA for human insulin (Coat-A-Count Insulin kit, Diagnostic Products Corp., Los Angeles, CA; Bossis et al., 1999) using bovine pancreatic insulin as the standard (Sigma Chemical Co., St. Louis, MO) and 0.2 mL sample volume. The intraassay CV was 6.7 %. Concentrations of IGF-I in plasma and FF were quantified by RIA (Echternkamp et al., 1990). The intraassay CV was 7.2 % after acid-ethanol extraction (16 h at 4 ^{o}C). Plasma and FF concentrations of progesterone were quantified with a solid phase RIA (Coat-A-Count Progesterone kit, Diagnostic Products Corp.; Vizcarra et al., 1997). The intraassay CV was 5.8% for plasma and 7.2 % for FF. Concentrations of estradiol 17-β in FF were quantified by RIA according to the method of Spicer and Enright (1991); the intraassay CV was 10.4 %. Concentrations of androstenedione were quantified according to the method of Stewart et al. (1996); the interassay CV was 11.0 %.

Ligand Blotting

Relative amounts of IGFBP in FF were assessed by one-dimensional SDS-PAGE as previously described (Stewart et al., 1996; Spicer et al., 2001). To summarized, 4 µl of FF was mixed with 21 µl of Laemmli sample buffer (Bio-Rad, Hercules, CA) and heat-denature (3 min at 100 ^{o}C). Samples were separated on 12% polyacrylamide gel 8 h at constant current (27 amperes) and varying voltage (36 volts for 8 h and 82 volts for 2 h). Following separation, proteins were transferred to nitrocellulose paper (Midwest Scientific, St. Louis, MO) for 2.5 h, and ligand-blotted for 12 h with ^{125}I-IGF-I and ^{125}I-IGF-II (1:1) at 4 ^{o}C. Gels were washed and exposed to X-ray film for 48 h at -80 ^{o}C. Intensity of protein bands was determined using scanning densitometry (Molecular Analyst, Bio-Rad) and values are expressed as arbitraty densitometric units (ADU/4 µl).

mRNA Analyses

Lysed granulosa cells were transferred to 1.5 mL eppendorf tubes and 0.1 mL of chloroform (Sigma Chemical Co., St. Louis MO) was added, then each sample was vortexed for 15 sec. Following a 3 min incubation at 22 ^{o}C, samples were centrifuged (3,500 x g) for 30 min at 4 ^{o}C. The upper aqueous phase was transferred to a new eppendorf tube and RNA was precipitated by addition of 0.25 mL of isopropyl alcohol (Pierce Chemical Company, Rockford, IL). Samples were gently mixed and then incubated at 22 ^{o}C for 10 min, followed by centrifugation at 3,500 x g for 10 min at 4 ^{o}C. The supernatant was removed, the RNA pellet was washed with 0.5 mL of 75 % ethanol, and the sample was

centrifuged at 3,500 g and 4°C as before for 5 min. The ethanol supernatant was removed and the RNA pellet was dried for 5 min at 22° C. The RNA was then dissolved in 0.03 mL of buffer (10 mM Tris-Cl, 1 mM EDTA; pH 7.4). Total RNA was quantified using the Nanodrop® ND-1000 Spectophotometer (NanoDrop Technologies, Inc., Montchanin, DE, USA) to determine the concentration of the total RNA extracted and to determine the amount of protein contamination. Optical density at 260 nm was used to quantify concentrations of RNAs. The 260/280 nm ratio was calculated to measure the amount of protein contamination which was close to two for all the samples. Samples were aliquoted and stored at -80 °C until analyzed for mRNA for aromatase, IGFBP-4, -5 and PAPP-A.

Quantitative RT-PCR.

Primer Express ™ software (Foster City, CA) was used to make primers and probes for quantitative RT-PCR as described by Voge et al. (2004). GenBank accession numbers that were used for PCR analysis of aromatase, IGFBP-4, IGFBP-5, and PAPP-A are in Table 1. High resolution electrophoresis was used to document that transcripts produced were of the molecular size predicted (Santiago et al., 2005).

Fluorescent real-time quantitative RT-PCR was used to determine mRNA expression for aromatase, IGFBP-4 and -5, and PAPPA-A in bovine granulosa cells. Expression was quantitated using a one-step RT-PCR reaction following the manufacture's specifications with modifications for Taqman® Gold RT-PCR

kit (P/N N808-0233; PE Biosystems, Foster City, CA) as described by Santiago et al. (2005).

Quantification of gene expression for aromatase, IGFBP-4 and -5 and PAPP-A mRNA expression was accomplished using the comparative threshold cycle (Ct) method (Hettinger et al., 2001; Ross et al., 2003 and Santiago et al., 2005).

Statistical Analyses

Data were analyzed as a completely randomized design with a 2 x 2 factorial treatment structure, using PROC MIXED (SAS Inst., Inc., Cary, NC). The model included the effect of BCS at calving and treatment as main effects, and the first order interaction. Pearson correlations were calculated to determine relationships among variables (PROC CORR, SAS).

RESULTS

EXPERIMENT 1

Prepartum BW and early postpartum BW were similar for G and M cows, however, at aspiration (72 ± 2 d post partum) G cows weighed 30 kg more that M cows (Figure 1; $P < 0.05$).

Body condition score was similar for G and M cows at calving, however, BCS at aspiration of the DF (72 d) was greater for G (5.1 ± 0.4) than M (4.4 ± 0.2) cows (Figure 2; $P < 0.003$). BCS was positively correlated with follicle size and IGF in FF ($r = 0.75$; $P < 0.01$ and ($r = 0.60$; $P < 0.04$; respectively (Table 4).

Days at aspiration of the DF were similar for both treatments (G, 72.8 ± 2.0 d; M, 71.3 ± 2.0 d). Maximum size of DF was greater ($P < 0.007$) for G (12.2 ± 0.4 d) than M (11.1 ± 0.7 d) cows (Figure 3). Interval after calving to luteal activity was longer for M cows (132.2 ± 12.8 d) compared with G (109.7 ± 15.2 d). Postpartum interval to luteal activity was negatively correlated ($r= - 0.58$; $P < 0.05$: Table 4) with BCS at aspiration.

Plasma concentrations of IGF-I prior to aspiration were greater in G (36.6 ± 3.5 ng/ml) than in M (24.7 ± 4.6 ng/ml) cows ($P < 0.01$; Figure 4). Concentrations of IGF-I in FF were also greater for G (35.1 ± 8.2 ng/ml) compared with M (23.9 ± 3.4 ng/ml) cows ($P < 0.01$; Figure 5). IFG-I before aspiration was positively correlated ($r= 0.68$; $P < 0.01$; Table 6) with insulin FF.

Concentrations of insulin in FF were greater ($P < 0.008$) for G (1.28 ± 0.27) than M (0.85 ± 0.16 ng/ml) cows, and G cows had greater ($P < 0.01$) insulin in plasma (1.40 ± 0.43) than M (0.80 ± 0.26 ng/ml) (Figure 6).

Concentrations of IGFBP-2, -3 and -5 in follicular fluid were not influenced by treatment. Concentration of IGFBP-4 and -5 in follicular fluid were greater in G than M cows ($P < 0.03$; Figure 7). IGFBP-3 was positively correlated with IGFBP-5 and IGFBP-4 ($r= 0.85$; $P < 0.001$ and $r= 0.79$; $P < 0.002$, respectively; Table 5).

Concentrations of progesterone in FF were greater ($P < 0.05$) for G (56.83 ± 4.56 ng/mL) compared with M (49.74 ± 5.29 ng/mL) cows. Androstenedione and estradiol in FF were not influenced ($P > 0.10$) by treatment (Table 2).

Abundance of mRNA for aromatase, IGFBP-4 and -5, and pregnancy-associated plasma protein-A were not affected by treatment (Table 7).

EXPERIMENT 2

Body weights 2 wk before calving and 2 wk after calving were similar for G and M cows, however, at aspiration (56 ± 9 d post partum) G cows weighed more that M cows (Figure 8; $P < 0.05$). Body condition score of H and M cows were similar at calving, however, BCS at aspiration of the DF was greater for H (4.8 ± 0.2) than M (4.3 ± 0.3) cows (Figure 9; $P < 0.01$).

Days after calving at aspiration of the DF were similar for G (56.6 ± 9.2 d) and M, (55.3 ± 8.5 d) cows. Maximum size of DF was not influenced by nutritional treatment (13.2 ± 1.6 mm; $P = 0.13$). Postpartum interval to luteal activity was longer for M cows (95 ± 24) than for H (80 ± 11d; $P < 0.05$). Interval from calving to luteal activity was negatively correlated ($r = -0.47$; $P < 0.01$) with BCS at aspiration of DF.

Concentrations of IGF-I in plasma 1 wk prior to aspiration of DF and at aspiration were greater ($P < 0.01$; Figure 10) in H compared with M cows (33.6 ± 11.7 ng/mL vs 18.6 ± 8.2 ng/mL). Concentrations of IGF-I in FF were also greater ($P < 0.01$; Figure 11) for H than M cows (34.0 ± 10.7 ng/mL vs 23.6 ± 8.5 ng/mL). Concentration of IGF-I in plasma and follicular fluid of G cows were correlated ($r= 0.62$; $P = 0.02$) but in M cows the correlation was not significant ($r = 0.26$; $P = 0.35$; Figure 12). BCS at aspiration was positively correlated with IGF-I in FF ($r = 0.46$, $P <0.01$; Table 8), and IGF-I in plasma was positively correlated with FF IGF-I ($r = 0.61$; $P <0.01$) (Table 8).

Similarly to concentrations of IGF-I, insulin in FF was greater ($P < 0.05$) for G (1.59 ± 0.22 ng/mL compared with M cows (0.97 ± 0.17 ng/mL) and H cows

had greater (P < 0.01) insulin in plasma (1.61 ± 0.17 ng/mL) than M (0.97 ± 0.17 ng/mL). Concentration of insulin in plasma and follicular fluid of G (r= 0.68; P = 0.0004; Figure 13) and M cows (r = 0.66; P = 0.0003; Figure 13) were correlated.

Concentrations of IGFBP-4 and -5 in plasma were 30% greater in G compared with M cows (P < 0.01; Figure 14). Concentrations of IGFBP-4 and -5 in FF were 68 and 48%, respectively, greater (P <0.05; Figure 15) for G compared with M cows. Concentrations of IGFBP-2 and -3 in plasma and FF were not influenced by treatment. FF IGFBP- 3 was correlated positively with IGFBP-2, IGFBP-5 and IGFBP- 4 (r= 0.40; P < 0.04; r= 0.41; P < 0.03 and r= 0.42; P < 0.03, respectively; Table 9). Concentration of IGFBP-2 and -5 in plasma at follicular aspiration were positively correlated with follicle size (P < 0.05). BCS at calving was positively correlated with IGFBP-2, and -3 (r= 0.58; P < 0.001 and (r= 0.60; P < 0.001; Table 10). Concentrations of IGF-I in plasma at aspiration were positively correlated with IGFBP-3 (r= 0.01; P < 0.01; Table 10).

Concentrations of progesterone, androstenedione, and estradiol in FF were not influenced (P > 0.10) by treatment (Figures 16, 17, 18).

Abundance of mRNA for aromatase IGFBP-4 and -5, aromatase, and pregnancy-associated plasma protein-A were not affected by treatment (Table 7).

Discussion

Experiments 1 and 2 were conducted under similar conditions in consecutive years. The main difference between the experiments was that follicles were aspirated on d 72 after calving in Exp. 1, and on d 56 in Exp. 2. The two experiments allowed evaluation of the effect of duration of nutritional treatments and days after calving on factors contributing follicular growth.

Reduced nutrient intake is associated with loss of body weight and BCS, decreased luteal activity, and cessation of estrous cycles (Richard et al., 1989; Bishop and Wettemann, 1993; Vizcarra et al., 1997). In the current experiments, weights prepartum and early after calving were similar for G and M cows, however, at aspiration in both experiments, G cows weighed more and had greater BCS compared with M cows ($P < 0.005$).

Increased postpartum nutrient intake induced fat deposition in G cows in both experiments. High-energy diets after calving increase fat deposition in mature cows (Perry et al., 1991; Stagg et al., 1995), primiparous cows (Ciccioli et al., 2003) and in growing heifers (Yelich et al., 1995). Increased BCS is required for the resumption of estrous cycles in nutritionally induced ovulatory heifers (Bossis et al., 2000) and cows (Richards et al., 1989). Primiparous cows on greater energy intake, compared with moderate energy intake after calving, partitioned a greater proportion of net energy to grow maternal tissue (Lalman et al., 2000).

Increased nutrient intake influenced some reproductive characteristics and metabolic hormones. Maximum size of DF was influenced by nutritional treatment in Exp. 1, but not in Exp. 2. Other studies (Armstrong et al., 2001; Murphy et al., 1991; Rutter and Manns, 1991; Lucy et al., 1992; Rhodes et al., 1995), also found that nutrient restriction decreased maximum size of DF. Cows fed rations supplying 100 % of energy requirements had more large follicles than cows fed low energy diets, and size of the largest follicle was greater in cows that received 100 % of energy requirement compared with cows that were fed 70 % of energy requirements (Grimard et al., 1995). Maximum size of bovine preovulatory dominant follicles was decreased in energy restricted cows at the first postpartum estrus (Ciccioli et al., 2003) and Hereford x Friesian heifers fed to gain for 10 wk had larger preovulatory DF compared with heifers that maintained or lost BW (Spicer et al., 1991). In Exp. 2, DF were aspirated at 56 d after calving, about 50 d before the expected first ovulation, and follicles were aspirated when growth plateaued. In contrast Ciccioli et al., 2003 measured preovulatory follicles.

Aspiration of DF at 56 d after calving in the second experiment could account for the lack of effect of nutritional treatment on follicle size. Similar to our results, increased energy, fat intake, or BCS did not alter size of bovine DF during postpartum anovulation or at the first postpartum ovulation (Stagg et al., 1995; Beam and Butler, 1998; White, 2004). Spicer et al. (1986) found that size of the DF did not differ between 7 and 56 d postpartum in suckled beef cows. Ovarian follicular development resumes 1 to 2 wk after calving in

beef cows (Murphy et al., 1990), but the interval to the first ovulation, is prolonged due to the failure of successive DF to ovulate (Stagg et al., 1995).

Postpartum interval to luteal activity was longer for M cows than for G in both experiments, and was negatively correlated with BCS at aspiration. Increased postpartum feed intake decreased the interval from calving to first estrus of primiparous cows (Ciccioli et al., 2003), and increased postpartum energy intake increased the number of cows in estrus during the breeding season (Spitzer et al., 1995). Nutritional management prepartum can also affect onset of ovarian activity in beef cows. Reduced energy intake prepartum delays the onset of estrus (Wiltbank et al., 1962; Dunn et al., 1969), and BCS at calving influenced pregnancy rates and postpartum interval to estrus in cows (Richards et al., 1986; Selk et al., 1988). In other studies (Wright et al., 1987; Whittier et al., 1988; Stagg et al., 1998), greater postpartum nutrient intake had no effect on the duration of the postpartum anovulatory interval. These discrepancies in the effect of nutrition on length of postpartum anestrus may be related to the many factors that influence reproduction in addition to nutrient intake, such as parity, breed, lactation, environment and endocrine status (Dunn and Kaltenbach, 1980).

Concentrations of IGF-I in plasma prior to aspiration of DF were greater in G than M cows in both experiments. Similarly, concentrations of IGF-I in plasma were directly related to nutrient intake in primiparous cows (Lalman et al., 2000; Ciccioli et al., 2003) and heifers (Yelich et al., 1996). Ciccioli et al. (2003) found that concentrations of IGF-I, and insulin did not

change during the 7 wk before first estrus in primiparous postpartum cows that were previously fed a high or moderate nutrient intake. Thus nutritional status (energy and protein intake relative to requirements) partially controls the synthesis and secretion of IGF-I (Thissen et al., 1994). Differences of the effect on nutrient intake on plasma IGF-I between studies could be due to the interval after calving at which nutrient intake was restricted.

Concentrations of IGF-I in FF were greater for G compared with M cows, and there was a positive relationship between concentration of IGF-I in plasma and IGF-I in FF in both experiments. Follicular dominance is associated with greater IGF-I concentrations in the FF of cattle (Webb et al., 1999). In the first experiment, concentrations of IGF-I in plasma and FF were positively correlated within M and G cows, however in the second experiment, the relationship was significant only in G cows. Rutter and Manns (1991) found concentrations of IGF-I in FF were not influenced by follicle size, dietary treatment, or day post partum at ovariectomy. Concentrations of IGF-I in FF were positively correlated with BCS. In these experiments, cows with greater BCS had greater energy and protein intake, so it cannot be determined if greater concentration of IGF-I in FF are stimulated by body fat reserves or nutrient intake.

Reduced nutrient intake uncouples the GH:IGF-I axis (Thissen et al., 1994). Inadequate nutrient intake increases GH secretion in cattle (Armstrong et al., 1993; Bossis et al., 1999) and serum concentrations of IGF-I and hepatic IGF-I mRNA are decreased (Vandehaar et al., 1995), probably

due to an insulin-dependant down-regulation of the GH receptor (Thissen et al., 1994; Kobayashi et al., 1999; Butler and Butler, 2001). Dietary restriction results in a loss of IGF-I responsiveness to exogenous GH treatment (see review by McGuire et al., 1992). The loss of responsiveness to GH in dietary restricted cattle may be due in part to decreased hepatic binding sites for GH (Breier et al., 1988). Increased GH in plasma is proposed to be associated with decreased negative feedback of IGF-I on hypothalamic-pituitary regulation of GH secretion resulting in increased pituitary synthesis and secretion of GH (Kirby et al., 1993).

Concurrent with increased concentrations of IGF-I in plasma, concentrations of insulin in plasma were greater for G than M. This is in agreement with previous studies (Lalman et al., 2000; Ciccioli et al., 2003) in primiparous beef cows. In both experiments, G cows had greater insulin in follicular fluid than M cows. Similarly, Landau et al. (2000) found that concentration of insulin in FF of cows fed a high energy diet was 26 % greater than in their counterparts fed corn gluten meal. Insulin and IGF-I are potent stimulators of progesterone secretion by bovine corpora lutea (Spicer and Echternkamp, 1995), and both hormones act synergistically with gonadotrophins to increase granulosal cell proliferation (Spicer and Echternkamp, 1995) and to enhance steroidogenesis in bovine granulosal (Spicer et al., 1993) and thecal cells (Spicer and Echternkamp, 1995; Spicer and Stewart, 1996).

Concentrations of progesterone, androstenedione, and estradiol in FF were not influenced by treatment. In contrast, White et al (2003) found that DF from the first follicular wave of an estrous cycle had 3.4-fold more estradiol and 7.6-fold greater androstenedione in FF than DF of anovulatory mature beef cows. In the current experiments DF was aspirated at least 50 d before the first postpartum ovulation. Similar to the anovulatory cows reported by White et al. (2003), DF of our cows produced minimal concentration of estradiol and androstenedione and were responsive to increase insulin and IGF-I associated with greater nutrient intake.

Insulin-like growth factor-I and its binding proteins have an integral function in energy metabolism and have been implicated as metabolic mediators of nutritional regulation of the reproductive axis in bovine females (Zulu et al., 2002). Concentrations of IGFBP-4 and -5 in plasma were 30 % greater in G than M cows in experiment 2 and concentrations of IGFBP-4 and -5 in FF were 68 % and 48 %, respectively, greater for G than M cows in experiment 2. Concentrations of IGFBP-2 and -3 in plasma and FF were not influenced by treatment. These results are contrary to the findings of Roberts et al. (1997), where amounts of IGFBP-2 in plasma at wk 2 post partum were greater and IGFBP-3 concentrations were less in cows that were anovulatory compared with cows that ovulated sooner after calving. Morevoer, greater undegradable intake protein (UIP) supplementation was associated with increased low-molecular weight IGFBP compared with supplementation with less UIP (Kane et al., 2004). Treatment of nutritionally induced anovulatory

beef cows with GnRH did not alter concentrations of IGFBP-5, 4, 3, or 2 in follicular fluid of large (5> mmm) or small (< 5 mm follicles) (Hamilton et al., 1999; Prado et al., 2002). Thus concentrations of IGFBPs in follicles may not change in response to gonadotropin stimulation of postpartum anovulatory cows.

Follicular growth and development are associated with decreased amounts of IGFBP-2, -4, and -5, whereas follicular atresia is characterized by increases in the relative abundance of these proteins (Echternkamp et al., 1994; de la Sota et al., 1996; Monget et al., 1996; Stewart et al., 1996; Mihm et al., 2000). Selection of DF is associated with increased granulosa cell aromatase activity followed by increased cAMP response to LH in follicular fluid (Rhodes et al., 2001). In addition, binding proteins may be important in the physiological regulation of FSH actions, probably by influencing the bioavailability of IGF-I or IGF-II and stimulating FSH-induced estradiol production by granulosa cells (Gutierrez et al., 1997). Proteolysis of specific IGFBP contribute to diminished binding activity in follicular fluid from humans (Chandrasekhar et al., 1995; Conover et al., 2001) and domestic species (Mazerbourg et al., 2000; Rivera et al., 2001; Spicer et al., 2001). Reduced concentrations of IGFBP-4 in preovulatory and dominant bovine follicles are associated with the presence of an IGFBP-4 protease (Mazerbourg et al., 2000; Rivera and Fortune, 2001; Spicer et al., 2001). In nutritionally induced anovulatory cows, IGF-I in FF is reduced, and IGF-I is necessary to activate pregnancy-associated plasma protein-A (PAPP-A), which is a zinc dependent

metalloproteinase that may be responsible for IGFBP proteolysis (Mazerbourg et al., 2001; Monget et al., 2003). The amount of IGFBP activity in follicles is a result of production, synthesis and degradation, specifically degradation for IGFBP-4.

Concentration of IGFBP-2 and -5 in plasma at follicular fluid aspiration were positively correlated with follicle size and BCS in Exp. 2($P < 0.05$). In addition, BCS at calving was positively correlated with IGFBP-4 in plasma at the time of aspiration of follicles. Concentrations of IGF-I in plasma at aspiration and in FF were positively correlated with amounts of IGBP-3 and -4 in FF.

Abundance of mRNA for aromatase, IGFBP-4 and -5, and pregnancy associated plasma protein–A were not affected by treatment in experiments 1 and 2. White et al. (2004) found that anovulatory and ovulatory cows had DF with similar amounts of aromatase mRNA in granulosa cells. Tian et al. (1995) found that aromatase mRNA in preovulatory DF did not increase with estradiol secretion. Amounts of mRNA for PAPP-A in DF were also similar for ovulatory cows and anovulatory cows with a postpartum interval > 58 d or < 58 d (White et al., 2004).

Variations in metabolic and endocrine function during negative balance of dairy cows are well documented, however this is the first study to determine the effect of greater nutrient intake after calving on insulin and

IGFBPs in DF and plasma. These results add to our understanding of nutritional influences on follicular growth and maturation.

Implications

Increased postpartum nutrient intake of primiparous beef cows increased BCS and increased concentrations of IGF-I and insulin in FF and plasma at 56 and at 72 d after calving. The nutritionally induced increased in concentrations of IGF-I and insulin could have direct and/or indirect effects on the length of the postpartum anestrous interval without affecting size of DF or concentrations of steroid hormones in FF. In addition, endocrine changes in DF may be associated with increased pregnancy rates at the first postpartum estrus in cows that receive greater nutrient intake. Although nutrient intake before calving has a greater effect on reproductive performance of primiparous cows compared with nutrient intake after calving, greater energy intake after calving can decrease the duration of postpartum anestrous and increase pregnancy rate during the breeding season.

Table 1. Primers and probes sequences and optimal reaction condition for target genes.

Gene	Sequence	GeneBank Accesion No.
Aromatase	FWD Primer 5' CCTGGCCTGGTGCGC (bp 645 to 659) REV Primer 5' TCCAGCCTGTCCAGATGCTT (bp 690 to 709) Probe 5' GGTGACCATCTGTGCTGATTCCATCA(bp 661 to 687)	Z32741
IGFBP-4	FWD Primer 5' GAGGAAAGAATGTGTATGTGCCTGATG (bp 1733 to 1757) REV Primer 5' GACCACAAACGGAGGAGGAA (bp 1808 to 1827) Probe 5' CATGCTGGGAGGTGAGGGACTTATCTATCTGG (bp 1772 to 1799)	S52770
IGFBP-5	FWD Primer 5' GTTTGCCTGAACGAAAAGAGCTA (bp 193 to 215) REV Primer 5' CGAGTAGGTCTCCTCTGCCATCT (bp 275 to 295) Probe 5' AGCCAAGATCGAAAGAGACTCCCGTGAGV (bp 225 to 252)	S52657
PAPP-A	FWD Primer 5' CAGATGTTGAGCAGCCCTGTAA (bp 557 to 578) REV Primer 5' GGGTTGACGGCTGAATTGG (bp 602 to 620) Probe 5' CCAGCCCGCACCTGGAGC (bp 581 to 600)	AF421141

Table 2. Concentrations of androstenedione, estradiol and progesterone (ng/mL) in follicular fluid of dominant follicles aspirated at 72 d postpartum.

Treatment	n	Androstenedione	Estradiol	Progesterone
High	6	42.1 ± 5.2	145.8 ± 59.89	56.83 ±4.56 [a]
Moderate	6	43.4 ± 4.9	119.1 ± 11.89	49.74 ± 5.29 [b]

[a, b] Means in a column with different superscripts differ $P < 0.05$

Table 3. Quantitative RT-PCR Analysis of gene expression for aromatase, PAPP-A, IGFBP-4 and IGFBP-5 in granulosa cells of dominant follicles aspirated at 72 d after calving.

Gene	Treatment	Target Gene Ct[a]	18 S Ct	ΔC_t[b]	$\Delta\Delta C_t$[c]	Fold Difference
Aromatase	Gain	24.70 ± 0.93	23.54 ± 1.03	1.15 ± 0.71	-2.08 ± 0.71	1.68 ± 0.57
	Maintain	27.02 ± 1.21	25.59 ± 1.33	1.43 ± 0.93	-1.80 ± 0.92	1.00 ± 0.73
IGFBP-4	Gain	29.59 ± 1.10	24.37 ± 0.95	5.21 ± 1.16	-5.35 ± 1.16	1.00 ± 1.10
	Maintain	31.56 ± 1.69	28.64 ± 1.45	2.92 ± 1.77	-7.65 ± 1.77	4.65 ± 1.69
IGFBP-5	Gain	32.43 ± 0.73	24.58 ± 1.02	7.85 ± 0.80	-2.99 ± 0.80	1.05 ± 0.57
	Maintain	37.06 ± 1.17	29.23 ± 1.56	7.83 ± 1.22	-3.02 ± 1.22	1.00 ± .088
PAPP-A	Gain	23.51 ± 1.18	24.42 ± 0.90	-0.90 ± 0.66	-3.25 ± 0.66	1.76 ± 0.55
	Maintain	25.22 ± 1.57	26.26 ± 1.20	-1.03 ± 0.87	-3.38 ± 0.87	1.00 ± 0.73

[a] C_t = cycle that its amplification plot crossed an arbitrary threshold assigned in the log-linear range of amplification.

[b] $\Delta_t Ct = C_t$ for target gene – C_t for normalization control, 18S.

[c] $\Delta\Delta C_t$ = Mean ΔC_t – highest mean ΔC_t

Fold difference = $2^{-\Delta\Delta C}$

Table 4. Partial correlation coefficients, among BCS at calving, BCS at aspiration, IGF before aspiration, IGF at aspiration follicle size, post partum interval, IGF follicular fluid (FF), androstenedione (A_4), estradiol (E_2), and progesterone (P_4) in follicular fluid (FF) at 72 d aspiration in postpartum primiparous cows in Exp. 1.

	BCS at Aspiration	IGF before Aspiration	IGF at Aspiration	Follicle size	Post partum interval	IGF FF	A_4 FF	E_2 FF	P_4 FF
BCS at Calving	0.53 0.08	0.30 0.35	0.47 0.12	0.54 0.07	-0.31 0.32	0.63 0.03	0.12 0.71	-0.02 0.95	0.42 0.17
BCS at Aspiration		0.49 0.10	0.63 0.03	0.60 0.04	-0.58 0.05	0.75 0.01	-0.28 0.39	0.29 0.36	0.43 0.17
IGF before Aspiration			0.68 0.01	0.44 0.15	-0.42 0.17	0.63 0.03	-0.16 0.62	0.34 0.29	0.67 0.02
IGF at Aspiration				0.58 0.05	-0.22 0.49	0.95 <.0001	-0.18 0.58	0.68 0.01	0.31 0.33
Follicle size					-0.28 0.38	0.61 0.03	0.22 0.48	0.32 0.31	0.36 0.24
Post partum interval						-0.34 0.28	0.18 0.57	0.32 0.32	-0.54 0.07
IGF FF							-0.09 0.79	0.49 0.10	0.40 0.20
A_4 FF								-0.28 0.38	-0.12 0.71
E_2 FF									-0.14 0.66

Table 5. Partial correlation coefficients, among insulin follicular fluid (FF), insulin plasma, follicular fluid (FF), insulin-like growth factor binding protein (IGFBP) IGFBP-3, IGFBP-2, IGFBP-5, IGFBP-4, and plasma IGFBP-3, IGFBP-2, IGFBP-5 and IGFBP-4 at 72 d aspiration in postpartum primiparous cows in Exp. 1.

	Insulin Plasma	FF IGFBP-3	FF IGFBP-2	FF IGFBP-5	FF IGFBP-4	Plasma IGFBP-3	Plasma IGFBP-2	Plasma IGFBP-5	Plasma IGFBP-4
Insulin FF	0.34 0.28	0.38 0.23	0.04 0.90	0.52 0.08	0.30 0.34	-0.15 0.65	0.20 0.54	0.48 0.11	-0.09 0.78
Insulin Plasma		0.17 0.60	0.08 0.80	0.28 0.39	0.20 0.53	-0.26 0.41	0.21 0.52	0.36 0.24	0.33 0.29
FF IGFBP-3			0.29 0.36	0.85 <0.001	0.79 0.002	0.003 0.99	0.26 0.42	0.26 0.41	0.01 0.98
FF IGFBP-2				0.12 0.71	0.50 0.10	0.46 0.14	0.42 0.18	0.20 0.54	-0.19 0.55
FF IGFBP-5					0.72 0.01	-0.01 0.96	0.46 0.13	0.36 0.25	0.30 0.35
FF IGFBP-4						-0.01 0.97	0.20 0.52	0.50 0.10	0.10 0.76
Plasma IGFBP-3							0.18 0.57	0.16 0.61	-0.45 0.14
Plasma IGFBP-2								0.15 0.64	0.51 0.09
Plasma IGFBP-5									0.06 0.85

Table 6. Partial correlation coefficients, among insulin follicular fluid (FF), insulin plasma, FF IGFBP-3, FF IGFBP-2, FF IGFBP-5, FF IGFBP-4, plasma IGFBP-3, plasma IGFBP-2, plasma IGFBP-5, plasma IGFBP-4, BCS at calving, BCS at aspiration, IGF before aspiration, IGF at aspiration, follicle size, post partum interval, IGF-I follicular fluid (FF), androstenedione (A_4), estradiol (E_2) and progesterone (P_4) in follicular fluid (FF) at 72d aspiration in postpartum primiparous cows in Exp. 1.

	Insulin FF	Insulin Plasma	FF IGFBP-3	FF IGFBP-2	FF IGFBP-5	FF IGFBP-4	Plasma IGFBP-3	Plasma IGFBP-2	Plasma IGFBP-5	Plasma IGFBP-4
BCS at Calving	0.34 0.28	0.06 0.85	0.19 0.56	0.34 0.29	0.12 0.71	-0.07 0.83	-0.03 0.94	0.28 0.38	-0.48 0.11	-0.21 0.50
BCS at Aspiration	0.55 0.07	0.56 0.06	0.31 0.33	0.38 0.22	0.28 0.38	0.05 0.87	0.12 0.72	0.30 0.34	0.20 0.54	-0.13 0.70
IGF before Aspiration	0.68 0.01	0.46 0.14	0.61 0.03	0.12 0.72	0.79 0.002	0.51 0.09	-0.13 0.68	0.39 0.21	0.43 0.17	0.15 0.65
IGF at Aspiration	0.72 0.01	0.31 0.32	0.46 0.13	0.16 0.63	0.55 0.07	0.29 0.36	0.12 0.71	0.10 0.77	0.31 0.33	-0.13 0.69
Follicle size	0.53 0.07	0.65 0.02	0.20 0.53	-0.07 0.84	0.23 0.48	-0.08 0.80	-0.24 0.44	0.24 0.44	0.05 0.87	0.11 0.73
Post partum interval	-0.42 0.17	-0.29 0.36	-0.32 0.31	-0.19 0.55	-0.36 0.26	-0.18 0.59	0.44 0.15	-0.48 0.11	-0.10 0.77	-0.43 0.16
IGF-I FF	0.74 0.01	0.40 0.19	0.41 0.19	0.34 0.28	0.47 0.12	0.29 0.37	0.10 0.77	0.18 0.57	0.22 0.50	-0.13 0.68
A_4 FF	-0.02 0.94	0.20 0.53	-0.55 0.07	-0.15 0.64	-0.51 0.09	-0.23 0.47	-0.52 0.08	-0.27 0.40	-0.15 0.63	0.08 0.81
E_2 FF	0.21 0.51	0.20 0.52	0.10 0.75	-0.17 0.60	0.25 0.43	-0.07 0.84	0.43 0.16	-0.13 0.69	0.28 0.38	-0.14 0.65
P_4 FF	0.62 0.03	0.23 0.47	0.35 0.26	0.35 0.26	0.54 0.07	0.29 0.35	0.03 0.93	0.82 0.001	0.30 0.35	0.19 0.55

Table 7. Quantitative RT-PCR Analysis of gene expression for aromatase, PAPP-A, IGFBP-4 and IGFBP-5 in granulosa cells of dominant follicles aspirated at 56 d after calving.

Gene	Treatment	Target Gene Ct[a]	18 S Ct	ΔC_t[b]	$\Delta\Delta C_t$[c]	Fold Difference
Aromatase	Gain	29.54 ±0.90	24.59 ±0.49	4.94 ± 0.80	-3.47 ±0.80	1.15 ± 0.40
	Maintain	29.21 ± 0.95	22.93 ±0.52	6.27 ± 0.85	-3.14 ±0.85	0.99 ± 0.42
IGFBP-4	Gain	37.68 ±0.41	26.92 ± 0.45	10.76 ±0.42	-4.02 ± 041	1.0 ± 0.18
	Maintain	36.58 ± 0.49	26.54 ± 0.54	10.08 ±0.50	-4.7 ± 0.49	1.17 ± 0.21
IGFBP-5	Gain	28.91 ±1.10	24.83 ±0.53	4.08 ±1.11	-7.72 ±1.11	1.00 ± 0.82
	Maintain	25.77 ± 1.17	23.22 ±0.56	2.54 ±1.18	-9.25 ±1.18	2.28 ± 0.87
PAPP-A	Gain	28.91 ±1.10	22.10 ±0.52	6.81±1.11	-7.72 ±1.11	1.00 ± 0.79
	Maintain	25.76 ± 1.17	20.43 ± 0.55	5.33 ±1.18	-9.20±1.18	2.19 ± 0.84

Ct[a] = cycle that its amplification plot crossed an arbitrary threshold assigned in the log-linear range of amplification.

ΔC_t[b] = C_t for target gene – C_t for normalization control, 18S.

$\Delta\Delta C_t$[c] = Mean ΔC_t – highest mean ΔC_t

Fold difference = $2^{-\Delta\Delta Ct}$

Table 8. Partial correlation coefficients, among BCS at calving, BCS at aspiration, IGF before aspiration, IGF at aspiration follicle size, post partum interval, IGF follicular fluid (FF), androstenedione (A_4), estradiol (E_2), and progesterone (P_4) in follicular fluid (FF) at 56 d aspiration in postpartum primiparous cows in Exp. 2.

	BCS at Aspiration	IGF before Aspiration	IGF at Aspiration	Follicle size	Post partum interval	IGF FF	A_4 FF	E_2 FF	P_4 FF
BCS at Calving	0.31 0.11	0.07 0.73	-0.10 0.61	0.15 0.43	-0.28 0.15	-0.10 0.60	-0.11 0.58	0.07 0.73	0.10 0.62
BCS at Aspiration		0.46 0.01	0.35 0.07	0.30 0.13	-0.47 0.01	0.46 0.01	0.07 0.72	-0.06 0.75	0.01 0.94
IGF before Aspiration			0.82 <.0001	0.45 0.02	-0.26 0.18	0.48 0.01	-0.12 0.53	0.14 0.49	-0.15 0.43
IGF at Aspiration				0.18 0.35	-0.03 0.87	0.61 0.001	-0.04 0.84	0.23 0.24	-0.13 0.50
Follicle size					-0.32 0.09	-0.09 0.64	0.12 0.54	0.17 0.39	-0.13 0.50
Post partum interval						-0.31 0.10	0.09 0.65	-0.13 0.51	0.04 0.84
IGF FF							-0.04 0.84	-0.07 0.74	-0.13 0.51
A_4 FF								0.25 0.21	-0.06 0.77
E_2 FF									-0.11 0.57

Table 9. Partial correlation coefficients, among insulin follicular fluid (FF), insulin plasma, follicular fluid (FF), insulin-like growth factor binding protein (IGFBP) IGFBP-3, IGFBP-2, IGFBP-5, IGFBP-4, and plasma IGFBP-3, IGFBP-2, IGFBP-5 and IGFBP-4 at 56 d aspiration in postpartum primiparous cows in Exp. 2.

	Insulin Plasma	FF IGFBP-3	FF IGFBP-2	FF IGFBP-5	FF IGFBP-4	Plasma IGFBP-3	Plasma IGFBP-2	Plasma IGFBP-5	Plasma IGFBP-4
Insulin	0.85	0.03	0.25	0.17	0.21	-0.10	0.02	0.15	0.21
FF	<.0001	0.89	0.21	0.40	0.28	0.60	0.91	0.45	0.28
Insulin		0.12	0.20	0.18	0.00	-0.04	0.02	0.03	0.04
Plasma		0.54	0.30	0.35	1.00	0.84	0.92	0.86	0.83
FF			0.40	0.41	0.42	0.02	0.31	0.33	-0.04
IGFBP-3			0.04	0.03	0.03	0.93	0.10	0.09	0.84
FF				0.72	0.51	0.05	-0.06	0.03	-0.18
IGFBP-2				<.0001	0.01	0.78	0.75	0.86	0.37
FF					0.60	-0.19	-0.11	-0.04	-0.08
IGFBP-5					0.001	0.34	0.56	0.83	0.69
FF						-0.33	-0.01	0.39	0.21
IGFBP-4						0.08	0.97	0.04	0.29
Plasma							0.55	0.11	-0.07
IGFBP-3							0.002	0.58	0.74
Plasma								0.48	0.26
IGFBP-2								0.01	0.17
Plasma									0.60
IGFBP-5									0.001

Table 10. Partial correlation coefficients, among insulin follicular fluid (FF), insulin plasma, FF IGFBP-3, FF IGFBP-2, FF IGFBP-5, FF IGFBP-4, plasma IGFBP-3, plasma IGFBP-2, plasma IGFBP-5, plasma IGFBP-4, BCS at calving, BCS at aspiration, IGF before aspiration, IGF at aspiration, follicle size, post partum interval, IGF-I follicular fluid (FF), androstenedione (A_4), estradiol (E_2) and progesterone (P_4) in follicular fluid (FF)at 56d aspiration in postpartum primiparous cows in Exp. 2.

	Insulin FF	Insulin Plasma	FF IGFBP-3	FF IGFBP-2	FF IGFBP-5	FF IGFBP-4	Plasma IGFBP-3	Plasma IGFBP-2	Plasma IGFBP-5	Plasma IGFBP-4
BCS at Calving	-0.05 0.79	-0.10 0.61	-0.02 0.94	-0.13 0.51	-0.28 0.16	-0.14 0.48	0.60 0.001	0.58 0.001	0.28 0.15	0.17 0.37
BCS at Aspiration	0.35 0.07	0.24 0.23	-0.06 0.78	0.12 0.56	0.12 0.56	0.28 0.15	0.04 0.85	0.19 0.33	0.04 0.84	0.26 0.18
IGF before Aspiration	0.39 0.04	0.36 0.06	0.33 0.08	0.23 0.24	0.26 0.18	0.19 0.34	0.35 0.06	0.35 0.07	0.15 0.46	0.01 0.97
IGF at Aspiration	0.26 0.18	0.38 0.05	0.49 0.01	0.18 0.36	0.26 0.18	0.15 0.43	0.24 0.22	0.31 0.11	0.06 0.76	-0.03 0.87
Follicle size	0.23 0.24	0.12 0.56	0.04 0.84	0.44 0.02	0.29 0.14	0.07 0.74	0.30 0.12	0.12 0.56	0.04 0.86	0.02 0.90
Post partum interval	-0.16 0.43	-0.10 0.63	-0.05 0.80	-0.17 0.39	-0.16 0.42	-0.31 0.11	0.01 0.95	-0.01 0.97	-0.40 0.04	-0.22 0.27
IGF-I FF	0.26 0.19	0.20 0.30	0.35 0.07	0.16 0.42	0.10 0.62	0.45 0.02	-0.13 0.50	0.20 0.30	0.25 0.20	0.16 0.42
A_4 FF	0.20 0.30	0.29 0.14	-0.22 0.26	0.16 0.40	-0.21 0.29	-0.10 0.62	-0.10 0.60	-0.26 0.18	-0.20 0.31	-0.18 0.37
E_2 FF	-0.003 0.99	0.07 0.73	<0.001 1.00	-0.05 0.81	-0.15 0.45	-0.07 0.74	0.10 0.62	0.01 0.96	0.19 0.32	0.07 0.73
P_4 FF	-0.06 0.78	-0.10 0.61	-0.15 0.44	0.12 0.53	0.27 0.17	-0.02 0.93	0.03 0.89	-0.01 0.95	-0.21 0.27	0.17 0.39

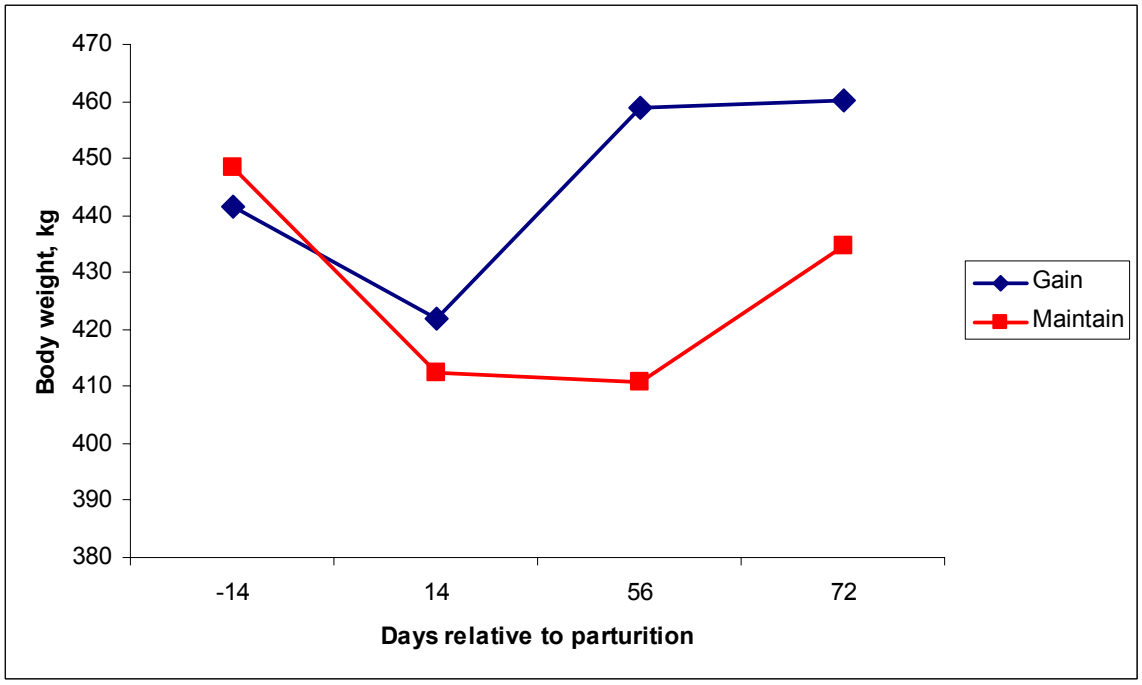

Figure 1. Body weight of primiparous cows prepartum (-14 d), early postpartum (14 d), late postpartum (56 d), and at aspiration of dominant follicles (72 d; $P < 0.05$).

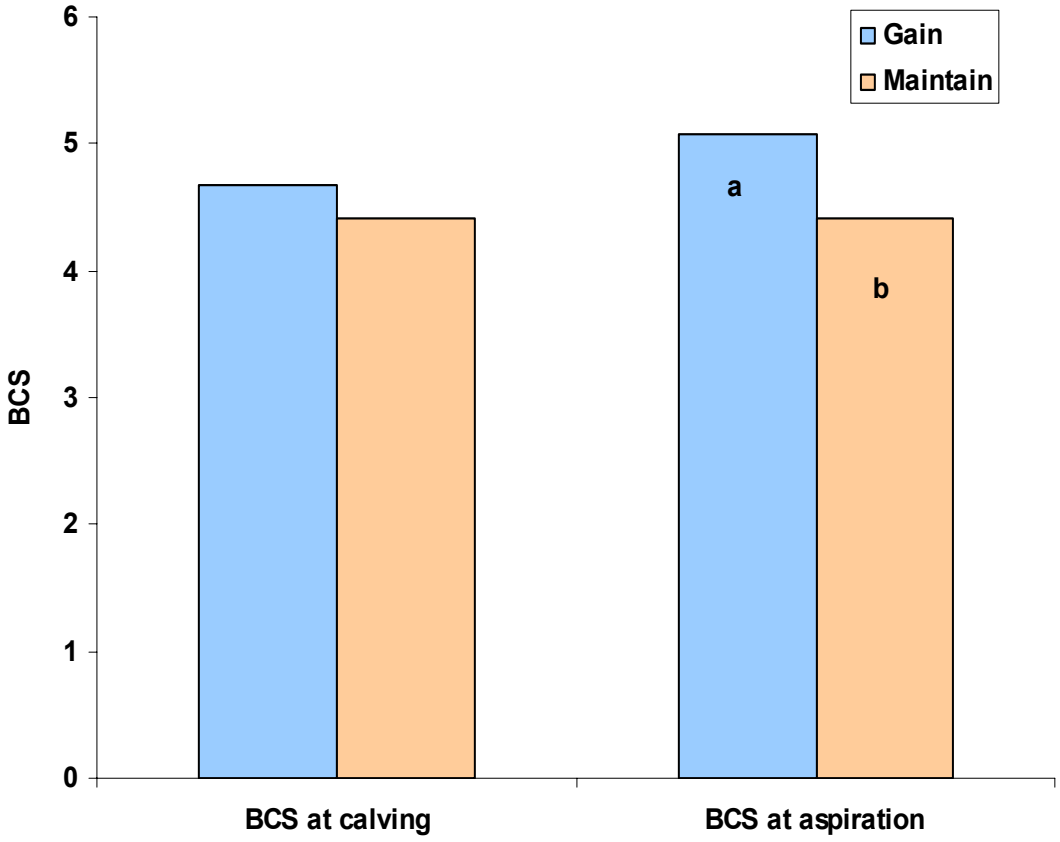

Figure 2. Effect of postpartum nutrition of beef cows on BCS at follicular aspiration [a, b] means with different superscripts differ ($P < 0.003$).

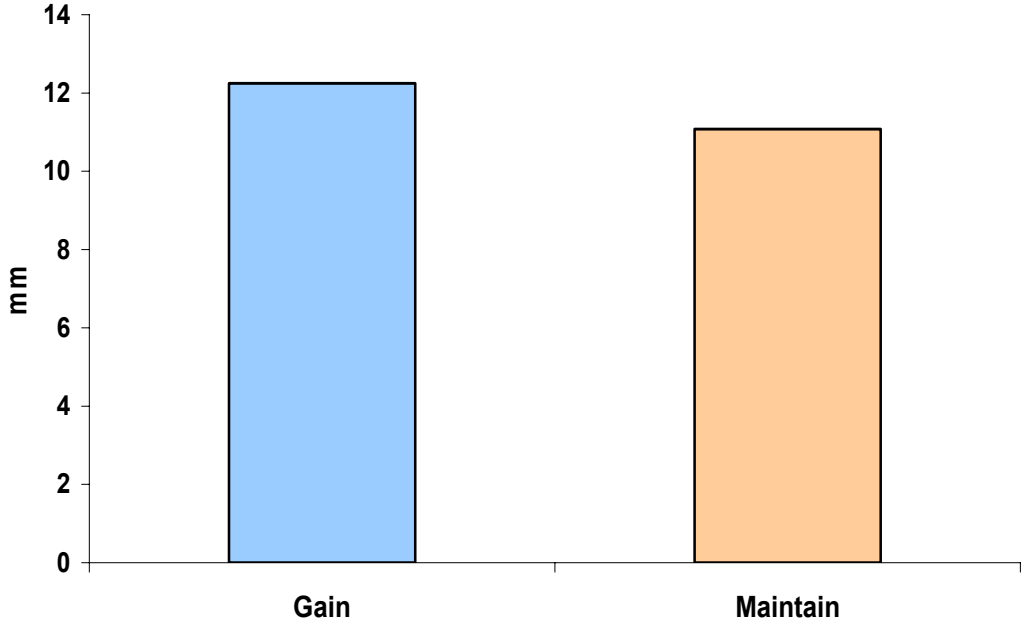

Figure 3. Effect of postpartum nutrition of beef cows on size of dominant follicles 72 d after calving ($P < 0.007$).

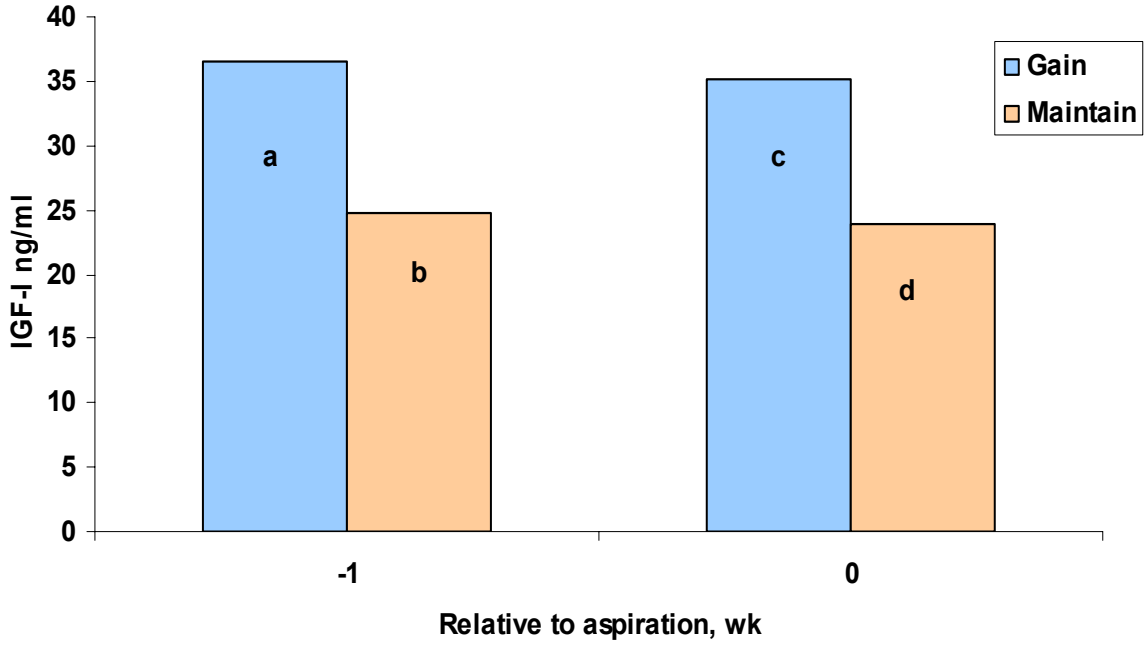

Figure 4. Effect of postpartum nutrition of beef cows on concentration of IGF-I in plasma 1 wk before (SE ± 0.6; ^{a,b} $P < 0.001$) and follicular aspiration at 72 d after calving (SE ± 2.55; $^{c, d}$ $P < 0.01$).

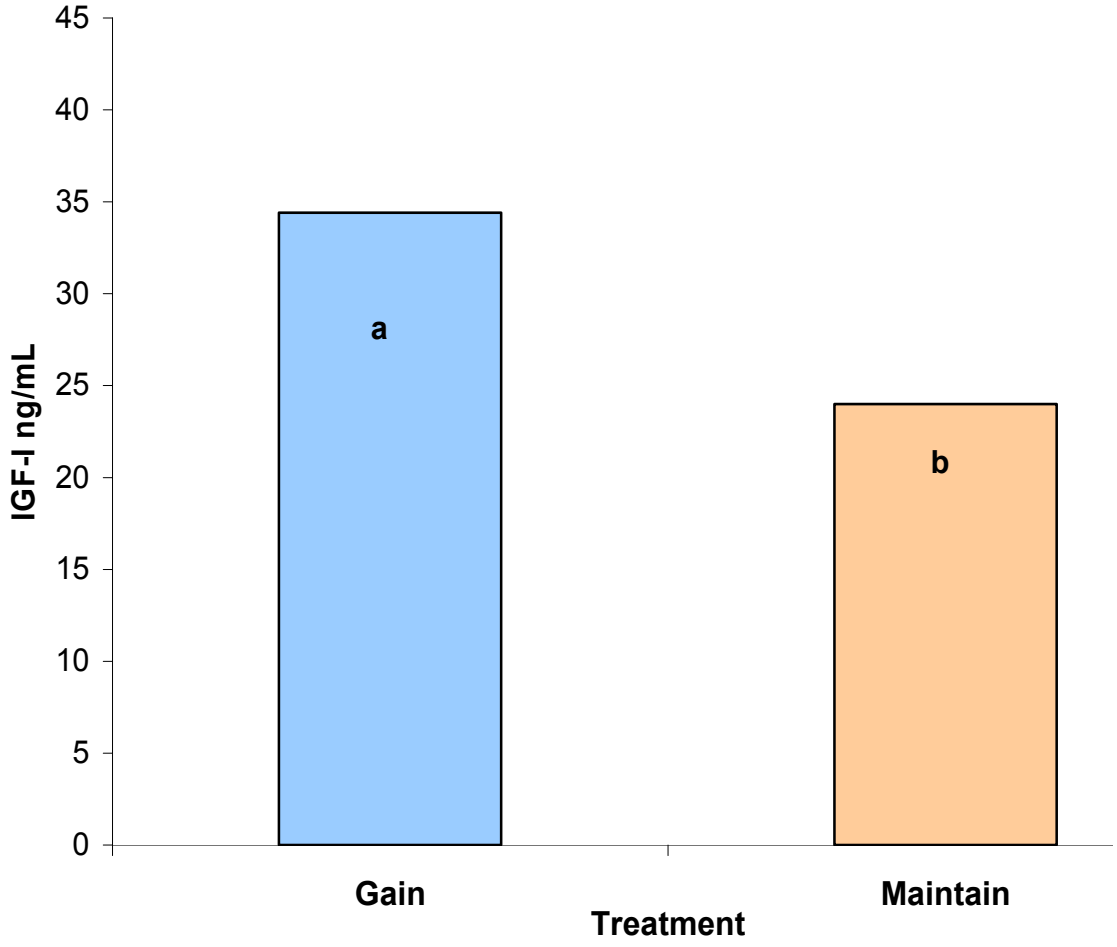

Figure 5. Effect of postpartum nutrition of beef cows on concentrations of IGF-I in follicular fluid at 72 d after calving (SE ± 2.3; P < 0.01).

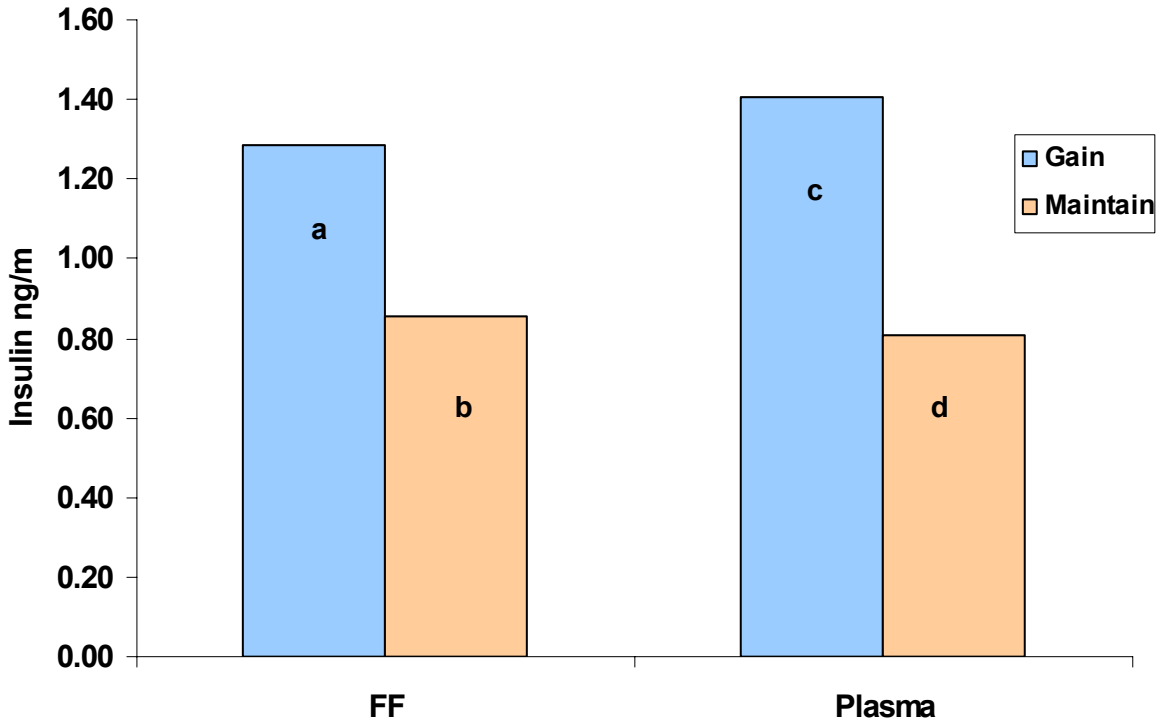

Figure 6: Effect of postpartum nutrition of beef cows on concentrations of insulin in plasma and follicular fluid at 72 d after calving.
[a, b] Bars with different letters within FF differ (P <0.008).
[c,d] Bars with different letters within FF differ (P <0.017).

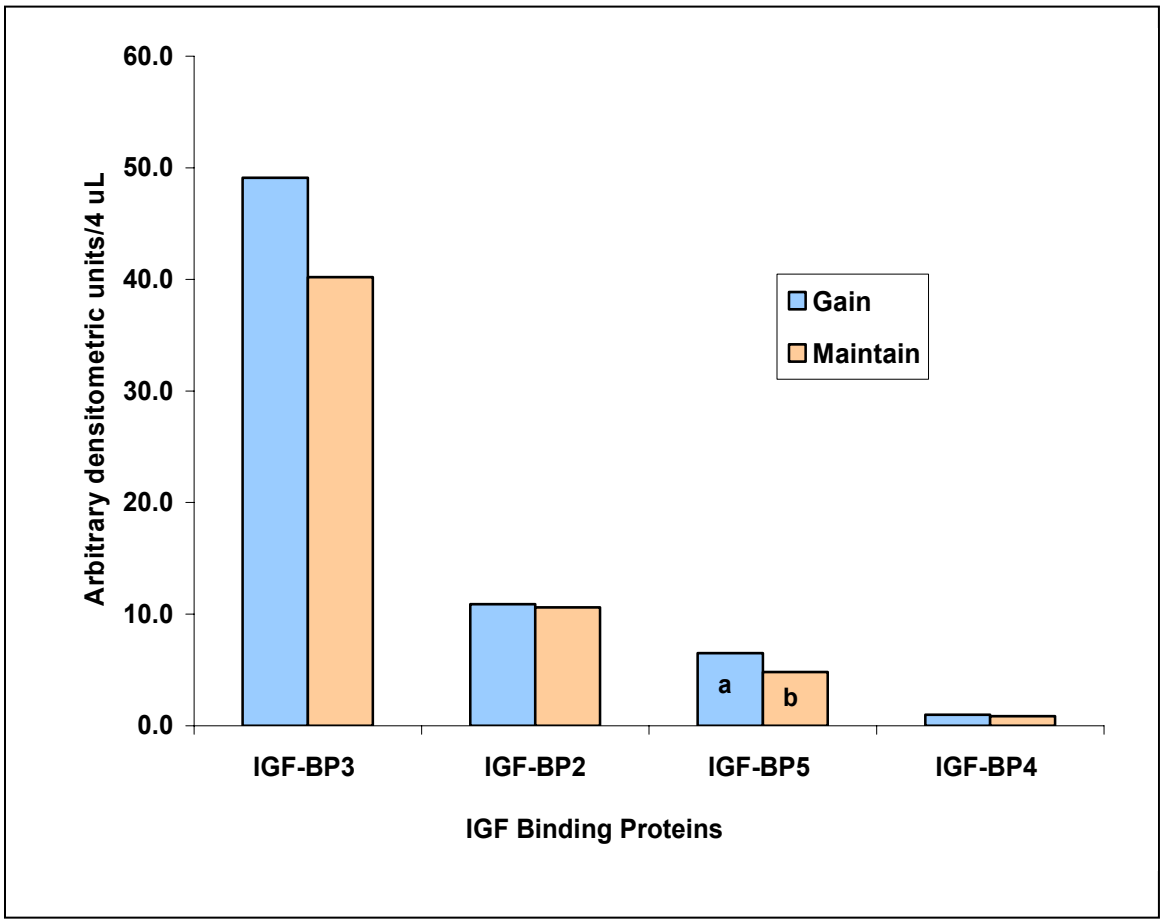

Figure 7. Influence of postpartum nutrition of primiparous beef cows on concentrations of IGF-I binding proteins (IGFBP) -3, -2, -5 and -4 in follicular fluid at 72 d after calving.
[a, b] Bars with different letters within a binding protein (BP) differ ($P < 0.03$).

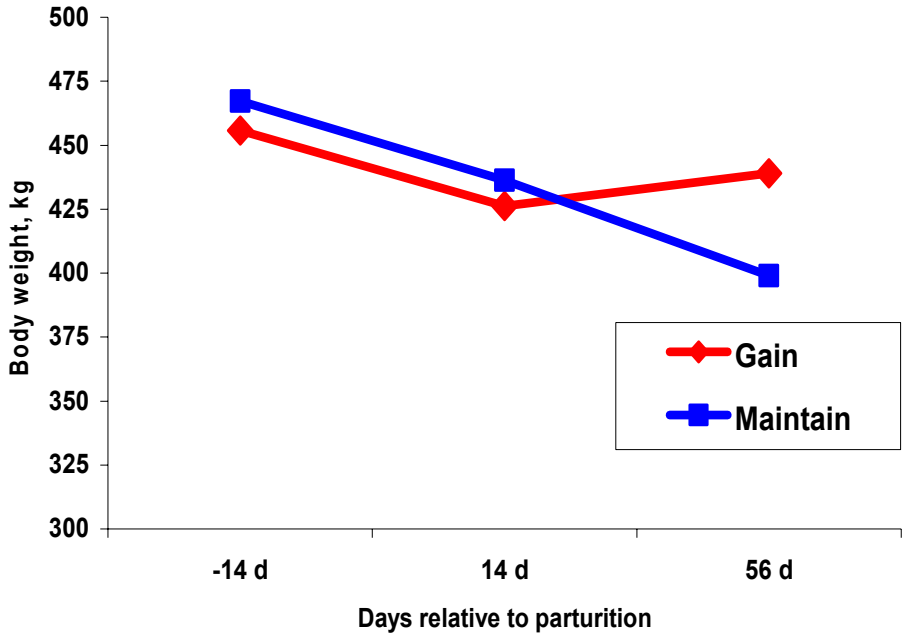

Figure 8. Body weight of primiparous cows prepartum (-14 d), early postpartum (14 d) and at aspiration of dominant follicles (56 days; $P < 0.05$).

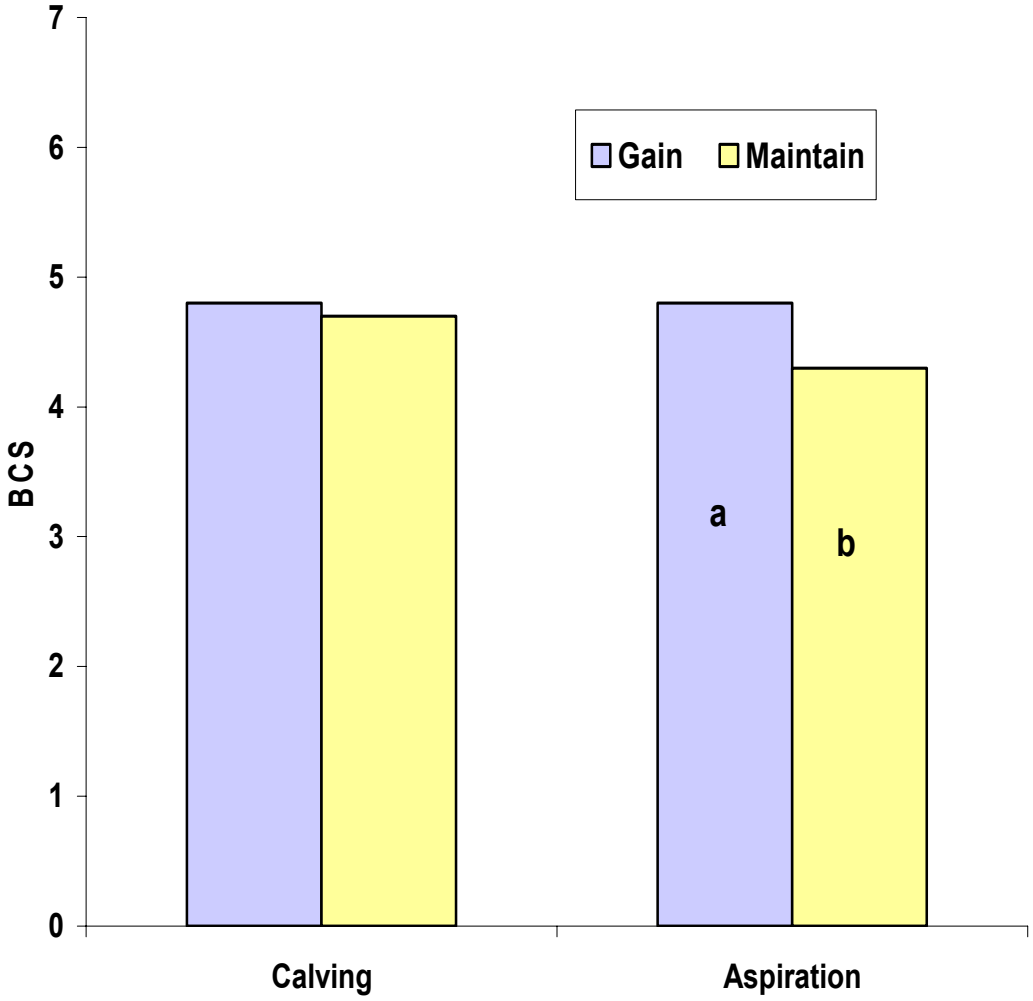

Figure 9: Effect of postpartum nutrition of beef cows on BCS at follicular aspiration.
[a,b] Bars with different superscripts differ ($P < 0.001$).

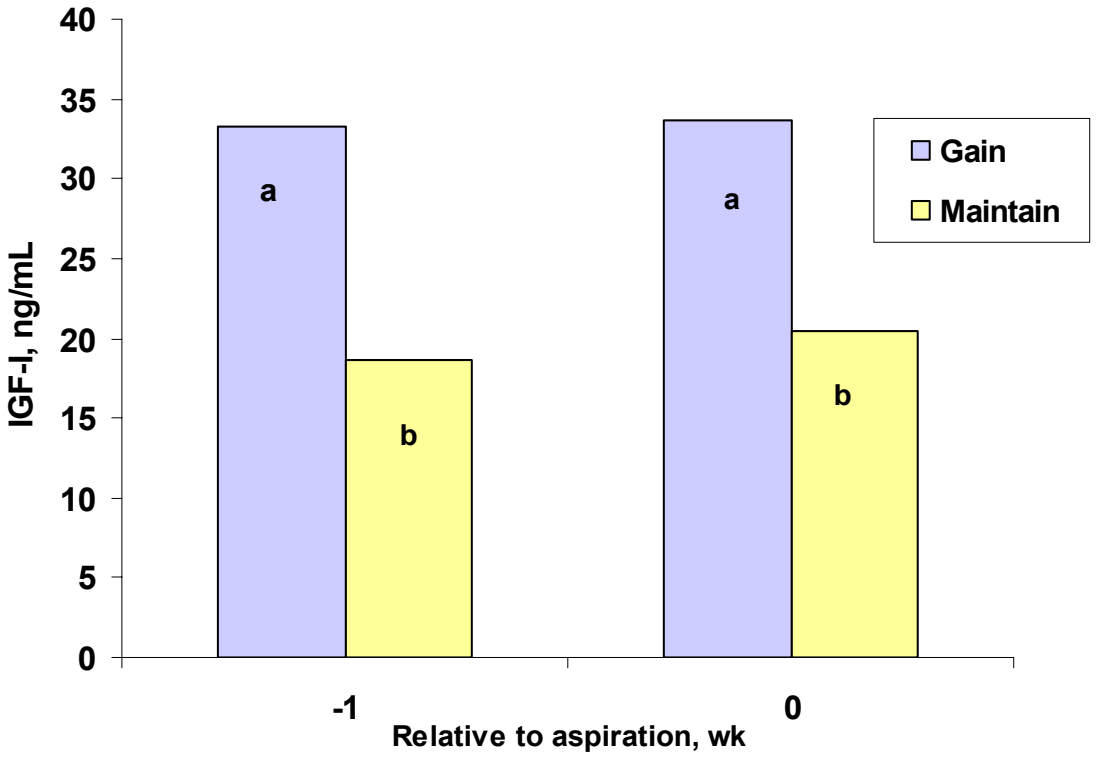

Figure 10. Effect of postpartum nutrition of beef cows on IGF-I in plasma 1 wk prior to and at the time of follicular aspiration (SE ± 2.5).
[a,b] Bars with different superscripts within a week differ (P < 0.01).

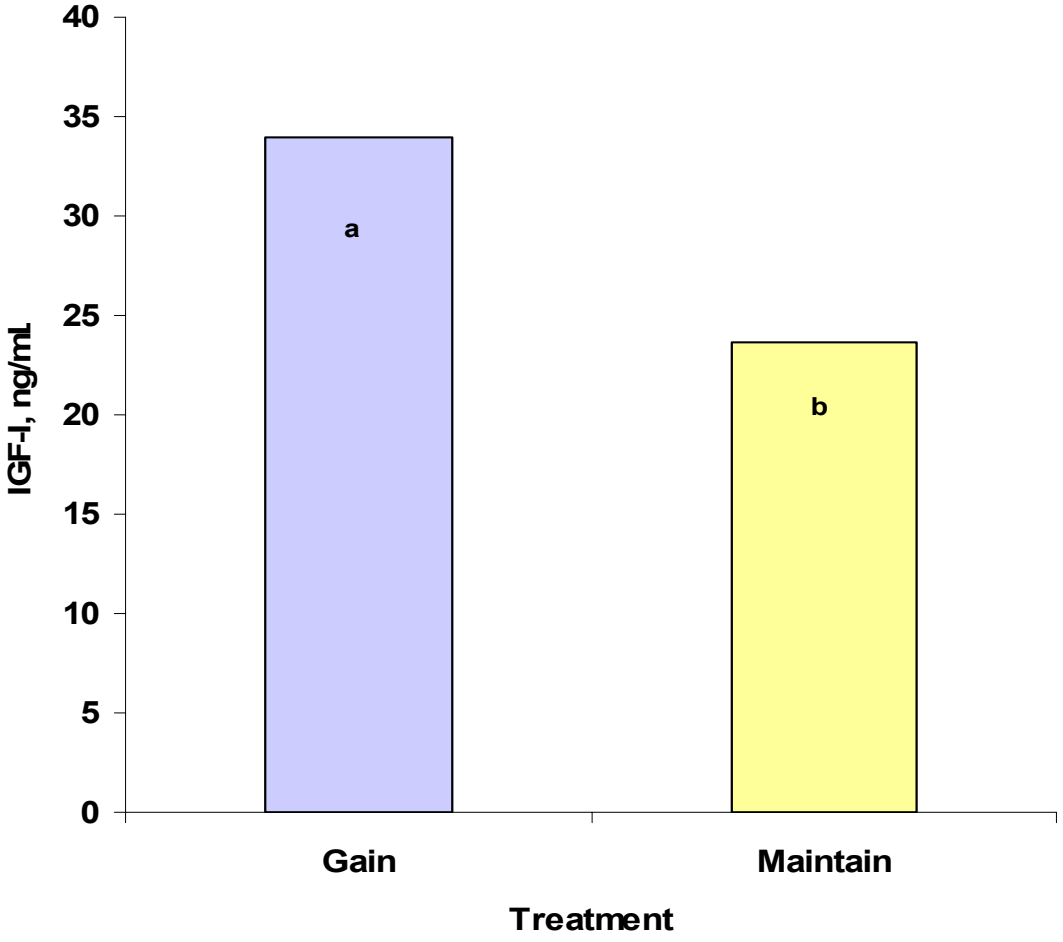

Figure 11: Effect of postpartum nutrition of beef cows on IGF-I in follicular fluid (SE ±2.7).
[a,b] Bars with different superscripts differ (P < 0. 01).

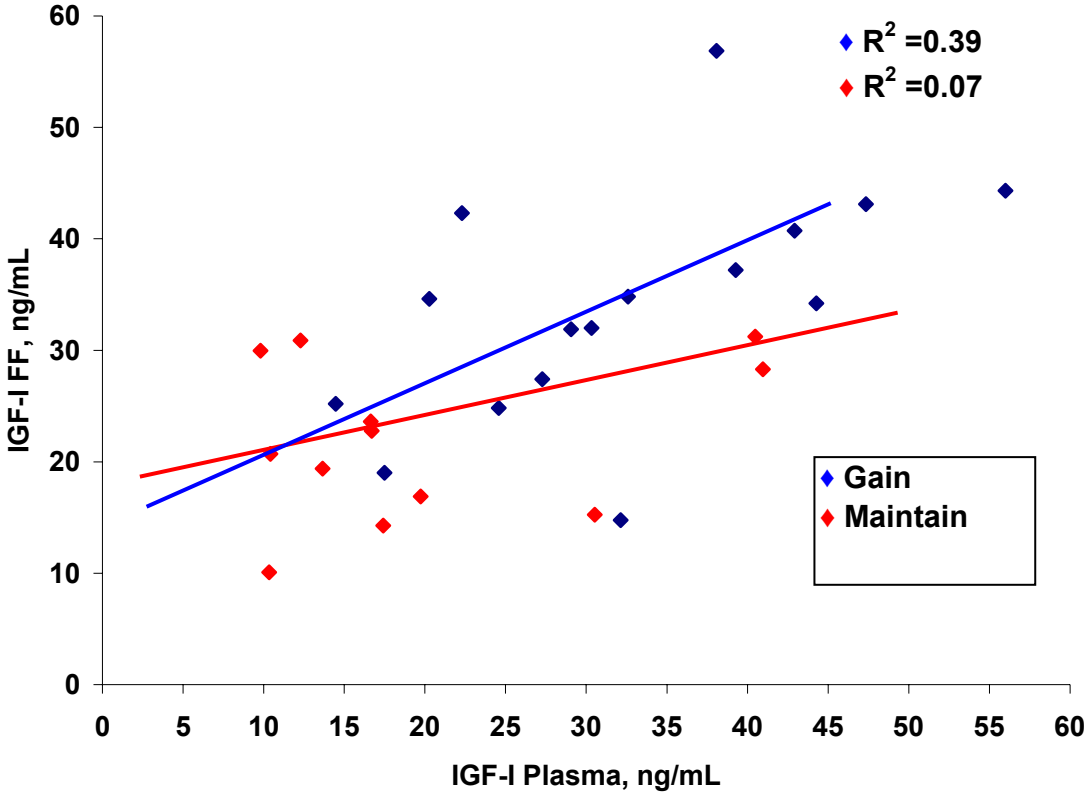

Figure 12. Relationship between IGF-I in plasma and follicular fluid.

Equation for ♦ Gain cows $IGF\text{-}I_{FF} = 14.98 + 0.56 * IGF\text{-}I_{Plasma}$ P = 0.02
Equation for ♦ Maintain cows $IGF\text{-}I_{FF} = 19.15 + 0.22 * IGF\text{-}I_{Plasma}$ P = 0.35

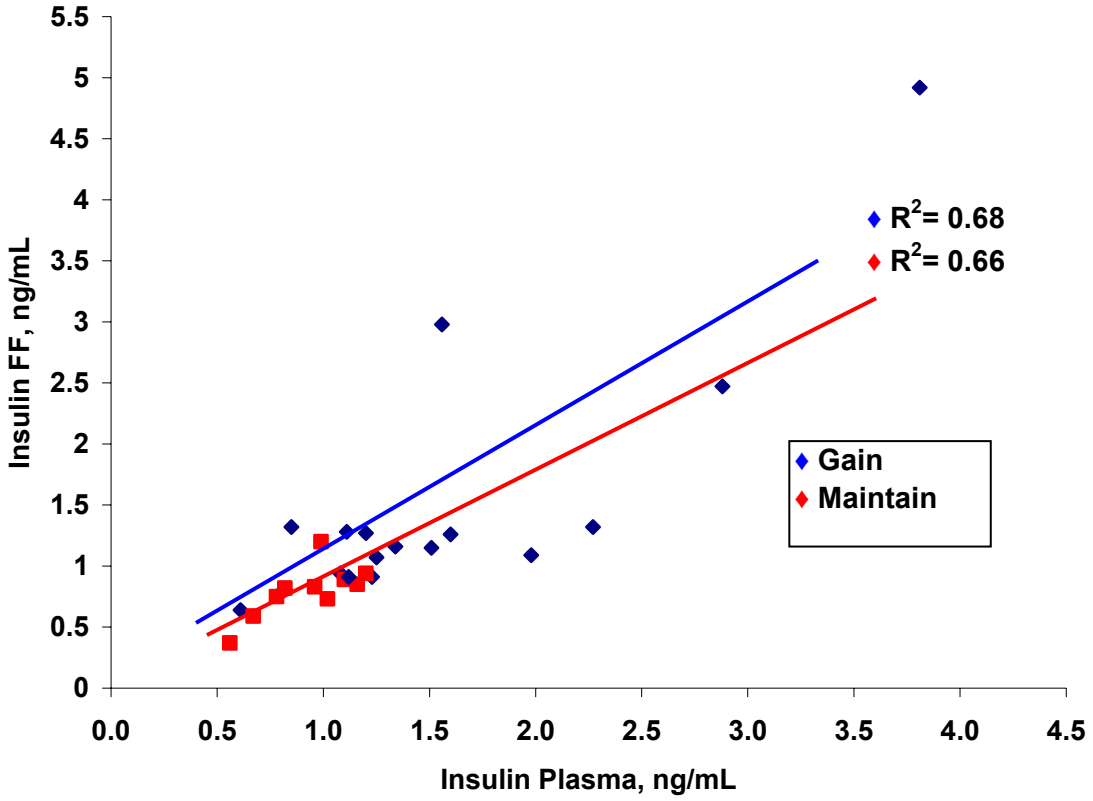

Figure 13. Relationship between Insulin in plasma and follicular fluid

Equation for ♦ Gain cows: $Insulin_{Plasma} = 0.6223 + 0.6233 * Insulin_{FF}$ P <0.0004
Equation for ♦ Maintain: $Insulin_{Plasma} = 0.2436 + 0.8576 * Insulin_{FF}$ P <0.0003

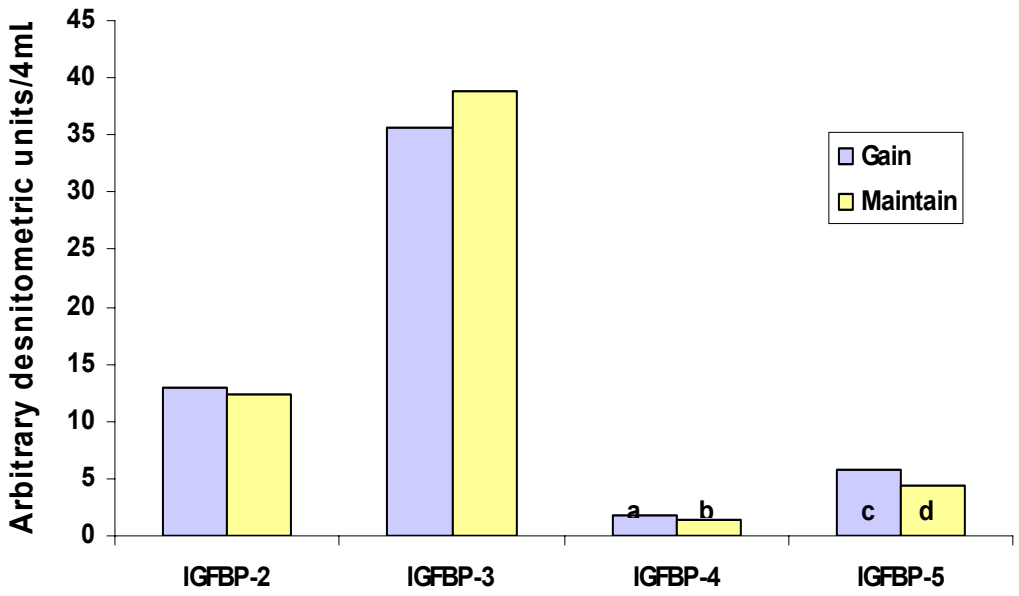

Figure 14. Influence of postpartum nutrition of primiparous beef cows on concentrations of IGF-I binding proteins (IGFBP) -2 (SE ± 1.1), -3 (SE ± 2.9), -4 (SE ± 0.1) and -5 (SE ± 0.4) in follicular fluid at 72 d after calving.

[a,b] Bars with different letters within a binding protein (BP) differ ($P < 0.05$).

[c,d] Bars with different letters within a binding protein (BP) differ ($P < 0.01$).

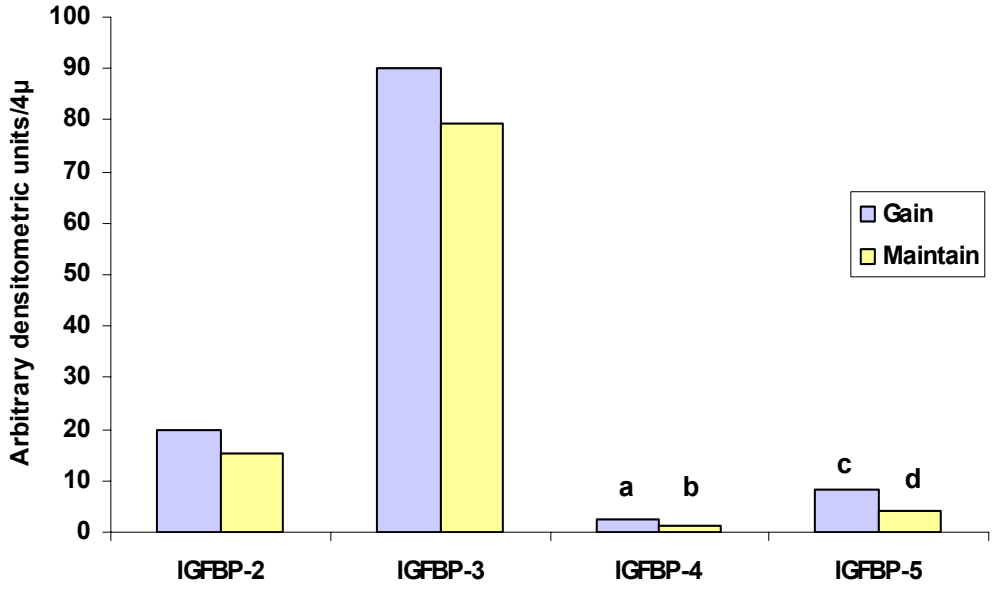

Figure 15. Influence of postpartum nutrition of primiparous beef cows on concentrations of IGF-I binding proteins (IGFBP) -2 (SE ± 2.0), -3 (SE ± 6.8), -4 (SE ± 0.3) and -5 (SE ± 1.1) in follicular fluid at 56 d after calving.

[a,b] Bars with different letters within a binding protein (BP) differ P < 0.05)

[c,d] Bars with different letters within a binding protein (BP) differ (P < 0.01)

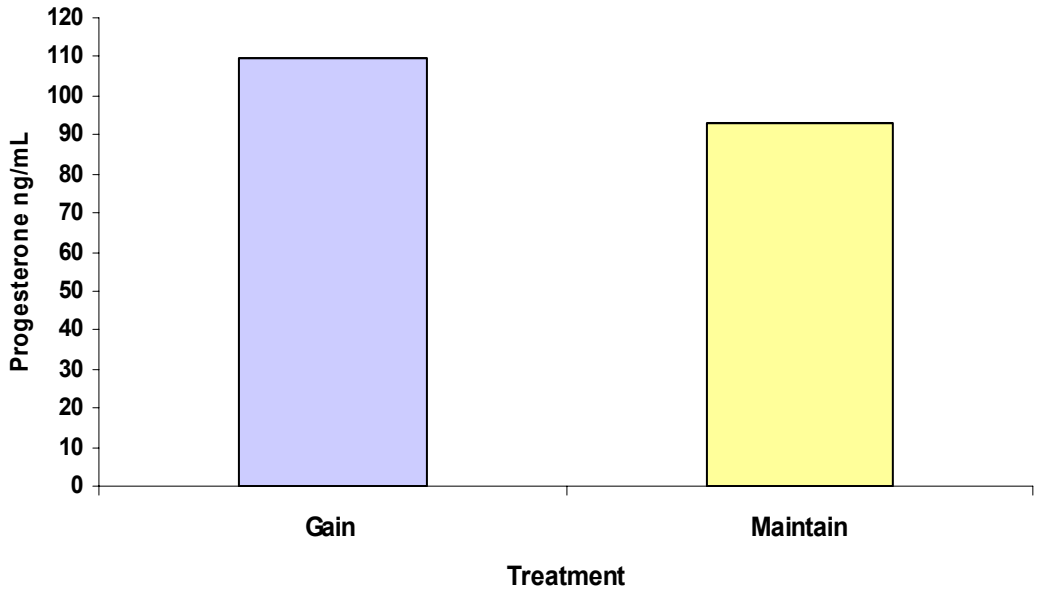

Figure 16. Effect of postpartum nutrition of beef cows on progesterone in follicular fluid at 56 d after calving (SE ± 30.4; P > 0.30).

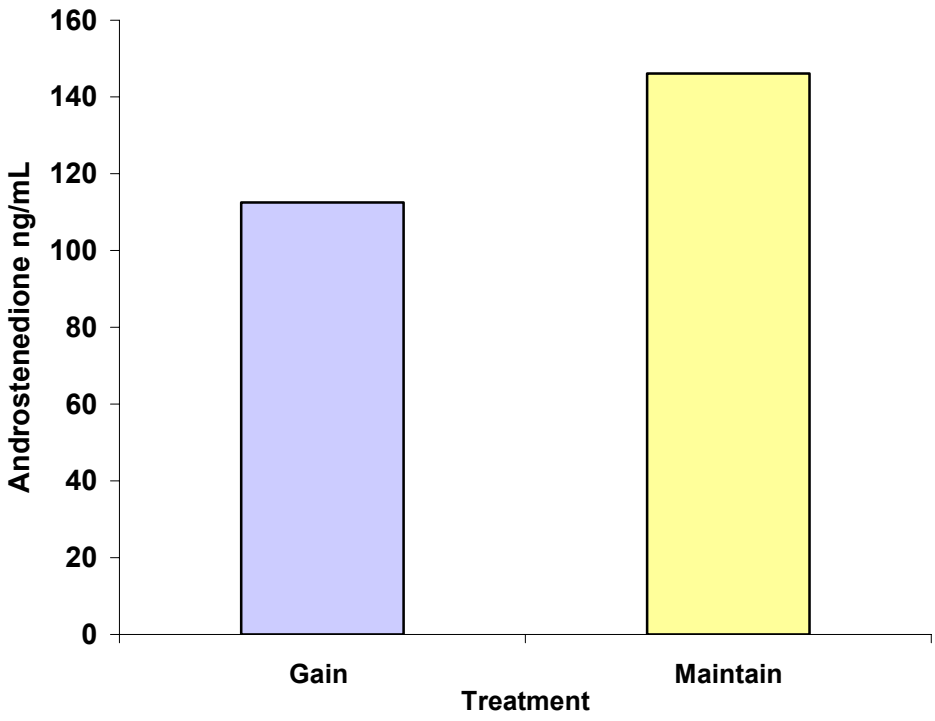

Figure 17: Effect of postpartum nutrition of beef cows on androstenedione in follicular fluid (SE ± 38.1; P > 0.30)

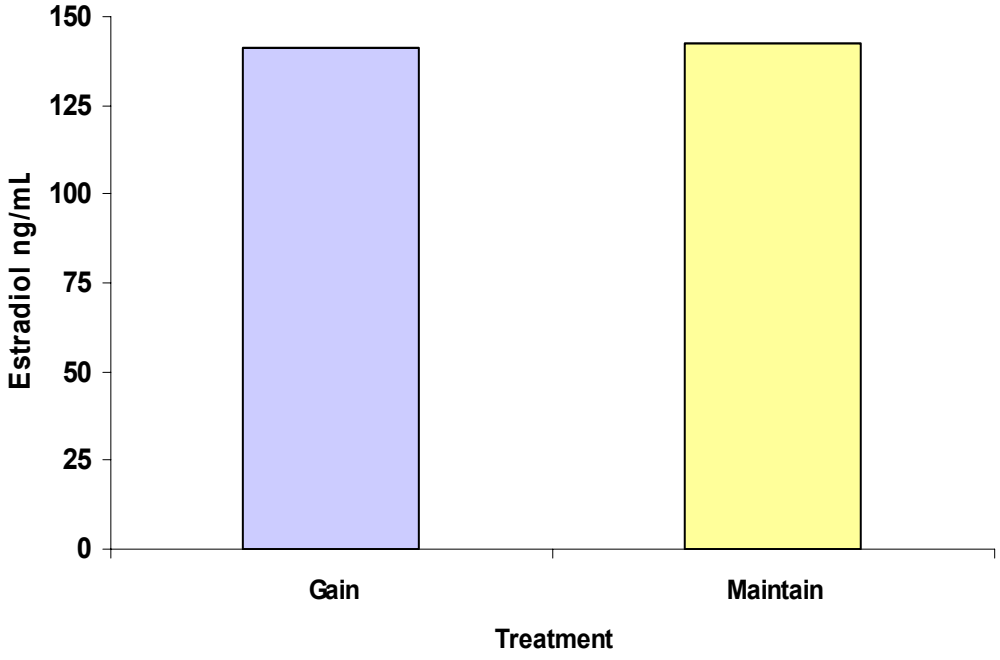

Figure 18. Effect of postpartum nutrition of beef cows on estradiol in follicular fluid (SE ± 30.4; P > 0.30).

CHAPTER IV

Influence of GnRH and estradiol on estrus and luteal activity of postpartum anestrous beef cows

ABSTRACT

The effect of treatment of postpartum anestrous beef cows with gonadotropin releasing hormone (GnRH) or estradiol on onset of first estrus and luteal activity was evaluated. Thirty-four cows were assigned based on body condition at calving and calving date to one of three treatments: GnRH, estradiol cypionate, or control. Ovarian follicles were evaluated by ultrasonography on two consecutive days at 40.5 ± 2.3 days) days after calving. Blood samples were collected twice a week, starting at 30-d after calving, and samples were taken on the day before treatment (d -1), d 0, d 3, d 6, and every 3 or 4 d until d 22 to determine luteal activity. Estrus was monitored with electronic mount detectors (HeatWatch) from d 30 until d 70 after calving. During 1 to 10 d after treatment, more GnRH cows (67%) had luteal activity than estradiol cows (25%), or control cows (0%). Treatment with GnRH increased ($P < 0.01$) the percentage of cows with luteal activity 11 to 20 d after treatment. Percentage of cows detected in estrus within 6 d after treatment was greater ($P < 0.05$) for estradiol (58%) than

GnRH (18%) or control cows (0%), and was similar for GnRH and control cows. The number of cows in estrus during 7 to 20 d after treatment was not influenced by treatment. Body condition score at calving did not influence the effect of treatment on estrus and luteal activity. Treatment of postpartum anestrous cows with GnRH initiated luteal activity without estrus, and treatment with estradiol increased the incidence of estrus without altering luteal activity.

Key Words: Estradiol, Estrus, GnRH, Luteal Activity, Postpartum Beef Cows

INTRODUCTION

A major cause of reduced reproductive efficiency in beef cows is an extended anestrous period after calving (Wettemann et al., 1980; Short et al., 1990, Wettemann et al., 2003). Cows must conceive within 85 d after calving to achieve the optimal 12 mo calving interval. Inadequate body condition score (BCS) at calving reduces pregnancy rates (Selk et al., 1988; Richards et al., 1989, Ciccioli et al., 2003). The number of follicular waves before the first ovulation was increased in thin cows (Murphy et al., 1990; Stagg et al., 1995). The first postpartum estrus in beef cows is usually preceded by a transient increase in plasma progesterone and is followed by a normal luteal phase (Perry et al., 1991; Looper et al., 2003). Treatment of anestrous cows with gonadotropin releasing hormone (GnRH) results in short-lived corpora lutea (CL; Kesler et al., 1980; Wettemann, 1982). The ability of dominant follicles to produce estradiol is limited during the postpartum anovulatory period (Spicer et al., 1986), and treatment of beef cows with estradiol did not alter the postpartum anestrous interval (Day et al., 1990). An understanding of the endocrine mechanisms that control postpartum anestrus is essential to decrease the interval from calving to conception. The objective of this study was to determine if treatment with GnRH or estradiol influences the onset of first estrus and luteal activity of postpartum anestrous beef cows.

MATERIALS AND METHODS

Body condition score (BCS; 1= emaciated, 9= obese; Wagner et al., 1988) of mature Hereford and Hereford x Angus anestrous cows was determined at calving and cows were classified as < 5 or ≥ 5. Thirty-two cows were stratified based on BCS and calving date, to one of three treatments; gonadotropin-releasing hormone (GnRH, 100 μg, i.m.; Cystorelin, Abbott Laboratories; n=11), estradiol cypionate (E; 1 mg; Pharmacia & Upjohn, n=12) or saline (control; i.m.; n=9). Ovaries of each cow were scanned by ultrasonography (Aloka 500-V ultrasound equipment with a 7.5-MHz probe; Corometrics Medical Systems, Wallingford, CT) on two consecutive d commencing at 40.5 ± 2.3 d after calving. Ultrasonography images were recorded with a VHD recorder (Panasonic PV-V4520; Matusushita Electric Corp. of America, Secuucus, NJ) and viewed at a later time to confirm the size of DF. Size of follicles was calculated as the mean of the longest and shortest diameters (Pierson and Ginther, 1988). Cows with a follicle at least 8 mm on the first day that increased in diameter by at least 0.5 mm on the second day, were assigned to treatment. Blood samples were collected twice a week before treatment, starting at 30 d after calving. Blood samples were collected on the day before treatment (d -1), d 0, d 3, d 6 and twice weekly until d 22 after treatment. Caudal vein blood was collected in vacutainers (10 mL) containing EDTA (0.1 ml of a 15% solution). Tubes were immediately placed on ice, centrifuged (2500 x g for 15 min) at 4 ^{o}C within 3 h after collection,

and plasma was recovered and stored at -20 ^{o}C until progesterone was quantified. Presence of luteal activity was determined when the concentration of progesterone was greater than 0.5 ng/mL. Estrus was monitored with electronic mount detectors (HeatWatch, DDx Inc., Denver, CO) from d 30 until d 70 postpartum and was defined as cows that received two or more mounts within 4 h (White et al., 2003). None of the treated cows exhibited estrus or had luteal activity before treatment. Only 7 of 39 cows had luteal activity before treatment and they were removed from the study.

Statistical Analysis

Percentage of cows in estrus within 6 d after treatment, and percentage with luteal activity during d 1 to 10 and d 11 to 20 after treatment were analyzed as a completely randomized design with a 2 x 3 factorial treatment structure using a generalized linear model (PROC GENMOD; SAS Inst., Inc., Cary, NC). The model included the effect of BCS at calving (< 5 or ≥ 5) and treatment as main effects, and the first order interaction. When treatment effects were significant, Fisher's exact test was used to compare response variables among treatments.

RESULTS

Body condition score at calving did not influence the effect of treatment on estrus and luteal activity ($P > 0.10$, Table 1). Means for the responses of thin (< 5 BCS) and moderate BCS (≥ 5) cows are reported.

Treatment of postpartum anestrous cows with estradiol increased the percentage of cows in estrus within 6 d after treatment (Figure 1). More cows treated with estradiol were in estrus within 6 d after treatment (58 %; $P < 0.01$) compared with cows treated with GnRH (18 %) or controls (0%). Treatment of cows with GnRH did not influence the percentage of cows in estrus compared with controls ($P > 0.10$).

The percentage of cows that had luteal activity within 10 d after treatment was greater ($P < 0.01$) for GnRH cows (66.7%) compared with cows treated with estradiol (33%), or control (0%) cows (Figure 2). The luteal response was similar ($P > 0.10$) for control and estradiol cows. The percentage of cows detected in estrus during d 7 to 20 d after treatment was not influenced by treatment (Table 2). In contrast, the percentage of cows with luteal activity during 11 to 20 d after treatment was greater ($P < 0.01$) for GnRH (66.7%) compared with estradiol (33.3%) or control (20%) cows.

Size of the dominant follicle at treatment of cows did not influence the response whether the dominant follicle was ≥ 11 mm in diameter or < 11 mm in diameter, the estrus and luteal responses were similar (Table 3).

DISCUSSION

Treatment of anestrous beef cows with GnRH initiated short luteal activity without estrus, indicating that GnRH caused an ovulatory surge of LH. Treatment with GnRH (100 µg) induces ovulation and luteinization of follicles in postpartum anestrous beef cows (Troxel et al., 1980; Wettemann et al., 1982). Infusion of GnRH (2 µg every hour for 13 d) in nutritionally induced anovulatory cows, stimulated LH secretion and induced resumption of luteal activity within 12 d in 75 % of the cows (Bishop and Wettemann, 1993; Vizcarra et al., 1997). Prado et al. (2002) quantified the dynamics of follicular growth in short- and long-term nutritionally induced anovulatory cows treated with GnRH. Concentrations of insulin-like growth factor-I were greater in large vs small follicles in cows that were anovulatory for 4 wk, but not in cows that were anovulatory for 18 wk. The percentage of cows initiating a new follicular wave was 75 % and 17 % for GnRH- and saline treated cows, respectively, during the 5-d treatment (Prado et al., 2002).

Release of gonadotropins in response to GnRH could vary due to several factors such as the reproductive status of the animal (Moss et al., 1981) or number of receptors in ewes (Moss et al., 1981) and cows (Wettemann et al., 1982; Looper et al., 2003). Wettemann et al. (1982) determined that the pituitary of postpartum suckled anestrous beef cow is responsive to GnRH and that maximum concentrations of LH in plasma occur about 2 hr after treatment.

Increases in sensitivity of the pituitary gland to GnRH and an increase secretion of GnRH are required for an ovulatory surge of LH in ewes (Nett et al., 1984). Wise et al. (1984) demonstrated that the density of GnRH receptors on gonadotropes determines their ability to respond to GnRH. Reduction of GnRH receptor numbers in ovariectomized ewes influenced tonic release of LH and maximal release was not affected unless the number of GnRH receptors was reduced by more than 50% (Wise et al., 1984). Number of GnRH receptors was increased in ovariectomized cows treated with estrogen (Looper et al., 2003). Furthermore, Rispoli and Nett (2005) indicated that regulation of GnRH receptor gene expression is influenced by many factors including steroid hormones, inhibin, activin and GnRH. These results indicate that pituitary responsiveness to GnRH is related to the number of receptors for GnRH.

Treatment with estradiol increased the incidence of estrus without altering luteal activity. Plasma concentration of estradiol is minimal during the luteal phase of the bovine estrous cycle (Wettemann et al., 1972; Glencross et al., 1973; Echternkamp and Hansel, 1973), increase during proestrus, and mediate the preovulatory LH release (Echternkamp and Hansel, 1973). Nancarrow et al. (1977), found that the positive feedback of estradiol on LH secretion was inhibited during the early postpartum period, however by the third week after calving most cows responded to treatment with estradiol and exhibited both estrus and an ovulatory surge of LH. There was a tendency for the time from estradiol treatment to maximal release of LH to be longer, and the maximum LH concentration to be less, in the early postpartum period than at later times

(Nancarrow et al., 1977). Most cows with suckling calves do not exhibit estrus or release LH in response to estradiol at 40 d after calving (Radford et al., 1978) whereas, estradiol induced an ovulatory-like surge of LH in most ovariectomized cows (Short et al., 1973; Short et al., 1979). When postpartum anestrous cows were implanted with estradiol, the incidence of short estrous cycles was reduced if ovulation occurred during the period of administration (Day et al., 1990). Ovariectomized cows (Forrest et al., 1981; Kesner and Covey, 1982) respond to exogenous estradiol, with preovulatory-like surges of LH. Schoenemann et al. (1985) found that estrogen can increase pituitary concentrations of LH. Estradiol induced a preovulatory-like surge of GnRH in the cerebrospinal fluid of ovariectomized cows, which was associated with a LH surge (Gazal et al., 1998). Looper et al. (2003), demonstrated that treatment of nutritionally induced anovulatory cows with estradiol, increased estradiol concentrations in plasma and increased the frequency and amplitude of LH pulses.

Venzke (1953), attempted to induce estrus and ovulation in ewes during the anestrous season by a single injection of estradiol cyclopentylpropionate. Although estrus was induced, ovulation did not occur. Similarly, estrous behavior was not induced in prepuberal heifers (Gonzalez-Padilla et al., 1975b) and in heifers (Swanson and McCarthy, 1978) treated with estradiol. Reames et al. (2005) found that if ovariectomized cows were treated with minimal doses of estradiol, the timing of onset of estrus was delayed relative to the time of the LH surge. Surges of LH were induced in ovariectomized cows when estradiol was

continuously infused in amounts to maintain concentrations of estradiol at 3 to 12 pg/mL. These amounts mimic concentrations that occur during proestrus.

Our results indicate that follicles did not ovulate in response to estradiol and probably the brain is refractory, and an ovulatory surge of LH is not induced at this stage postpartum in anestrous beef suckling cows. However, GnRH treatment induced ovulation or luteinization of dominant follicles, without estrous behavior.

IMPLICATIONS

Further studies are needed to determine factors that regulate GnRH neuron response to estradiol. Although the pituitary of postpartum anestrous beef cows is responsive to GnRH and releases LH, the hypothalamus probably does not respond to estradiol and release GnRH. Estrus can be induced in postpartum cows by treatment with estradiol but ovarian function is not initiated.

Table 1. Influence of BCS at calving on estrus (1-6 d), and luteal activity (1-10 d) after treatment of postpartum anestrous beef cows with estradiol or GnRH.

	Estrus		Luteal Activity	
Treatment	< 5 [a]	≥ 5	< 5	≥ 5
Control	0	0	0	0
Estradiol	58	60	28	40
GnRH	17	20	83	60

[a] Body Condition Score

Table 2. Incidence of estrus and luteal activity in postpartum beef cows within d 1-20 after treatment with estradiol or GnRH.

Treatment	Cows, no	Estrus during d 7-20 after trt, %	Luteal activity d 11-20 after trt, %
Control	9	40.0	20.0[a]
Estradiol	12	16.7	33.3[a]
GnRH	11	25.0	66.6[b]

[a,b] means in column with different superscript differ (P < 0.01)

Table 3. Influence of size of dominant follicle on estrus (%) during 1 to 6 d after treatment and luteal activity (%) within 10 d after treatment of anestrous beef cows with estradiol or GnRH.

	Estrus		LA	
Treatment	< 11 mm	≥ 11 mm	< 11 mm	≥ 11 mm
Saline	0	0	0	0
ECP	8.3	66.7	0	25
GnRH	25	33.3	25	41

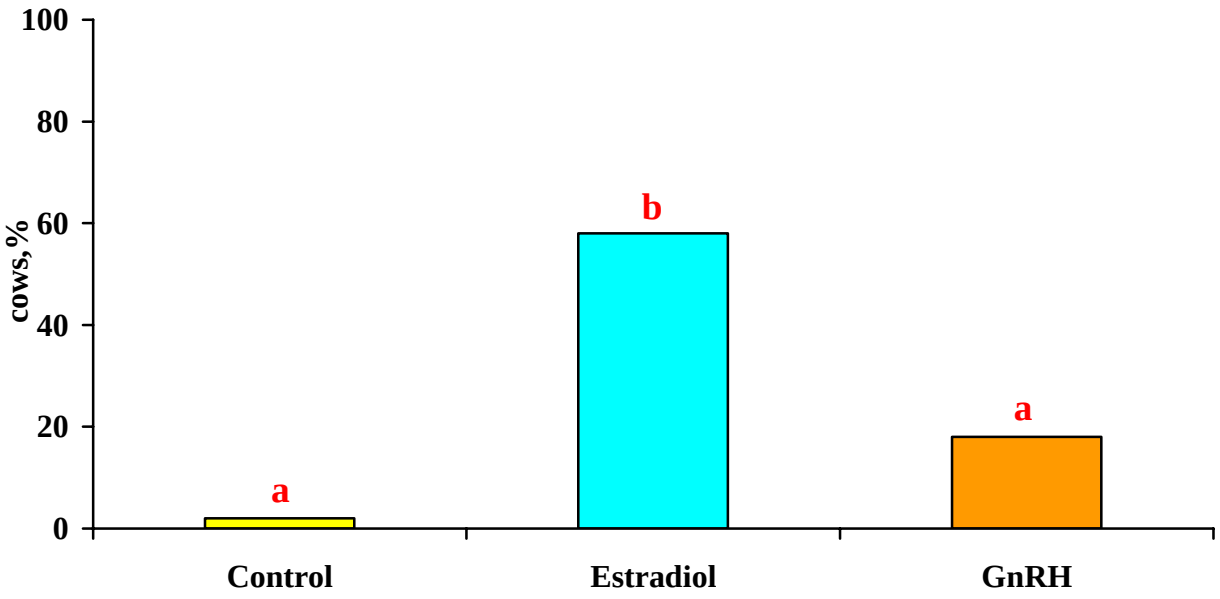

Figure 1: Incidence of estrus in postpartum beef cows within 6 d after treatmentwith estradiol or GnRH [a, b] Means differ (P < 0.01).

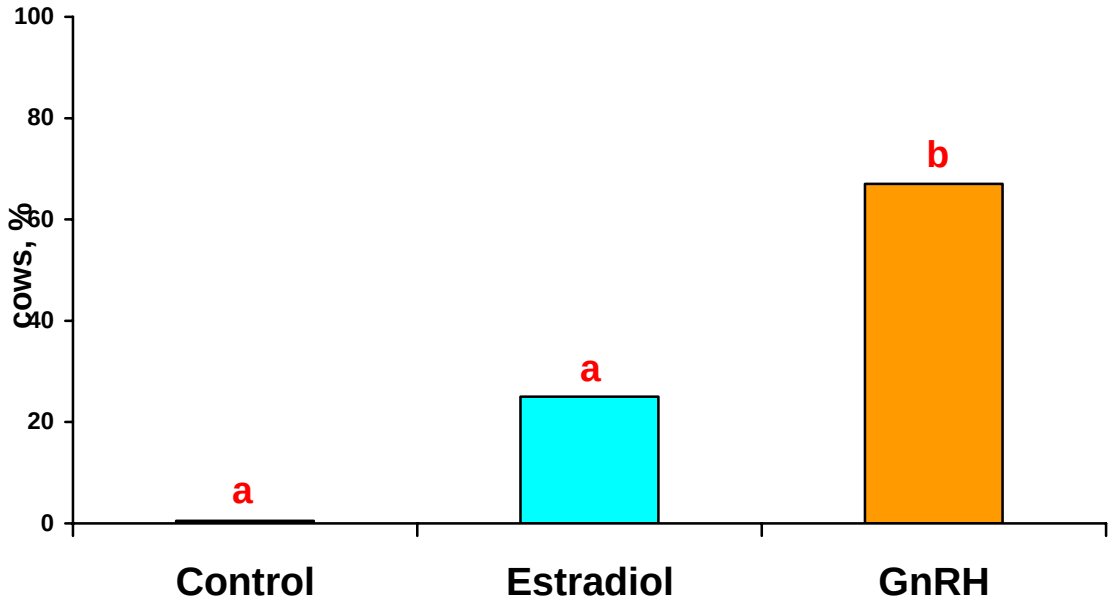

Figure 2. Luteal activity in postpartum beef cows within 10 d after treatment with estradiol or GnRH. [a, b] Means differ ($P < 0.01$).

CHAPTER V
SUMMARY AND CONCLUSIONS

Effects of nutrition on insulin-like growth factor-I (IGF-I) and insulin in plasma and dominant follicles (DF) were evaluated in two experiments at 72 ± 2d and at 56 ± 9 d after calving in anovulatory primiparous Angus x Hereford cows. Body condition score (BCS) at calving was 4.5 ± 0.1 in experiment 1 and 4.8 ± 0.2 in experiment 2. Cows were stratified based on BCS and calving date and randomly assigned to one of two nutritional treatments at calving: maintain (M), 2.27 kg of a 40% CP supplement per day and ad libitum hay; or gain (G), *ad libitum* access to a 50 % concentrate diet and hay. Body condition score at aspiration of the DF was greater for H than M cows and postpartum interval to luteal activity was longer for M cows than for H. Maximum size of DF was influenced by nutritional treatment at 72 d after calving but not at 56 d. Concentrations of IGF-I in FF were greater for H than M cows and plasma concentrations of IGF-I prior to aspiration were also greater in G than in M cows. Concentrations of insulin in FF and plasma were greater for G than M cows. Concentrations of IGFBP-4 and -5 in plasma were 30% greater ($P<0.01$) in G than M cows. Concentrations of IGFBP-4 and -5 in FF were 68 and 48%, respectively, greater for G than M cows and concentration of IGFBP-2 and -5 in plasma at follicular aspiration were positively correlated with follicle size. BCS at

calving was positively correlated with IGFBP-2, -4 and -5 in plasma at aspiration of follicles. Concentration of IGF-I in plasma at aspiration and in FF was positively correlated with IGFBP-3 and -4 in FF. Abundance of mRNA for aromatase, IGFBP-4 and -5, and for pregnancy-associated plasma protein-A were not affected by treatment.

The effect of treatment of postpartum anestrous beef cows with gonadotropin releasing hormone (GnRH) or estradiol on onset of first estrus and luteal activity was evaluated. Thirty-four cows were assigned based on body condition at calving and calving date to one of three treatments: GnRH, estradiol cypionate, or control. During 1 to 10 d after treatment, more GnRH cows had luteal activity than estradiol cows, or control cows. Treatment with GnRH increased the percentage of cows with luteal activity 11 to 20 d after treatment. Percentage of cows detected in estrus within 6 d after treatment was greater for estradiol than GnRH or control cows, and was similar for GnRH and control cows. The number of cows in estrus during 7 to 20 d after treatment was not influenced by treatment. Body condition score at calving did not influence the effect of treatment on estrus and luteal activity. Treatment of anestrous beef cows with GnRH initiated short luteal activity without estrus, indicating that GnRH caused an ovulatory surge of LH.

Treatment with estradiol increased the incidence of estrus without altering luteal activity. Our results indicate that follicles did not ovulate in response to estradiol and probably the brain was refractory and an ovulatory surge of LH was not induced at this stage postpartum in anestrus beef suckling cows

In conclusion, concentrations of IGF-I and insulin in FF may be related to follicular functions and changes in follicular fluid IGFBP concentrations rather than local translational regulation may have a role in dietary induced changes in postpartum follicular growth. The nutritionally induced increases in concentrations of IGF-I and insulin could have direct and/or indirect effects on the length of the postpartum anestrous interval. In addition, endocrine changes in DF may be associated with increased pregnancy rates at the first postpartum estrus in cows that receive greater nutrient intake.

Further studies are needed to determine factors that regulate secretion of GnRH in response to estradiol. Although the pituitary of postpartum anestrous beef cows is responsive to GnRH and releases LH, the hypothalamus does not respond to estradiol and release GnRH.

Elucidaton of (1) the mechanisms by which nutrient intake and body energy reserves regulate hypothalamo-hypophyseal-ovarian function in beef cows and (2) factors that influence the effects of estradiol on behavioral estrus and the ovulatory surge of LH, will result in development of management systems and/or treatments to decrease the interval from calving to conception in beef cows.

LITERATURE CITED

Acosta, B., G.K. Tarnavsky, T.E. Platt, D.L. Hamernik, J.L. Brown, H.M. Schoenemann, and J.J. Reeves. 1983. Nursing enhances the negative effect of estrogen on LH release in the cow. J. Anim. Sci. 57: 1530-1536.

Adamo, M., D. LeRoith, J. Simon, and J. Roth. 1988. Effect of altered nutritional states on insulin receptors. Ann. Rev. Nutr. 8: 149-166.

Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. H. Ko, and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertility 94: 177-188.

Ahima, R.S. and J.S. Flier. 2000. Leptin. Annu. Rev. Physiol. 62: 413-437.

Ahima, R. S., J. Kelly, J. K. Elmquist, and J. S. Flier. 1999. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. Endocrinology 140: 4923-4931.

Allrich, R. D. 1994. Endocrine and neural control of estrus in dairy cows. J. Dairy Sci. 77: 2738-2744.

Amstalden, M., R. Garcia, S.W. Williams, R.L. Stanko, S.E. Nizielski, C.D. Morrison, D.H. Keisler, and G. L. Williams. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: Relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63: 127-133.

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

Arase, K., J. S. Fisler, N. S. Shargill, D.A. York and G. A. Bray. 1988. Intracerebroventricular infusions of 3-OHB and insulin in a rat model of dietary obesity. Am. J. Physiol. 255: 974-981.

Arias, P., M. Rodriguez, B. Szwarcfar 115 Sinay, and A. J. Moguilevsky. 1992. Effect of insulin on LHRH release by perifused hypothalamic fragments. Neuroendocrinology 56: 415-418.

Armstrong, D.G., G. Baxter, C.G. Gutierrez, C. O. Hogg, A. L. Glazyrin, B.K. Campbell, T. A. Bramley, and R. Webb. 1998. Insulin-like growth factor binding protein -2 and -4 messenger ribonucleic acid expression in bovine ovarian follicles: effect of gonadotropins and developmental status. Endocrinology 139: 2146-2154.

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

Armstrong, J. D., and A. M. Benoit. 1996. Paracrine, autocrine, and endocrine factors that mediate the influence of nutrition on reproduction in cattle and swine. J. Anim. Sci. 74 (Suppl. 3): 18-35.

Armstrong, D.G., T. G. McEvoy, G. Baxter, J. J. Robinson, C. O. Hogg, K.J. Woad, R. Webb, and K. D. Sinclair. 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: Associations with the ovarian insulin-like growth factor system. Biol. Reprod. 64: 1624-1632.

Armstrong, D. G., G. Baxter, C. O. Hogg, and K. J. Woad. 2002. Insulin-like growth factor (IGF) system in the oocyte and somatic cells of bovine preantral follicles. Reproduction 123: 789-797.

Armstrong, D. G., J. G. Gong, and R. Webb. 2003. Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reprod. Suppl. 61: 403-414.

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

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

Bagley, C. P. 1993. Nutritional management of replacement beef heifers: A review. J. Anim. Sci. 71: 3155-3163.

Baile, C.A, M. A. Della-Fera, R. J. Martin. 2000. Regulation of metabolism and body fat mass by leptin. Annu. Rev. Nutr. 20: 105-127.

Baird, D. T. 1977. Evidence in vivo for the two-cell hypothesis of oestrogen synthesis by the sheep Graffian follicle. J. Reprod. Fertil. 50: 183-185.

Baird, D. T., and B. K. Campbell. 1998. Follicle selection in sheep with breed differences in ovulation. Mol. Cell. Endocrinol. 145: 89-95.

Bao, B., and J. A. Garverick. 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: A review. J. Anim. Sci. 76: 1903-1921.

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

Bauman, D. E. 1999. Bovine somatrotropin and lactation: From basic science to commercial application. Domest. Anim. Endocr. 17: 101-116.

Baxter, R. C., N. Hizuka, K. Takamo, S. R. Holman, and K. Asakawa K. 1993. Responses of insulin-like growth factor binding protein-1 (IGFBP-1) and the IGFBP-3 complex to administration of insulin-like growth factor-I. Acta Endocrinol. (Copenh.) 128: 101-118.

Beam, S. W., and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56: 133-142.

Beam, S. W., and W. R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81: 121-131.

Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 54: 411-424.

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

Bell, D. J., J. C. Spitzer, and G. L. Burns. 1998. Comparative effects of early weaning or once-daily suckling on occurrence of postpartum estrus in primiparous beef cows. Theriogenology 50: 707-715.

Bellows, R. A., R. E. Short, J. J. Urick, and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiple or single. J. Anim. Sci. 39: 589-596.

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

Besnard, N., C. Pisselet, D. Monniaux, and P. Monget. 1997. Proteolytic activity degrading insulin-like growth factor-binding protein-2, -3, -4, and -5 in healthy growing and atretic follicles in the pig ovary. Biol. Reprod. 56: 1050-1058.

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

Bishop, D. K., R. P. Wettemann, and L. J. Spicer. 1994. Body energy reserves influences the onset of luteal activity after early weaning of beef cows. J. Anim. Sci. 72: 2703-2708.

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

Boland, N. I., P. G. Humpherson, H. J. Leese, and R. G. Gosden. 1993. Pattern of lactate production and steroidogenesis during growth and maturation of mouse ovarian follicles in vitro. Biol. Reprod. 48: 798-806.

Bonnet, M., I. Gourdou, C. Leroux, Y. Chilliard, and J. Djiane. 2002. Leptin expression in the ovine mammary gland: Putative sequential involvement of adipose, epithelial, and myoepithelial cells during pregnancy and lactation. J. Anim. Sci. 80: 723-728.

Borger, M. L., W. A. Greene, and D. L. Grooms. 1996. Electronic pressure sensing system as an alternative to visual observation in beef cows with or without syncro-mate B treatment. J. Anim. Sci. 74 (Suppl 1): 244 (Abstr).

Bossis, I., R. P. Wettemann, S. D. Welty, J. A. Vizcarra, L. J. Spicer, and M. G. Diskin. 1999. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function preceding cessation of ovulation. J. Anim. Sci. 77: 1536-1546.

Bossis, I., R. P. Wettemann, S. D. Welty, J. Vizcarra, and L. J. Spicer. 2000. Nutrionally induced anovulation in beef heifers: Ovarian and endocrine function during realimentation and resumption of ovulation. Biol. Reprod. 62: 1436-1444.

Breier, B. H., P. D. Gluckman, and J. J. Bass. 1988. Influence of nutritional status and oestradiol-17β on plasma growth hormone, insulin-like growth factors-I and -II and the response to exogenous growth hormone in young steers. J. Endocrin. 118: 243-250.

Burns, K. H., and M. M. Matzuk. 2002. Minireview: Genetic models for the study of gonadotropin actions. Endocrinology 143: 2823-2835.

Busby, W. H., D. K. Snyder, and D. R. Clemmons. 1988. Radioinmmunoassay of a 26,000-dalton plasma insulin-like growth factor-binding protein: Control by nutritional variables. J. Clin. Endocrinol. Metab. 67: 1225-1230.

Butler, S. T., A. L. Marr, S. H. Pelton, R. P. Radcliff, M. C. Lucy, and W. R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J. Endocrinol. 176: 205-217.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60-61: 449-457.

Campbell, B. K., R. J. Scaramuzzi, and R. Webb. 1995. Control of antral follicular development and selection in sheep and cattle. J. Reprod. Fertil. Suppl. 49: 335-350.

Canfield, R. W. and W. R. Butler. 1990. Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Domest. Anim. Endocrinol. 7: 323-330.

Cao, G. Y., R. V. Considine, and R. B. Lynn. 1997. Leptin receptors in the adrenal medulla of the rat. Am. J. Physiol. 273: 448-452.

Cara, J. F., and R. L. Rosenfield. 1988. Insulin-like growth factor I and insulin potentiate luteinizing hormone- induced androgen synthesis by rat ovarian thecal-intertitial cells. Endocrinology 123: 733-739.

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

Cataldo, N. A., and L. C. Giudice. 1992. Insulin-like growth factor binding protein profiles in human ovarian follicular correlate with follicular functional status. J. Clin. Endocrinol. Metab. 74: 821-829.

Chamberlain, C. S., and L. J. Spicer. 2001. Hormonal control of ovarian cell production of insulin-like growth factor binding proteins. Mol. Cell. Endocrinol. 182: 69-81.

Chandrasekher, Y. A., H. J. H. M. V. Dessel, B. C. J. M. Fauser, and L. C. Giudice. 1995. Estrogen- but not androgen-dominant human ovarian follicular fluid contain an IGFBP-4 protease. J. Clin. Endocrinol. Metab. 80: 2734-2739.

Chehab, F. F., K. Mounzih, R. Lu, and M. E. Lim. 1997. Early onset of reproductive function in normal female mice treated with leptin. Science 275: 88-90.

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

Cheung, C. C., J. E. Thornton, J. L. Kuijper, D. S. Weigle, D. K. Clifton, and R.A. Steiner. 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 138: 855-858.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Leroux, J. Djiane, and F. Bocquier. 2001. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest. Anim. Endocr. 21: 271-295.

Chun, S-Y., H. Billig, J.L. Tilly, I. Furuta, A. Tsafriri, A.J.W. Hsueh. 1994. Gonadotropin suppression of apoptosis in cultured preovulatory follicles: Mediatory role of endogenous insulin-like growth factor-I. Endocrinology 135: 1845-1853.

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. W. White, D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81: 3107-3120.

Cioffi, J. A., J. Van Blerkom, M. Antaczak, A. Shafer, S. Wittmer, and H. R. Snodgrass. 1997. The expression of leptin and its receptors in pre-ovulatory human follicles. Mol. Hum. Reprod. 3: 467-472.

Clemmons, D. R. A. Klibanski, L. E. Underwood, J. W. McArthur, E. C. Rigway, I. Z. Beitins, and J. J. Van Wyk. 1981. Reduction of plasma immunoreactive somatomedin-C during fasting in humans. J. Clin. Endocrinol. Metab. 53: 1247-1250.

Clemmons, D. R., D. K. Snyder, and W. H. Busby. 1991. Variables controling secretion of insulin-like growth factor binding protein-2 in normal human subjects. J. Clin. Endocrinol. Metab. 73: 727-733.

Clemmons, D. R., and L. E. Underwood. 1991. Nutritional regulation of IGF-I and IGF binding proteins. Ann. Rev. Nutr. 11: 393-404.

Clemmons, D. C. 1993. IGF binding proteins and their functions. Mol. Reprod. Dev. 35: 368-375.

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

Conover, C. A., G. F. Faessen, K. E. Ilg, Y. A. Chandrasekher, M. Christiansen, M. T. Overgaard, C. Oxvig, and L. C. Giudice. 2001. Pregnancy-associated plasma protein-a is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. Endocrinology 142: 2155-2158.

Conover, C. A., C. Oxvig, M. T. Overgaard, M. Christiansen, and L. C. Giudice. 1999. Evidence that the insulin-like growth factor binding protein-4 protease in human ovarian follicular fluid is pregnancy associated plasma protein-A. J. Clin. Endocrinol. Metab. 84: 4742-4745.

Corah, L. R., T. G. Dunn, and C. C. Kaltenbach. 1975. Influence of prepartum nutrition on the reproductive performance of beef females and the performance of their progeny. J. Anim. Sci. 41: 819-824.

Counts, D. R., H. Gwirtsman, L. M. S. Carlsson, M. Lesem, and G. B. Cutler. 1992. The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. J. Clin. Endocrinol. Metab. 75: 762-767.

Cunningham, M. J., D. K. Clifton, and R. A. Steiner. 1999. Leptin's actions on the reproductive axis: Perspectives and mechanisms. Biol. Reprod. 60: 216-222.

Cutshaw, J.L., J. F. Hunter, and G.L., Williams. 1991. Effects of transcutaneous thermal and electrical stimulation of the teat on pituitary luteinizing hormone prolactin and oxytocin secretion in ovariectomized estradiol treated beef cows following acute weaning. Theriogenology **37:** 915–934.

Cwyfan-Hughes, S. C., H. D. Mason, S. Franks, and J. M. Holly. 1997. The insulin-like growth factors (IGFs) in follicular fluid are predominantly bound in the ternary complex. J. Endocrinol. 155: R1-R4.

Daniel, J.A., M.G. Thomas, C.S. Hale, J.M. Simmons, and D.H. Keisler. 2000. Effect of cerebroventricular infusion of insulin and (or) glucose on hypothalamic expression of leptin receptor and pituitary secretion of LH in diet-restricted ewes. Domest. Anim. Endocr. 18: 177-185.

Darwash, A.O., G.E. Lamming, and L.M. Hicking. 2001. Oestrus in relation to peak oestradiol level in ovariectomized Galloway cows. Anim. Sci. 72: 401-405.

Davoren, J. B., and A. J. Hsueh. 1984. Insulin enhances fsh-stimulated steroidogenesis by cultured rat granulosa cells. Mol. Cell Endocrinol. 35: 97-105.

Day, M. L., R. M. Dyer, G. W. Wilson, and W.F. Pope. 1990. Influence of estradiol on duration of anestrus and incidence of short estrous cycles in postpartum cows. Domest. Anim. Endocr. 7: 19-25.

de la Sota, R., F. Simmen, T. Diaz, and W. Thatcher. 1996. Insulin-like growth factor system in bovine first-wave dominant and subordinate follicles. Biol. Reprod. 55: 803-812.

DeRouen, S. M., D.E. Franke, D.G. Morrison, W.E. Wyatt, D.F. Coombs, T.W. White, P.E. Humes, and B.B. Greene. 1994. Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. J. Anim. Sci. 72: 1119-1125.

Dhyvetter, D. V., and J. S. Caton. 1996. Manipulation of reproduction and lactation with supplementation in beef cattle. In: Proc 3rd Grazing Livest Nutr Conf, Proc West Sec Amer Soc Anim Sci 47 (Suppl. 1). 83-93.

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

Diskin, M. G., D. R. Mackey, J. F. Roche, and J. M. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci. 78: 345-370.

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

Ducy, P., M. Amling, S. Takeda, M. Priemel, A. F. Schilling, F. T. Beil, J. Shen, C. Vinson, J. M. Rueger, and G. Karsenty. 2000. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 100: 197-207.

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

Dunn, T. G., and C. C. Kaltenbach. 1980. Nutrition and the postpartum interval of the ewe, sow and cow. J. Anim. Sci. 51 (Suppl 2): 29-39.

Dyer, C. J., J. M. Simmons, R. L. Matteri, and D. H. Keisler. 1997. Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions in well-fed and feed-restricted ewes. Domest. Anim. Endocr. 14: 119-128.

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: 1362-1370.

Echternkamp, S.E., H.J. Howard, A.J. Roberts, J. Grizzle, and T. Wise. 1994. Relationships among concentrations of steroids, insulin-like growth factor-I, and insulin-like growth factor binding proteins in ovarian follicular fluid of beef cattle. Biol. Reprod. 51: 971-981.

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

El-Hefnawy, T., S. Ioffe, and M. Dym. 2000. Expression of the leptin receptor during germ cell development in the mouse testis. Endocrinology 141: 2624-2630.

El-Roeiy, A., X. Chen, V. J. Roberts, S. Shimasaki, N. Ling, and D. LeRoith. 1994. Expression of the genes encoding the insulin-like growth factors, the IGF and insulin receptors, and IGF-binding proteins-1-6 and the localization of their gene products in normal and polycystic ovary syndrome ovaries. J. Clin. Endocrinol. Metab. 78: 1488-1496.

El- Roeiy, A., X. Chen, V. J. Roberts, D. LeRoith, C. T. Roberts and S.S.C. Yen. 1993. Expression of insulin-like growth factor-I (IGF-I) and IGF-II and the IGF-I, IGF-II, and insulin receptor genes and localization of the gene products in the human ovary. J. Clin. Endocrinol. Metab.77: 1411-1418.

Eppig, J. J. 2001. Oocyte control of ovarian follicular development and function in mammals. Reproduction 122: 829-838.

Etherton, T. D., and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev. 78: 745-761.

Farris, E. J. 1954. Activity of dairy cows during estrus. J.A.V.M.A. 25: 117-120.

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

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

Finn, P. D., M. J. Cunningham, K. Y. Pau, H. G. Spies, D. K. Clifton, and R. A. Steine. 1998. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology 139: 4652-4662.

Floyd, L. N. 2001. Effect of the number of cows in estrus and confinement area on estrous behavior of beef cows. MSc, Oklahoma State University, Stillwater Oklahoma.

Foote, R. H. 1975. Estrus detection and estrus detection aids. J. Dairy Sci. 58: 248-256.

Forrest, D. W., C.C. Kaltenbach, and T. G. Dunn. 1981. Estriol- and estradiol-17β- induced luteinizing hormone release in ovariectomized cows and ewes. J. Anim. Sci. 52: 1106-1113.

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

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

Fortune, J. E., J. Sirois, and S. M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology 29: 95-107.

Fortune, J. E., J. Sirois, A. M. Turzillo, and M. Lavoir. 1991. Follicle selection in domestic ruminants. J. Reprod. Fertil. Suppl. 43: 187-198.

Fortune, J. E., G. M. Rivera, A. C. O. Evans, and A. M. Turzillo. 2001. Differentiation of dominant versus subordinate follicles in cattle. Biol. Reprod. 65: 648-654.

Fortune, J. E., G. M. Rivera, and M. Y. Yang. 2004. Follicular development: The role of the follicular microenvironment in selection of the dominant follicle. Anim. Reprod. Sci. 82-83: 109-126.

Frisch, R. H. 1980. Pubertal adipose tissue: Is it necessary for normal sexual maturation? Evidence from the rat and human female. Fed. Proc. 39: 2395-2400.

Frühbeck, G. J., J. Gomez-Ambrosi and J. A. Martinez. 1999. Pre- and postprandial expression of the leptin receptor splice variants OB-Ra and ON-Rb in murine peripheral tissues. Physiol. Res. 48: 189-195.

Funston, R. N., J. G. E. Seidel, J. Klindt, and A. J. Roberts. 1996. Insulin-like growth factor-I and insulin-like growth factor binding proteins in bovine serum and follicular fluid before and after the preovulatory surge of luteinizing hormone. Biol. Reprod. 55: 1390-1396.

Gadsby, J. E., J. A. Lovdal, S. Samaras, J. A. Barber, and J. M. Hammond. 1996. Expression of mRNA or IGF-I and IGFBP in porcine corpora lutea. Biol. Reprod. 54: 339-346.

Garcia, M. R., Amstalden, M., Williams, S.W., Stanko, R.L. Morrison, C.D., Keisler, D.H., Nizielski, S.E., and G.L. Williams. 2002. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J. Anim. Sci. 80: 2158-2167.

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

Ge, J., W. E. Nicholson, D. M. Plotner, C. E. Farin, and J. E. Gadsby. 2000. IGF-I receptor mRNA and protein expression in pig corpora lutea. J. Reprod. Fertil. 120: 109.

Glencross, R. G., I. B. Munro, B. E. Senior and G. S. Pope. 1973. Concentrations of oestradiol-17 beta, oestrone and progesterone in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta Endocrinol. (Copenh.) 73: 374-378.

Glister, C., D. S. Tannetta, N. P. Groome, and P. G. Knight. 2001. Interactions between follicle-stimulating hormone and growth factors in modulating secretion of steroids and inhibin-related peptides by nonluteinized bovine granulosa cells. Biol. Reprod. 65: 1020-1028.

Gomez, E., J. J. Tarin, and M. D. Pellicer. 1993. The role of gonadotropins and growth factors. Fertil. Steril. 60: 40-46.

Gong, J. G., B. K. Campbell, T. A. Bramley, and R. Webb. 1996. Treatment with recombinant bovine somatotropin enhances ovarian follicle development and increases the secretion of IGF-I by ovarian follicles in the ewes. Anim. Reprod. Sci. 41: 13-26.

Gong, J. G., D. McBride, T. A. Bramley, and R. Webb. 1994. Effects of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on bovine granulosa cell steroidogenesis in vitro. J. Endocrinol. 143: 157-164.

Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postparutm period. Reproduction 123: 419-427.

Gonzalez-Padilla, E., G. D. Niswender, and J. N. Wiltbank. 1975. Puberty in beef heifers. II. Effect of injections of progesterone and estradiol-17b on serum LH, FSH and ovarian activity. J. Anim. Sci. 40: 1105-1109.

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

Granger, A. L., W. E. Wyatt, W. M. Craig, D. L. Thompson, and F. G. Hembry. 1989. Effects of breed and wintering diet on growth, puberty and plasma concentrations of growth hormone and insulin-like growth factor-I in heifers. Domest. Anim. Endocr. 6: 253.

Griffith, M.K., and G.L. Williams. 1996. Roles of maternal vision and olfaction in suckling-mediated inhibition of luteinization hormone secretion, expression of maternal selectivity, and lactational performance of beef cows. Biol. Reprod. 54: 761-767.

Grimes, R. W., J.A. Barber, S. Shimasaki, N. Ling, and J. M. Hammond. 1994. Porcine ovarian granulosa cells secrete insulin-like growth factor-binding proteins-4 and -5 and express their messenger ribonucleic acids: regulation by follicle-stimulating hormone and insulin-like growth factor-I. Biol. Reprod. 50: 695-701.

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

Gruaz, N. M., M. Lalaoui, D. D. Pierroz, P. Englaro, P. C. Sizonenko, W. F. Blum, and M. L. Aubert. 1998. Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food-restricted female rats. Neuroendocrinology 10: 627-633.

Guedon, L., J. Saumande, and B. Desbals. 1999. Relationships between calf birth weight, prepartum concentrations of plasma energy metabolites and resumption of ovulation postpartum in limousine suckled beef cows. Theriogenology 52: 779-789.

Gutierrez, C. G., J. Oldham, T. A. Bramley, J. G. Gong, B. K. Campbell, and R. Webb. 1997. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. J. Anim. Sci. 75: 1876-1884.

Gutierrez, C. G., A. L. Glazyrin, G. W. Robertson, B. K. Campbell, J. G. Gong, T. A. Bramley and R. Webb. 1997. Ultra-structural characteristics of bovine granulosa cells associated with maintenance of oestradiol production in vitro. Mol. Cell. Endocrinol. 134: 51-58.

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

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

Hagemann, L. J. 1999. Influence of the dominant follicle on oocytes from subordinate follicles. Theriogenology 51: 449-459.

Hamilton, T. D., J. A. Vizcarra, R. P. Wettemann, B. E. Keefer, and L. J. Spicer. 1999. Ovarian function in nutritionally induced anestrous cows: Effects of exogenous gonadotrophin-releasing hormone in vivo and effect of insulin and insulin-like growth factor I in vitro. J Reprod. Fertil. 117: 179-187.

Hammond, J. 1949. Physiology of reproduction in relation to nutrition. Br. J. Nutr. 3: 79-83.

Hansel, W. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. J. Reprod. Fertil. Suppl. 1: 33-48.

Hardie, L., P. Trayhurn, D. Abramovich, and P. Fowler. 1997. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clin. Endocrinol. (Oxf.) 47: 101-106.

Harrison, L. M., and R. D. Randel. 1986. Influence of insulin and energy intake on ovulation rate, luteinizing hormone and progesterone in beef heifers. J. Anim. Sci. 63: 1228-1235.

Havrankova, J. J. Roth, and M. J. Brownstein. 1979. Concentrations of insulin and insulin receptors in the brain are independent of peripheral insulin levels. Studies of obese and streptozotocin-treated rodents. J. Clin. Invest. 64: 636-642.

Hawkins, D. E., M. K. Petersen, M. G. Thomas, J. E. Sawyer, and R. C. Waterman. 1999 Can beef heifers and young postpartum cows be physiologically and nutritionally manipulated to optimize reproductive efficiency? Proc. Am. J. Anim. Sci. http://www.asas.org/jas/symposia/proceedings/0928.pdf. Accessed Dec. 8, 2005.

Helmer, S.D., and J.H. Britt. 1985. Mounting behavior as affected by stage of estrous cycle in Holstein heifers. J. Dairy Sci. 68: 1290-1296.

Heiman, M. L, R. S. Ahima, L. S. Craft, B. Schoner. T.W. Stephens, and J. S. Flier. 1997. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 138: 3859-3863.

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

Hernandez, E. R., C. T. Roberts, D. Leroit, and E. Y. Adashi. 1989. Rat ovarian IGF-I gene expression is granulosa cell selective: 5'-untranslated mRNA variant representation and hormonal regulation. Endocrinology 125: 572-575.

Hess, B. W., S. L. Lake, E. J. Scholljegerdes, T. R. Weston, V. Nayigihugu, J. D. C. Molle, and G. E. Moss. 2005. Nutritional controls of beef cow reproduction. J. Anim. Sci. 83 (E. Suppl.): E90-E106.

Hettinger, A. M., M. R. Allen, B. R. Zhang, D. W. Goad, J. R. Malayer, and R. D. Geisert. 2001. Presence of the acute phase protein, bikunin, in the endometrium of gilts during estrous cycle and early pregnancy. Biol. Reprod. 65: 507-513.

Hileman, S. M., K. K. Schillo, and J. B. Hall. 1993. Effects of acute, intracerebroventricular administration of insulin on serum concentrations of luteinizing hormone, insulin and glucose in ovariectomized lambs during restricted and ad libitum feed intake. Biol. Reprod. 48: 117-124.

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

Houseknecht, L. K., and C. P. Portocarrero. 1998. Leptin and its receptors: Regulators of whole-body energy homeostasis. Dom. Anim. Endocr. 15: 457-475.

Hughes, S. C., H. D. Mason, S. Franks, and J. M. Holly. 1997. The insulin-like growth factors (IGFs) in follicular fluid are predominantly bound in the ternary complex. J. Endocrinol. 155: R1-4.

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

Hwa, V., Y. Oh, and R. G. Rosenfeld. 1999. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr. Rev. 20: 761-787.

Imakawa, K., M. L. Day, M. Garcia-Winder, D. D. Zalesky, R. J. Kittok, B. D. Schanbacher, and J. E. Kinder. 1986. Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. J. Anim. Sci. 63: 565-571.

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

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

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

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

Ireland, J. J., and J. F. Roche. 1983. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: Changes in concentration of hormones in follicular and specific binding of gonadotropins to follicles. J. Anim. Sci. 57: 157-167.

Ireland, J. J., and J. F. Roche. 1983. Growth and differentiation of large antral follicle after spontaneous luteolysis in heifers: Changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J. Anim. Sci. 57: 157-167.

Jia, X. C., J. Kalmijn, and A. J. Hsueh. 1986. Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. Endocrinology 118: 1401-1409.

Jo, M., C. M. Komar, and J. E. Fortune. 2002. Gonadotropin surge induces two separate increases in messenger RNA for progesterone receptor in bovine preovulatory follicles. Biol. Reprod. 67: 1981-1988.

Jones, E. J., J. D. Armstrong, and R. W. Harvey. 1991. Changes in metabolites, metabolic hormones, and luteinizing hormone before puberty in Angus, Braford, Charolais, and Simmental heifers. J. Anim. Sci. 69: 1607-1615.

Kalra, S. P., and P. S. Kalra. 1996. Nutritional infertility-the role of the interconnected hypothalamic neuropeptide Y-galanin-opiod network. Front. Neuroendocrinol. 17: 371-401.

Kane, K. K., D. E. Hawkins, G. D. Pulsipher, D. J. Dennniston, C. R. Krehbiel, M. G. Thomas, M. K. Petersen, D. M. Hallford, M. D. Remmenga, A. J. Roberts, and D. H. Keisler. 2004. J. Anim. Sci. 82: 283-291.

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

Keisler, D. H., and M. C. Lucy. 1996. Perception and interpretation of the effects of undernutrition on reproduction. J. Anim. Sci. 74(Suppl.3): 1-17.

Keisler, D. H., J. A. Daniel, and C. D. Morrison. 1999. The role of leptin in nutritional status and reproductive function. J. Reprod. Fertil. Suppl. 54: 425-435.

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

Kesner J. J., and E. M. Convey. 1982. Interaction of estradiol and luteinizing hormone releasing hormone on follicle stimulating hormone release in cattle. J. Anim. Sci. 54: 817-821.

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

Kershaw, E. E., and J. S. Flier. 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 89: 2548-2556.

Kiddy, C. A. 1977. Variation in physical activity as an indication of estrus in dairy cows. J. Anim. Sci. 60: 235-243.

King, G.J., J.F. Hurnik, and H.A. Robertson. 1976. Ovarian function and estrus in dairy cows during early lactation. J. Anim. Sci. 42: 688-692.

Kirby, C. J., J. D. Armstrong, B. G. Huff, R. L. Stanko, R. W. Harvey, E. P. Heimer, and R. M. Campbell. 1993. Changes in serum somatotrophin, somatotrophin mRNA, and serum and follicular insulin-like growth factor-I in response to feed restriction in cows actively immunized against growth hormone-releasing factor. J. Anim. Sci. 71: 3033-3042.

Ko, J. C. H., J. P. Kastelic, M. R. D. Campo, and O. J. Ginther. 1991. Effects of a dominant follicle on ovarian follicular dynamics during the oestrous cycle in heifers. J. Reprod. Fertil. 91: 511-519.

Kobayashi, Y., C.K. Boyd, C.J. Bracken, W.R. Lamberson, D.H. Keisler, and M.C. Lucy. 1999. Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by a specific down-regulation of GHR 1A that is associated with decreased insulin-like growth factor I. Endocrinology 140: 3947-3954.

Kobayashi, Y., C. K. Boyd, B. L. McCormack, and M. C. Lucy. 2002. Reduced insulin-like growth factor-i after acute feed restriction in lactating dairy cows is independent. J. Dairy Sci. 85: 748-754.

Krisher, R. L. The effect of oocyte quality on development. 2004. J. Anim. Sci. E-Suppl. 82: E14-23.

Kulick, L. J., K. Kot, M. C. Wiltbank, and O. J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 52: 913-921.

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

Lalman, D. L., J. E. Williams, B. W. Hess, M. G. Thomas, and D. H. Keisler. 2000. Effect of dietary energy on milk production and metabolic hormones in thin, primiparous beef heifers. J. Anim. Sci. 78: 530-538.

Lamb, G.C., B.L. Miller, J.M. Lynch, K.E. Thompson, J. S. Heldt, C.A. Loest, D.M. Grieger, and J.S. Stevenson. 1999. Twice daily suckling but not milking with calf presence prolongs postpartum anovulation. J. Anim. Sci. 77: 2207-2218.

Landau, S., R. Braw-Tal, M. Kaim, A. Bor, and I. Bruckental. 2000. Preovulatory follicular status and diet affect the insulin and glucose content of follicles in high-yielding dairy cows. Anim. Reprod. Sci. 64: 181-197.

Lawrence, J. B., C. Oxvig, M.T. Overgaard, L. Sottrup-Jensen, G.J. Gleich, L.G. Hays, J.R. Yates III, and C.A. Conover. 1999. The insulin-growth factor-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc. Natl. Acad. Sci. U.S.A. 96: 3149-3153.

Leeuwenberg, B. R., N. L. Hudson, L. G. Moore, P. R. Hurst and K.P. McNatty. 1996. Peripheral and ovarian IGF-I concentration during the ovine estrous cycle. J. Endocrinol. 148: 281-289.

Lehrer, A. R., G. S. Lewis, and E. Aizinbud. 1992. Estrus detection in cattle: Recent developments. Anim. Reprod. Sci. 28: 355-361

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

Lents, C. A. R.P. Wettemann, F.J. White, I. Rubio, N.H. Ciccioli, L.J. Spicer, D.H. Keisler and M.E. Payton. 2005. Influence of nutrient intake and body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. J. Anim. Sci. 83: 586-596.

Lents, C. A., F. J. White, D. L. Lalman, and R. P. Wettemann. 2000. The effects of body condition and protein supplementation of postpartum beef cows on estrous behavior and follicle size. J. Anim. Sci. 78 (Suppl. 1): 205 (Abstr.).

Looper, M. L. 1999. Body energy reserves and steroids regulate gonadotropins and reproduction in beef cows. PhD Thesis. Oklahoma State University, Stillwater, Oklahoma.

Looper, M. L. , J.A. Vizcarra, R.P. Wettemann, J.R. Malayer, T.D. Bradens, R.D. Geisert, and G.L. Morgan. 2003. Influence of estradiol, progesterone, and nutrition on concentrations of gonadotropins and GnRH receptors, and abundance of mRNA for GnRH receptors and gonadotropin subunits in pituitary glands of beef cows. J. Anim. Sci. 81: 269-278

Lucy, M. C., J. Beck, C. R. Staples, H. H. Head., R. L. de la Sota, and W. W. 1992. Follicular dynamics, plasma metabolites, hormones and insulin-like growth factor I (IGF-I) in lactating cows with positive or negative energy balance during the preovulatory period. Reprod. Nutr. Dev. 32: 331-341.

Macmillan, K. L., and R. J. Curnow. 1977. Tail painting-a simple form of oestrus detection in New Zealand dairy herds. N. Z. J. Exp. Agric 5: 357-361.

Malven, P.V., J.R. Parfet, D.W. Gregg, R.D. Allrich, and G.E. Moss. 1986. Relationships among concentrations of four opioid neuropeptides and luteinizing hormone-releasing hormone in neural tissues of beef cows following early weaning. J. Anim. Sci.. 62: 723-733.

Marston, T. T., K. S. Lusby, R. P. Wettemann, and H. T. Purvis. 1995. Effects of feeding energy or protein supplements before or after calving on perfomance of spring-calving cows grazing native range. J. Anim. Sci. 73: 657-664.

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

Mason, H. D., S. Cwyfan-Hughes, J. M. P. Holy, and S. Franks. 1998. Potent inhibition of human ovarian steroidogenesis by insulin-like growth factor binding protein-4 (IGFBP-4). J. Clin. Endocrinol. Metab. 83: 284-287.

Mathew, S. R., W. P. McCaughey, A. D. Kennedy, N. J. Lewis, and G. H. Crow. 1999. Electronic monitoring of mounting behavior in beef cattle on pasture. Can. Vet. J. 40: 796-798.

Matamoros, I. A., N. M. Cox, and A. B. Moore. 1991. Effects of exogenous insulin and body condition on metabolic hormones and gonadotrophin-induced follicular development in prepubertal gilts. J. Anim. Sci. 69: 2081-2091.

Mazerbourg, S., M. T. Overgaard, C. Oxvig, M. Christiansen, C. A. Conover, I. Laurendeau, M. Vidaud, G. Tosser-Klopp, J. Zapf, P. Monget. 2001. Pregnancy-associated plasma protein-A (PAPP-A) in ovine, bovine, porcine, and equine ovarian follicles: Involvement in IGF binding protein-A proteolytic degradation and mRNA expression during follicular development. Endocrinology 142: 5243-5253.

Mazerbourg, S. J. Zapf, R.S. Bar, D.R. Brigstock, L.C. Binnoux and P. Monget 1999. Insulin-like growth factor binding protein-4 proteolytic degradation in ovine preovulatory follicles: Studies of underlying mechanisms. Endocrinology 140: 4175-4184.

Mazerboug, S., J. Zapf, R. S. Bar, D. R. Brigstock, and P. Monget. 2000. Insulin-like growth factor (IGF)-binding protein-4 proteolytic degradation in bovine, equine, and porcine preovulatory follicles: regulation by IGFs and heparin-binding domain-containing peptides. Biol. Reprod. 63: 390-400.

McArdle, C. A., C. Kohl, K. Rieger, I. Groner, and U. Wehrenberg. 1991. Effects of gonadotropins, insulin and insulin-like growth factor I on ovarian oxytocin and progesterone production. Mol. Cell Endocrinol. 78: 211-220.

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

McDougall, S., C.R. Burke, K.L. MacMillan, and N.B. Williamson. 1992. The effect of pretreatment with progesterone on the oestrous response to oestradiol- 17β benzoate in the post-partum dairy cow. In: Proc. N. Z. Soc. Anim. Prod. 52: 2901-2910.

McGowan, M. K., K. M. Andrews, J. Kelly, and S. P. Grossman. 1990. Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. Behav. Neurosci. 104: 373-385.

McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci. 70: 2901-2910.

McGuire, M. A., D. E. Bauman, D. A. Dwyer, and W. S. Cohick. 1995. Nutritional modulation of the somatotropin/insulin-like growth factor system: Response to feed deprivation in lactating cows. J. Nutr. 125: 493-502.

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: 1151-1157.

Mihm, M., E.J. Austin, T.E.M. Good, J.L.H. Ireland, P.G. Knight, J.F. Roche and J.J. Ireland. 2000. Identification of potential intrafollicular factors involved in selection of dominant follicles in heifers. Biol. Reprod. 63: 811-819.

Miklos, G. L. G. 2005. The human cancer genome project-one more misstep in the war on cancer. Nature Biotechnology 23: 535-537.

Minegishi, T., T. Hirakawa, H. Kishi, K. Abe, Y. Abe, T. Mizutani, and K. Miyamoto. 2000. A role of insulin-like growth factor I for follicle-stimulating hormone receptor expression in rat granulosa cells. Biol. Reprod. 62: 325-333.

Monget, P., N. Besnard, C. Huet, C. Pisselet, and D. Monniaux. 1996. Insulin-like growth factor-binding proteins and ovarian folliculogenesis. Hormone Res. 45: 211-217.

Monget, P., S. Mazerbourg, T. Delpuech, M. C. Maurel, S. Maniere, J. Zapf, G. Lalmanach, C. Oxvig, and M. T. Overgaard. 2003. Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation. Biol. Reprod. 68: 77-86.

Monget, P., D. Monniaux, C. Pisset and P. Durand. 1993. Changes in insulin-like growth factor-I (IGF-I), IGF-II, and their binding proteins during growth and atresia of ovine ovarian follicles. Endocrinology 132: 1438-1446.

Monniaux, D., C. Pisselet, and J. Fontaine. 1994. Uncoupling between proliferation and differentiation of ovine granulosa cells in vitro. Biol. Reprod. 46: 497-510.

Morrison, D. G., J. C. Spitzer, and J. L. Perkins. 1999. Influence of prepartum body condition score change on reproduction in multiparous beef cows calving in moderate body condition. J. Anim. Sci. 77: 1048-1054.

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

Muhlhausler, B. S., C. T. Roberts, B. S. Yuen, E. Marrocco, H. Budge, M. E. Symonds, J. R. McFarlane, K. G. Kauter, P. Stagg, J. K. Pearse, and I.C. McMillen. 2003. Determinants of fetal leptin synthesis, fat mass, and circulating leptin concentrations in well-nourished ewes in late pregnancy. Endocrinology 144: 4947-4954.

Murphy, L. J., G. T. Bell, and H. G. Friesen. 1987. Tissue distribution of IGF-I and 2 mRNA in adult rats. Endocrinology 120: 1279-1282.

Murphy, M.G., M.P. Boland, and J.F. Roche. 1990. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J. Reprod. Fertil. 90: 523-533.

Murphy, M.G., W.J. Enright, M.A. Crowe, K. McConnell, L.J. Spicer, M.P. Boland, and J.F. Roche. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. J. Reprod. Fert. 92: 333-338.

Myers, T.R., D.A. Myers, D.W. Gregg, and G.E. Moss. 1989. Endogenous opioid suppression of release of luteinizing hormone during suckling in postpartum anestrous beef cows. Domest. Anim. Endocr. 6: 183-190.

Nancarrow, C. D., H. M. Radford, R. J. Scaramuzzi and T. B. Post. 1977. Responses to injected oestrogen in suckled cows. Theriogenology 8: 192.

Nebel, R. L., W. L. Walker, C. L. Kosek, and S. M. Pandolfi. 1995. Integration o an electronic pressure sensing system for the detection of estrus into daily reproductive management. J. Dairy Sci. 78 (Suppl. 1): 225.

Nebel, R. L., and S. M. Jobst. 1998. Evaluation of systematic breeding programs for lactating dairy cows: A review. J. Dairy Sci. 81: 1169-1174.

Nett, T.M., M.E. Crowder and M.E. Wise. 1984. Role of estradiol in inducing an ovulatory-like surge of luteinizing hormone in sheep. Biol. Reprod. 30: 1208-1215.

Nicholas, B., R. K. Scougall, D. G. Armstrong, and R. Webb. 2002. Changes in insulin-like growth factor binding protein (IGFBP) isoforms during bovine follicular development. Reproduction 124: 439-446.

Nugent, R. A., T. G. Jenkins, A. J. Roberts, and J. Klindt. 1993. Relationship of post-partum interval in mature beef cows with nutritional environment, biological type and serum IGF-I concentrations. Anim. Prod. 56: 193.

NRC. 1996. Nutrient Requirements of Beef Cattle (7th Rev. Ed.). National Academy Press, Washington, D. C.

Nuttick, F. G., Charpigny, P. Mermillod, H. Loosfelt, G. Meduri, S. Freret, B. Grimard and Y. Heyman et al. 2004. Expression of components of the insulin-like growth factor system and gonadotropin receptors in bovine cumulus-oocyte complexes during oocyte maturation. Domest. Anim. Endocr. 27: 179-195.

O'Connell, C. M., R. P. Wettemann, and D. K. Bishop. 1990. LH in serum of heifers immunized against GnRH and pulsed with a GnRH analog. J. Anim. Sci.. 68 (Suppl. 1): 416 (Abstr.).

Orlowski, C. C., A. L. Brown, G. T. Ooi, Y. W. Yang, L. Y. H. Tseng, and M. M. Rechler. 1990. Tissue, developmental, and metabolic regulation of messenger ribonucleic acid encoding a rat insulin-like growth factor-binding protein. Endocrinology 126: 644-652.

Osoro, K., and I. A. Wright. 1992. The effect of body condition, live weight, breed, age, calf performance, and calving date on reproductive performance of spring-calving beef cows. J. Anim. Sci. 70: 1661-1665.

Oxvig, C., O. Sand, T. Kristensen, G. J. Gleich, and L. Sottrup-Jensen. 1993. Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. J. Biol. Chem. 268: 12243-11246.

Pehrson, B., K. Plym Forshell, and J. Carlsson. 1992. Zentralbl Veterinarmed A. 39: 187-192.

Pennington, J. A., J. L. Albright, and C. J. Callahan. 1986. Relationships of sexual activities in estrous cows to different frequencies of observation and pedometer measurements. J. Dairy Sci. 69: 2925-2934.

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

Pierson, R. A., and O. J. Ginther. 1988. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 29: 21-37.

Poretsky, L., and M. Kalin. 1987. The gonadotropic function of insulin. Endocr. Rev. 8: 132-141.

Poretsky, L., N. A. Cataldo, Z. Rosenwaks, and L. C. Giudice. 1999. The insulin-related ovarian regulatory system in health and disease. Endocr. Rev. 20: 535-582.

Prado, T. M., R. P. Wettemann, L. J. Spicer, J. A. Vizcarra, and G. L. Morgan. 2002. Influence of exogenous gonadotropin-releasing hormone on ovariafunction in beef cows after short- and long-term nutritionally induced anovulation. J. Anim. Sci. 80: 3268-3276.

Rabiee, A. R., I. J. Lean, J. M. Gooden and B. G. Miller. 1999. Relationships among metabolites influencing ovarian function in the dairy cow. J. Dairy Sci. 82: 39-44.

Radcliff, R. P. M.J. VandeHaar, Y. Kobayashi, B.K. Sharma, H.A. Tucker, M.C. and Lucy. 2004. Effect of dietary energy and somatotropin on components of the somatotropic axis in Holstein heifers. J. Dairy Sci. 87: 1229-1235.

Radford, H.M., C. D. Nancarrow and P.E. Mattner. 1978. Ovarian function in suckling and non-suckling beef cows postpartum. J. Reprod. Fertil. 54: 49-56.

Rae, D. O., P. J. Chenoweth, M. A. Giangreco, P. W. Dixon, and F. L. Bennett. 1999. Assessment of estrus detection by visual observation and electronic detection methods and characterization of factors associated with estrus and pregnancy in beef heifers. Theriogenology 51: 1121-1132.

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

Rakestraw, J., K. S. Lusby, R. P. Wettemann, and J. J. Wagner. 1996. Postpartum weight and body condition loss and performance of fall-calving cows. Theriogenology 26: 461-473.

Randel, R. D. 1990. Nutrition and postpartum rebeeding in cattle. J. Anim. Sci. 1990: 853-862.

Rausch, M. I.,M. W. Tripp, K. E. Govoni, W. Zang, W. J. Weber, B. A. Crooke, T. A. Hoagland, and S. A. Zinn. 2002. The influence of level of feeding on growth and serum insulin-like growth factor i and insulin-like growth factor-binding proteins in growing beef cattle supplied with somatotropin. J. Anim. Sci. 80: 94-100.

Reames, P.S., T.B. Hatler and W.J. Silvia. 2005. Behavioral and endocrine responses to estradiol-17β in Holstein Cows. J. Anim. Sci. Suppl. 1. 83: 37 (Abstr).

Rechler, M. M., and S. P. Nissley. 1990. Insulin-like growth factors. Handbook of Experimental Pharmacology 95: 263-281.

Reimers, T. J., R. D. Smith, and S. K. Newman. 1985. Management factors affecting reproductive performance of dairy cows in the northeastern united states. J. Dairy Sci. 68: 963-973.

Reynolds, C. K., and H. F. Tyrrell. 2000. Energy metabolism in lactating beef heifers. J. Anim. Sci. 78: 2696-2705.

Rhodes, F. M., L. A. Fitzpatrick, K. W. Entwistle, and G. De'ath. 1995. Sequential changes in ovarian follicular dynamics in Bos indicus heifers before and after nutritional anoestrus. J. Reprod. Fertil. 104: 41-49.

Rhodes, F. M., A. J. Peterson, and P. D. Jolly. 2001. Gonadotrophin responsiveness, aromatase activity and insulin-like growth factor binding protein content of bovine ovarian follicles during the first follicular wave. Reproduction 122: 561-569.

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

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

Richards, M. W., R. P. Wettemann, L. J. Spicer, and G. L. Morgan. 1991. Nutritionally anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biol. Reprod. 44: 961-966.

Richards, M. W., L. J. Spicer, and R. P. Wettemann. 1995. Influence of diet and ambient temperature on bovine serum insulin-like growth factor-i and thyroxine: Relationships with non-esterified fatty acids, glucose, insulin, luteinizing hormone and progesterone. Anim. Reprod. Sci. 37: 267-279.

Rispoli, L. A., and T. M. Nett. 2005. Pituitary gonadotropin-releasing hormone (GnRH) receptor: Structure, distribution and regulation of expression. Anim. Reprod. Sci. 88: 57-74.

Rivera, G. M., Y. A. Chandrasekher, A. C. Evans, L. C. Giudice, and J. E. Fortune. 2001. A potential role for insulin-like growth factor binding protein-4 proteolysis in the establishment of ovarian follicular dominance in cattle. Biol. Reprod. 102-111.

Rivera, G. M., and J. E. Fortune. 2003. Proteolysis of insulin-like growth factor binding proteins -4 and -5 in bovine follicular fluid: Implications for ovarian follicular selection. Endocrinology 144: 2977-2987.

Roberts, A. J., R. A. N. III, J. Klindt, and T. G. Jenkins. 1997. Circulating insulin-like growth factor I, insulin-like growth factor binding proteins, growth hormone, and resumption of estrus in postpartum cows subjected to dietary energy restriction. J. Anim. Sci. 75: 1909-1917.

Roberts, A. J., J. Klindt, and T. G. Jenkins. 2005. Effects of varying energy intake and sire breed on duration of postpartum anestrus, insulin like growth factor-1, and growth hormone in mature crossbred cows. J. Anim. Sci. 83: 1705-1714.

Robinson, J. J. 1990. Nutrition in the reproduction of farm animals. Nutr. Res. Rev. 3: 253-276.

Robinson, J.J. 1996. Nutrition and reproduction. Anim. Reprod. Sci. 42 25–34.

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

Rosenbaum, M., M. Nicolson, J. Hirsch, S.B. Heymsfield, D. Gallagher, F. Chu, and R.L. Leibel. 1996. Effects of gender, body composition, and menopause on plasma concentrations of leptin. J. Clin. Endocrinol. Metab. 81: 3424-3427.

Rosenfeld, R. G., V. Hwa, L. Wilson, A. Lopez-Bermejo, C. Buckway, C. Burren, W.K. Choi, G. Devi, A. Ingermann, D. Graham, G. Minniti, A. Spagnoli, and Y. Oh. 1999. The insulin-like growth factor binding protein superfamily: New perspectives. Pediatrics 104: 1018-1020.

Rosenfeld, C. S., X. Yuan, M. Manikkam, M. D. Calder, H. A. Garverick, and D. B. Lubahn. 1999. Cloning, sequencing, and localization of bovine estrogen receptor-β within the ovarian follicle. Biol. Reprod. 60: 691-697.

Ross, J.W., M. D. Ashworth, A. G. Hurst, J. R. Malayer, and R. D. Geisert. 2003. Analysis and characterization of differential gene expression during rapid trophoblastic elongation in the pig using suppression substactive hybridization. Reprod. Biol. Endocrinol. 1: 13-

Rothwell, N. J., and M. J. Stock. 1988. Insulin and thermogenesis. Int. J. Obes. 12: 93-102.

Rowlands, T. M., J. M. Symonds, R. Farrookhi, and O. W. Blaschuk. 2000. Cadherins: Crucial regulators of structure and function in reproductive tissues. Rev. Reprod. 5: 53-61.

Russel, A. J. F., and I. A. Wright. 1983. The use of blood metabolites in the determination of energy status in beef cows. Anim. Prod. 37: 335-343.

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

Rutter, L. M., and J. G. Manns. 1991. Insulin-like growth factor i in follicular development and function in postpartum beef cows. J. Anim. Sci. 69: 1140-1146.

Rutter, L. M. and R. D. Randel. 1984. Postpartum nutrient intake and body condition: effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58: 265-274.

Rutter, L.M. and R.D. Randel. 1986. Nonpuberal estrus in beef heifers. J. Anim. Sci.. 63: 1049-1053.

Samoto, T., T. Maruo, C.A. Ladines-Llave, H. Matsuo, J. Deguchi, E.R. Barnea, and M. Mochizuk. 1993. Insulin receptor expression in follicular and stromal compartments of the human ovary over the course of follicular growth, regression and atresia. Endocr. J. 40: 715-726.

Santiago, C. A., J. L. Voge, P. Y. Aad, D. T. Allen, D. R. Stein, J. R. Malayer, L. J. Spicer. 2005. Pregnancy-associated plasma protein-a and insulin-like growth factor binding protein mRNAs in granulosa cells of dominant and subordinate follicles of preovulatory cattle. Domest. Anim. Endocr. 28: 46-63.

SAS. 2003. SAS/STAT User's Guide (Release 8.0). SAS Inst Inc., Cary, N.C.

Sawyer, G. L., I. D. Russell-Brown, and J. K. Silcock. 1986. A comparison of three methods of oestrus detection in commercial dairy herds verified by serum progesterone analysis. Anim. Reprod. Sci. 10: 1-10.

Schallenberger, E. 1985. Gonadotrophins and ovarian steroids in cattle. III. Pulsatile changes of gonadotrophin concentrations in the jugular vein postpartum. Acta Endocrinol. (Copenh) 109: 37-43.

Schams, D., B. Berisha, M. Kosmann, R. Einspanier, and W. M. Amselgruber. 1999. Possible role of growth hormone, IGFs, and IGF-binding proteins in the regulation of ovarian function in large farm animals. Domest. Anim. Endocr. 17: 279-285.

Schams, D., and B. Berisha. 2002. Steroids as local regulators of ovarian activity in domestic animals. Domest. Anim. Endocr. 23: 53-65.

Shively, T.E., and G.L. Williams. 1989. Patterns of tonic luteinizing hormone release and ovulation frequency in suckled anestrous beef cows following varying intervals of temporary weaning. Domest. Anim. Endocr. 6: 379-387.

Schoenemann, H.M., W.D. Humphrey, M.E. Crowder, T.M. Nett, and J.J. Reeves. 1985. Biol. Reprod. 32: 574-583.

Schoppee, P. D., J. D. Armstrong, R. W. Harvey, M. D. Whitacre, and R. M. Campbell. 1996. Immunization against growth hormone releasing factor or chronic feed restriction initiated at 3.5 months of age reduces ovarian response to pulsatile administration of GnRH at 6 months of age and delays onset of puberty in heifers. Biol. Reprod. 55: 87-98.

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

Short, R. E., R. D. Randel, R. B. Staigmiller, and R. A. Bellows. 1979. Factors affecting estrogen-induced LH release in the cow. Biol. Reprod. 21: 683-689.

Schrick, F. N., J. C. Spitzer, T. Gimenez, D. M. Henricks, T. C. Jenkins, and B. B. Plyler. 1992. Is nutritional anestrus precipitated by subfunctional corpora lutea in beef cows? Domest. Anim. Endocr. 9: 187-197.

Schwartz, M. W., D. P. Figlewicz, D. G. Baskin, S. C. Woods, and D. Porte. 1992. Insulin in the brain: A hormonal regulator of energy balance. Endocr. Rev. 13: 387-414.

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: 3153-3159.

Senger, P. L. 1994. The estrus detection problem: New conceptus, technologies, and possibilities. J. Dairy Sci. 77: 2745-2753.

Sharma, B. K., M. J. Vandehaar, and N. K. Ames. 1994. Expression of insulin-like growth factor-I in cows at different stages of lactation and in late lactation cows treated with somatotropin. J. Anim. Sci. 77: 2232-2241.

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., R. A. Bellows, E. L. Moody, and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. J. Anim. Sci. 34: 70-78.

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: 799-816.

Sierra-Honigmann M. R., A. K. Nath, C. Murakami, G. Garcia-Cardena, A. Papapetropoulos, W. C. Sessa, L. A. Madge, J. S. Schechner, M. B. Schwabb, P. J. Polverini, and J. R. Flores-Riveros. Biological action of leptin as an angiogenic factor. 1998. Science 281: 1683-1286.

Silveira, P.A., R.A. Spoon, and G.L. Williams. 1993. Evidence for maternal behavior as a requisite link in suckling-mediated anovulation in cows. Biol. Reprod. 49: 1338-1346.

Silvia, W. J., R. W. Hemken, and T. B. Hatler. 2002. Timing of onset of somatotropin supplementation on reproductive performance in dairy cows. J. Dairy Sci. 85: 384-389.

Simpson, R. B., C.C. Chase Jr , L.J. Spicer, R.K. Vernan, A.L. Hammond, and D.O. Rae. 1994. Effects of exogenous insulin on plasma and follicular insulin like growth factor I, insulin like growth factor binding activity, follicular oestradiol and progesterone and follicular growth in superovulated Angus and Brahman cows. J. Reprod. Fertil. 102: 483-492.

Sinclair, K. D., P. J. Broadbent, and J. S. M. Hutchinson. 1994. The effect pre- and post-partum energy and protein supply on the blood metabolites and reproductive performance of single- and twin-suckling beef cows. Anim. Prod. 59: 391-400.

Singh, B. and Armstrong, D. T. 1997. Insulin-like growth factor-1, a component of serum that enables porcine cumulus cells to expand in response to follicle-stimulating hormone in vitro. Biol. Reprod. 56: 1370-1375.

Smith, M. F., W.C. Burrell, L.D. Shipp, L.R. Sprott, W.N. Songster, and J.N. Wiltbank. 1979. Hormone treatments and use of calf removal in postpartum beef cows. J. Anim. Sci. 48: 1285-1294.

Smith, W. J., L. E. Underwood, and D. R. Clemmons. 1995. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J. Clin. Endocrinol. Metab. 80: 443-449.

Snyder, J.L., J.A. Clapper, A. J. Roberts, D. W. Sanson, D. L. Hamernik, and G. E. Moss. 1999. Insulin-like growth factor-I, insulin-like growth factor-binding proteins, and gonadotropins in the hypothalamic-pituitary axis and serum of nutrient-restricted ewes. Biol. Reprod. 61: 219-224.

Soliman, A. T., A.E. Hassan, M. K. Aref, R. L. Hintz, R. G. Rosenfeld, and A. D. Rogol. 1986. Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. Pediatr. Res. 20: 1122-1130.

Soldani, R., A. Cagnacci, and S. S. Yen. 1994. Insulin, insulin-like growth factor I (IGF-I) and IGF-II enhance basal and gonadotrophin-releasing hormone-stimulated luteinizing hormone release from rat anterior pituitary cells in vitro. Eur. J. Endocrinol. 131: 641-645.

Spicer, L. J. 2001. Leptin: a possible metabolic signal affecting reproduction. Domest. Anim. Endocrinol. 21: 251-270.

Spicer, L. J. 2004. Proteolytic degradation of insulin-like growth factor binding proteins by ovarian follicles: A control mechanism for selection of dominant follicles. Biol. Reprod. 70: 1223-1230.

Spicer, L. J. 2001. Receptors for insulin-like growth factor-I and tumor necrosis factor-α are hormonally regulated in bovine granulosa and thecal cells. Anim. Reprod. Sci. 67: 45-58.

Spicer, L. J., E. M. Convey, H. A. Tucker, and S. E. Echternkamp. 1986. Effects of intermittent injections of LHRH on secretory patterns of LH and FSH and ovarian follicular growth during postpartum anovulation in suckled beef cows. J. Anim. Sci. 62: 1317-1323.

Spicer, L. J., and S. E. Echternkamp. 1986. Ovarian follicular growth, function and turnover in cattle: A review. J. Anim. Sci. 62: 428-451.

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

Spicer, L. J., and W. J. Enright. 1991. Concentrations of insulin-like growth factor I and steroids in follicular fluid of preovulatory bovine ovarian follicles: effects of daily injections of a growth hormone-releasing factor analog and (or) thyrotropin-releasing hormone. 69: 1133-1139.

Spicer, L. J. and Stewart. 1996. Interaction among bovine somatotropin, insulin, and gonadotropins on steroid production by bovine granulosa and thecal cells. J. Dairy Sci. 79: 813-821.

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, ovarian activity, and estrous behavior. J. Dairy Sci. 73: 929-937.

Spicer, L. J., W. J. Enright, M. G. Murphy, and J. F. Roche. 1991. Effect of dietary intake on concentrations of insulin-like growth factor-I in plasma and follicular fluid, and ovarian function in heifers. Domest. Anim. Endocr. 8: 431-437.

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

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

Spicer, L. J., E. Alpizar, and R. E. Stewart. 1994. Evidence for an inhibitory effect of insulin-like growth factor-I and -II on insulin-stimulated steroidogenesis by nontransformed ovarian granulosa cells. Endocrine 2: 735-739.

Spicer, L. J., P. Alvarez, T. M. Prado, G. L. Morgan, and T. D. Hamilton. 2000. Effects of intraovarian infusion of insulin-like growth factor-i on ovarian follicular function in cattle. Domest. Anim. Endocr. 18: 265-278.

Spicer, L. J., C. S. Chamberlain, and G. L. Morgan. 2001. Proteolysis of insulin-like growth factor binding proteins during preovulatory follicular development in cattle. Domest. Anim. Endocr. 21: 1-15.

Spicer, L. J., J. C.C. Chase, and L. M. Rutter. 2002. Relationship between serum insulin-like growth factor-I and genotype during the postpartum interval in beef cows. J. Anim. Sci. 80: 716-722.

Spicer, L. J., C. A. Santiago, T. R. Davidson, T. S. Bridges, and C. S. Chamberlain. 2005. Follicular fluid concentrations of free insulin-like growth factor (IGF)-I during follicular development in mares. Domest. Anim. Endocr. 29: 573-581.

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

Stagg, K., M. G. Diskin, J. M. Sreenan, and J. F. Roche. 1995. Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. Anim. Reprod. Sci. 38: 49-61.

Stagg, K., L. J. Spicer, J. M. Sreenan, J. F. Roche, and M. G. Diskin. 1998. Effect of calf isolation on follicular wave dynamics, gonadotropin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. Biol. Reprod. 59: 777-783.

Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73: 938-947.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81: 856-871.

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

Stewart, R.E., L.J. Spicer, T.D. Hamilton, B.E. Keefer, L.J. Dawson, G.L. Morgan, and S.E. Echternkamp. 1996. Levels of insulin-like (IGF) growth factor binding proteins, luteinizing hormone, and IGF-I receptors, and steroids in dominant follicles during the first follicular wave in cattle exhibiting regular estrous cycle. Endocrinology 137: 2842-2850.

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

Swanson, L.V., and S. K. McCarthy. 1978. Estradiol treatment and luteinizing hormone (LH) response of prepuberal Holstein heifers. Biol. Reprod. 18: 475-480.

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

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

Sunderland, S. J., M. A. Crowe, M. P. Boland, J. F. Roche, and J. J. Ireland. 1994. Selection, dominance and atresia of follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 101: 547-555.

Taylor, V.J., D. E. Beever, M. J. Bryant, and D. C. Wathes. 2004. First lactation ovarian function in dairy heifers in relation to prepubertal metabolic profiles. 2004. J. Endocrinol. 180: 63-75.

Teirmaa, T., V. Luukkaa, J. Rouru, M. Koulu, and R. Huupponen. 1998. Correlation between circulating leptin and luteinizing hormone during menstrual cycle on normal-weight women. Europ. J. Endocrinol. 139: 190-194.

Tian, X. C., A. K. Berndtson, and J. E. Fortune. 1995. Differentiation of bovine preovulatory follicles during the follicular phase is associated with increases in messenger ribonucleic acid for cytochrome P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase, and P450 17α- hydroxylase, but not P450 aromatase. Endocrinology 136: 5102-5110.

Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocr. Rev. 15: 80-101.

Trout, W.E., and P. V. Malven. 1988. Quantification of naloxone binding sites in brains from suckled beef cows during postpartum anestrus and resumption of estrous cycles. J. Anim. Sci. 66: 954-960.

Tsai, S.-J., and M. C. Wiltbank. 1998. Prostaglandin f2a regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. Biol. Reprod. 58: 346-352.

Turzillo, A. M., and J. E. Fortune. 1990. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J. Reprod. Fertil. 89: 643-653.

Tseng, L. Y., G. T. Ooi, A. L. Brown, D. S. Straus, and M. M. Rechler. 1992. Transcription of the insulin-like growth factor-binding protein-2 gene is increased in neonatal and fasted adult rat liver. Mol. Endocrinol. 6: 1195-1201.

Urban, R.J., M. A. Shupnik, and Y. H. Bodenburg. 1994. Insulin-like growth factor-I increases expression of the porcine P-450 cholesterol side chain cleavage gene through a GC-rich domain. J. Biol. Chem. 269: 25761-25769.

Vailes, L. D., S. P. Washburn, and J. H. Britt. 1992. Effects of various steroid milieus or physiological states on sexual behavior of Holstein cows. J. Anim. Sci. 70: 2094-2103.

Van Dessel, H.J., I. Schipper, T. D. Pache, van Geldorp, H., de Jong, F. H. and B. C. Fauser. 1996. Normal human follicle development: an evaluation of correlations with oestradiol, androstenedione and progesterone levels in individual follicles. Clin. Endocrinol. (Oxf.) 44: 191-198

Van Houten, M., B. I. Posner, B. M. Kopriwa, and J. R. Brawer. 1979. Insulin-binding sites in the rat brain: in vivo localization to the circumventricular organs by quantitative autoradiography. Endocrinology 105: 666-673.

Vandehaar, M. J., B. K. Sharma, and R. L. Fogwell. 1995. Effect of dietary energy restriction on the expression of insulin-like growth factor 1 in liver and corpus luteum. J. Dairy Sci. 78: 832-841.

Venzke, W. G. 1953. Efficacy of estradiol cyclopentylpropionate (ECP) in anestrous ewes. Am. J. Vet. Res. 52: 411-414.

Vestergaard, M., S. Purup, P. Henckel., E. Tonner, D. J. Flint, L. R. Jensen, K. Sejrsen. 1995. Effects of growth hormone and ovariectomy on performance, serum hormones, insulin-like growth factor-binding proteins, and muscle fiber properties of prepubertal Friesian heifers. J. Anim. Sci.. 73: 3574-3584.

Vicini, J.L., F.C. Buonomo, J.J. Veehuizen, M.A. Millar, D.R. Clemmons, R.J. Collier. 1991. Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. J. Nutr. 121: 1656-1164.

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

Vizcarra, J. A., R. P. Wettemann, J. C. Spitzer, and D. G. Morrison. 1996. Body condition at parturition and postpartum weight gain influence luteal activity, glucose, insulin and NEFA concentrations in primiparous beef cows. J. Anim. Sci. 74 (Suppl. 1): 12.

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

Voge, J. L., C. A. T. Santiago, P. Y. Aad, D. W. Goad, J. R. Malayer, J. R. and L. J. Spicer. 2004. Quantification of insulin-like growth factor binding protein mRNA using real-time PCR in bovine granulosa and theca cells: Effect of estradiol, insulin, and gonadotropins. Domest. Anim. Endocr. 26: 241-258.

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

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

Wang, J., R. Liu, M. Hawkins, N. Barzilai, and L. Rosseti. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature. 393: 684-688.

Wang, X., Y. Yanzhu, M. L. Adamo, X. Wang, and Y. Z. Yang. 1997. Characterization of the rat IGF-I gene promoters and identification of a minimal exon-2 promoter. Endocrinology 138: 1528-1536.

Warnick, A. C., D. D. Hargrove, F. M. Peacock, and H. L. Chapman. 1981. Effects of pre-wintering condition score and winter feed levels on pregnancy rate of Brahman cows and calf weaning weight. J. Anim. Sci. 53 (Suppl.1).

Wathes, D. C., C. M. Perks, A. J. Davis, and P. A. Denning-Kendall. 1995. Regulation of IGF-I and progesterone synthesis by insulin and growth hormone in bovine ovary. Biol. Reprod. 52: 882-889.

Webb, R., P. C. Garnsworthy, J. G. Gong, R. S. Robinson, and D. C. Wathes. 1999. Consequences for reproductive function of metabolic adaptation to load metabolic stress in dairy cows. Occasional Publication 24 Brit. Soc. Anim. Sci. Pencaitland, U.K.: 99-122.

Webb, R., P. C. Garnsworthy, J.-G. Gong, and D. G. Armstrong. 2004. Control of follicular growth: Local interactions and nutritional influences. J. Anim. Sci. 82 (Suppl.): E63-E74.

Webb, R., B. Nicholas, J. G. Gong, B. K. Campbell, C. G. Gutierrez, H. A. Garverick, and D. G. Armstrong. 2003. Mechanisms regulating follicular development and selection of the dominant follicle. Reprod. Suppl. 61: 71-90.

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

Wettemann, R.P. 1980. Postpartum endocrine function of cattle, sheep and swine. J. Anim. Sci. 51 (Suppl. 2): 2-14.

Wettemann, R.P. 1994. Precalving nutrition/birth weight interaction and rebreeding efficiency. Oklahoma State University (Ed.), Anim. Sci. Res. Report.

Wettemann, R. P., and I. Bossis. 2000. Energy intake regulates ovarian function in beef cattle. Proc. Am. Soc. Anim. Sci., 1999. Available at: htpp://asas.org/jas/symposia/proceedings/0938.pdf. Accessed Dec. 8, 2005.

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

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

Wettemann, R. P., C. A. Lents, N. H. Ciccioli, F. J. White, and I. Rubio. 2003. Nutritional- and suckling-mediated anovulation in beef cows. J. Anim. Sci. 81 (E.Suppl.2): E48-E59.

Wettemann, R.P., K.S. Lusby, J.C. Garmendia, M.W. Richards, G.E. Selk, and R.J. Rasby. 1986. Postpartum nutrition, body condition and reproductive performance of first calf heifers. Okla. Agric. Exp. Sta. Res. Rep. MP-118: 314-315.

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

Whisnant, C.S., T. E. Kiser, F. N. Thompson, and C. R. Barb. 1986b. Opiod inhibition of luteinzing hormone secretion during the postpartum period in suckled beef cows. J. Anim. Sci. 63: 1445-1448.

Whisnant, C.S., F. N. Thompson, T. E. Kiser, and C. R. Barb. 1986c. Effect of Naloxone on serum luteinizing hormone, Cortisol and prolactin concentrations in anestrous beef cows. J. Anim. Sci.. 62: 1340-1345.

White, F. J. 2004. Effects of body condition score and postpartum interval on ovarian function of anestrous beef cows. PhD Thesis. Oklahoma State University, Stillwater, Oklahoma.

White, F. J., C. A. Lents, N. H. Ciccioli, R. P. Wettemann, and L. J. Spicer. 2001. Concentrations of leptin and insulin like growth factor-I (IGF-I) during acute nutritionally induced anovulation and realimentation. J. Anim. Sci. 79 (Suppl. 1): 34 (Abstr.).

White, F. J., I. Rubio, C. A. Lents, N. H. Ciccioli, R. P. Wettemann, and L. J. Spicer 2003. Insulin like growth factor-I (IGF-I), IGF binding proteins (IGFBP) and steroids in dominant follicles of postpartum beef cows. J. Anim. Sci. 81 (Supp. 1): 119 (Abstr.).

White, F. J., R. P. Wettemann, M. L. Looper, T. M. Prado, and G. L. Morgan. 2002. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. J. Anim. Sci. 80: 3053-3059.

Whitley, N C., M. Thomas, J. L. Ramirez, A. B. Moore, and N. M. Cox. 2002. Influences of parity and level of feed intake on reproductive response to insulin administration after weaning in sows. J. Anim. Sci. 80: 1038-1043.

Williams, G. L. 1990. Suckling as a regulator of postpartum rebreeding in cattle: A review. J. Anim. Sci. 68: 831-852.

Williams, G.L., and M. K. Griffith. 1995. Sensory and behavioral control of gonadotrophin secretion during suckling-mediated anovulation in cows. J. Reprod. Fertil. Suppl. 49: 463-475.

Williams, G.L., J. Kotwica, W. D. Slanger, D. K. Olson, J. E. Tilton, and L. J. Johnson. 1982. Effect of suckling on pituitary responsiveness to gonadotropin-releasing hormone throughout the early postpartum period of beef cows. J. Anim. Sci. 54: 594-602.

Williams, G. L., F. Talavera, B. J. Petersen, 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 of systematic progesterone. Biol. Reprod. 29: 362-373.

Willis, D., H. Mason, C. Gilling-Smith, and S. Franks. 1996. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. J. Clin. Endocrinol. Metab. 81: 302-309.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1962. Effect of energy level on reproductive performance phenomena on mature Hereford cows. J. Anim. Sci. 21: 219-225.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, and D. R. Zimmerman. 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23: 1049-1053.

Wiltbank, M. C., A. Gumen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology 57: 21-52.

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: 1007-1013

Woody, C. O. N. L. First, and A. I. Pope. 1967. Effect of exogenous progesterone on estrous cycle length. J. Anim. Sci. 26: 139-141.

Wright,I.A., .M. Rhind, J.F. Russel, T.K. Whyte, A.J. McBean, and S.. McMillen. 1987. Effects of body condition, food intake and temporary calf separation on the duration of the post-partum anoestrus period and associated LH, FSH, and prolactin concentrations in beef cows. Anim. Prod. 45: 395-402.

Wright, I. A., S. M. Rhind, A. J. Smith, and T. K. Whyte. 1992. Effects of body condition and estradiol on luteinizing hormone secretion in post-partum beef cows. Domest. Anim. Endocr. 9: 305-312.

Xu, Z. Z., D. J. McKnight, R. Vishwanath, C. J. Pitt, and L. J. Burton. 1997. Estrus detection using radiotelemetry or visual observation and tail painting of dairy cows on pasture. J. Dairy Sci. 81: 2890-2896.

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

Yelich, J. V., R. P. Wettemann, T. T. Marston, and L.J.Spicer. 1996. Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. Domest. Anim. Endocr. 13: 325-338.

Yonekura, S., K. Kitade, G. Furukawa, K. Takahashi, N. Katsumata, K. Katoh,Y. and Obara. 2002. Effects of aging and weaning on mRNA expression of leptin and CCK receptors in the calf rumen and abomasum. Domest. Anim. Endocr. 22: 25-35.

Yonekura, S., T. Senoo, Y. Kobayashi, T. Yonezawa, K. Katoh, and Y. Obara. 2003. Effects of acetate and butyrate on the expression of leptin and short- form leptin receptor in bovine and rat anterior pituitary cells. Gen. Com. Endocrinol. 133: 165-172.

Zalesky, D. D., D.W. Forrest, N. H. McArthur, J. M. Wilson, D. L. Morris, and P. G. Harms. 1990. Suckling inhibits release of luteinizing hormone-releasing hormone from the bovine median eminence following ovariectomy. J. Anim. Sci. 68: 444-448.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425-431.

Zieba, D. A., M. Amstalden, and G. L. Williams. 2005. Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest. Anim. Endocr. 166-185.

Zulu, V. C., Y. Sawamukai, K. Nakada, K. Kida, and M. Moriyoshi. 2002. Relationship among insulin-like growth factor-I, blood metabolites and postpartum ovarian function in dairy cows. J. Vet. Med. Sci. 64: 879-885.

VITA

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Scope and Method of Study: Effects of nutrition on insulin-like growth factor-I (IGF-I) and insulin in plasma and dominant follicles (DF), and IGF binding proteins in DF were evaluated in anovulatory primiparous Angus x Hereford cows after calving. Cows were assigned to one of two postpartum nutritional treatments: maintain (M), 2.27 kg of a 40% CP supplement per day and ad libitum hay; or gain (G), ad libitum access to a 50 % concentrate diet and hay. Ovarian follicles were aspirated by ultrasonography guided needle at 72 ± 2d (n= 12) and at 56 ± 9 (n= 28d) after calving. The effect of treatment of postpartum anestrous beef cows with gonadotropin releasing hormone (GnRH) or estradiol on onset of first estrus and luteal activity was evaluated. Thirty-four cows were assigned to one of three treatments: GnRH, estradiol cypionate, or control. Estrous behavior and ovarian luteal activity were evaluated.

Findings and Conclusions: Body condition score at aspiration of the DF was greater for H than M cows and postpartum interval to estrus with luteal activity was longer for M than for H cows. Maximum size of DF was influenced by nutritional treatment. Concentrations of IGF-I and insulin in FF and plasma were greater for H than M. Concentrations of insulin in FF and plasma were greater for G than M cows in Exp. 1 and Exp 2. These results indicate that concentrations of IGF-I and insulin in FF are influenced by nutritional intake and may be related to follicular function. Concentration of IGF binding proteins -4 and -5 were greater in G than M cows. Changes in FF IGF binding proteins may have a role in dietary induced changes in postpartum follicular growth. More cows treated with GnRH had luteal activity during 1 to 10 d after treatment. Percentage of cows detected in estrus within 6 d after treatment was greater for estradiol than GnRH or control cows, and was similar for GnRH and control cows. Body condition score at calving did not influence the effect of treatment on estrus and luteal activity. Treatment of anestrous beef cows with GnRH initiated short luteal activity without estrus, indicating that GnRH caused an ovulatory surge of LH. Treatment with estradiol increased the incidence of estrus without altering luteal activity. Our results indicate that concentrations of IGF-I and insulin in FF are influenced by nutritional intake and may be related to follicular function. Follicles did not ovulate in response to estradiol and an ovulatory surge of LH was not induced at this stage postpartum in anestrous beef cows.

ADVISER'S APPROVAL: Dr. Robert P. Wettemann